

SUBMISSION:

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THE CLINICAL AND LABORATORY EFFECTS OF INTRAVENOUS FLUIDS ON HAEMOSTASIS

ABSTRACT

The intravenous administration of fluids in shock is life saving. However, there is increasing controversy as to the rationale for the normalisation of blood pressure in the treatment of shock with either colloids or crystalloids.

Increasing the blood pressure by either the administration of colloids or crystalloids may also impair the formation of new blood clots and also dislodge existing ones and thus contribute to ongoing haemorrhage. The main aim of this thesis was to investigate the intrinsic and volumetric effect that these different intravenous resuscitation fluids have on the haemostatic process and bleeding.

A variety of studies including in vitro mechanistic investigations, electron microscopy, randomised trials, randomised and double blinded trials were carried out. These were supported by epidemiological and systematic literature reviews to clarify the controversy.

INTRODUCTION:

The work and publications, which I am submitting for my MD, was carried out between 1993 and 2002. At all stages I was the main instigator of planning and research. However, a number of people helped me with different aspects of the research.

The work began initially when I was a Research Fellow in the Trauma Centre at Johannesburg Hospital. I subsequently continued with this on-going research at both the Leicester University Hospitals NHS Trust and the Department of Haemostasis and Thrombosis at the Queen's Medical Centre, Nottingham.

When I started the research, there was a limited body of knowledge on how intravenous fluids affect haemostasis in trauma. Following my initial and subsequent publications, there have been further developments and a growth in the knowledge and interest in this field. As a consequence I have modified my research by utilising this new knowledge and technology as it has become available. The findings in my publications should therefore be considered in the context of the state of knowledge at the time the work was carried out.

The work submitted investigated both the laboratory and clinical effects that intravenous fluids, Haemaccel, Gelofusine, albumin and normal saline have on the haemostatic process. My research has specifically focused on the gelatin colloids, particularly Haemaccel, with its high calcium content (6.25 mmol/L) comparing its effects with the similar non-calcium containing gelatin, Gelofusine, and the physiological colloid albumin and isotonic normal saline.

My in vitro work was developed to elucidate the mechanisms by which these fluids affect the various parts of the haemostatic process during trauma, and as a prelude to a randomised clinical trial.

As knowledge developed and clinical practice evolved, I undertook a number of epidemiological studies to ensure that my laboratory and clinical research remained focused and up to date. This ensured that the scope of the research was relevant to my on-going investigations and current practice.

My initial hypothesis was that a large volume of fluid replacement and the type of colloid administered may have a detrimental effect on clinical outcome, and thus hypotensive resuscitation could be a more favourable alternative. Epidemiological surveys, meta-analysis and systematic review of literature were carried out to test this hypothesis, and where possible the findings were applied to my laboratory and clinical studies.

BACKGROUND OF THE RESEARCH:

Intravenous fluid administration to correct haemorrhage and hypovolaemia has been a long established way of maintaining the blood pressure, improving tissue perfusion and treating shock.¹ In the early part of the century, Ringer (1834-1910), and later Hartmann (1898-1964) were the first to develop isotonic crystalloid solution. This initial work was the beginning of the development of fluid replacement regimes for the future.^{2, 3, 4, 5, 6, 7}

Following the establishment of isotonic crystalloid solution, further progress in this field led to the development of iso-oncotic colloids such as the synthetic Dextrans and physiological human albumin.^{8,9} Subsequently, there were further improvements and modifications of these fluids leading to the development of the new synthetic iso-oncotic colloids such as the new generation starches and the gelatin solutions.^{10,11,12,13,14,15} These new gelatin based iso-oncotic colloids (Gelofusine and Haemaccel) were thought to give the clinician a much improved fluid which would both rapidly expand intravascular volume and maintain blood pressure and tissue perfusion for a longer period of time.^{16, 17}

Until the 1980's there were no definite guidelines for either the volume or type of fluid to be administered in a shocked patient. Nevertheless the general consensus was that in a shocked patient, a volume of fluid should be given in such a way as to normalise the blood pressure and pulse rate rapidly, and fluid administration should be ongoing so as to maintain the blood pressure until there was general evidence of adequate tissue perfusion.¹⁸

Review of the literature prior to the 1980's showed that the main focus of research was on the beneficial effects that colloids or crystalloids had over each other in improving the physiological parameters in haemorrhagic shock.^{19, 20, 21} Despite the fact that these treatment regimes favoured larger volumes of fluid replacement in shock, there was a paucity of evidence to support that these larger volumes of fluids administered had a deleterious effect on either the haemodynamic or the intrinsic haemostatic processes. The limited available evidence was related to large volume dilutional coagulopathies, with little evidence at the time of the possibility that these fluids in themselves could have more marked detrimental intrinsic properties, which could independently affect clot quality.^{22, 23} The studies there were in haemostatic impairment were limited in both extent and nature.

In the late 1980's the whole field of large volume replacement was brought into question when the American College of Surgeons brought out their advanced trauma life support (ATLS) guidelines on fluid replacement.^{24, 25} These guidelines stated that in shocked patients the maximum amount of fluid given initially should be two litres of crystalloid to be followed by immediate surgical intervention should there be evidence of on-going haemorrhage.

This was the first time that there was a change in the focus and it was realised that giving a fixed and limited amount of fluid in a bolus form was imperative in preventing the adverse effects of large volume replacement. On the other hand, there had been a steady growth of knowledge over time, showing that both the type and volume of fluid given could also have a detrimental intrinsic effect in itself by impairing haemostasis and clot quality, and thus, prolong on-going haemorrhage.^{26,}

^{27, 28, 29, 30}

During the mid 1990's clinicians began to observe, both experimentally and clinically, that various intravenous fluids had a detrimental effect by prolonging haemorrhage, which resulted in a poor clinical outcome.^{31, 32} It was for this reason that the Americans recommended use of crystalloids followed by blood as crystalloid solutions were thought to have a lower intrinsic effect. In contrast European countries continued to use both crystalloids and colloids. This has led to the universal crystalloid/colloid debate in terms of fluid management and their safety.^{33, 34}

UK Accident and Emergency Departments use four different types of intravenous fluid, namely, isotonic crystalloids, the calcium containing iso-oncotic gelatin Haemaccel, the similarly non-calcium containing iso-oncotic fluid Gelofusine and the physiologically and naturally occurring solution albumin in the care of the critically injured. Hence the research emphasis throughout this thesis was based mainly on Haemaccel, Gelofusine, albumin and normal saline.

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BRIEF SYNOPSIS OF THE PAPERS:

1. EVALUATION OF THE EFFECT OF COLLOID (HAEMACCEL) ON THE BLEEDING TIME IN THE TRAUMA PATIENT.

(Journal Royal Society of Medicine 1996; 89: 101P-104P)

INTRODUCTION:

In 1992 there were anecdotal reports that traumatised patients who had been resuscitated with the calcium containing colloid Haemaccel for shock showed a greater tendency for increased bleeding in the peri- and post-operative period compared to those who had been resuscitated with crystalloid.

However, a full review of the literature at the time showed that there was very little evidence to confirm that Haemaccel had a detrimental effect on the haemostatic mechanism or bleeding time.

The evidence in the literature was limited and inconclusive and was based on a combination of in vitro work and animal studies. Review of the limited clinical studies at that time showed that the research had focused mainly on the beneficial effects that gelatins played in improving physiological parameters when compared to crystalloids. Similar to the animal studies, the information obtained was limited and inconclusive.

Following these concerns, I undertook a research post at the Johannesburg Trauma Centre (Category I Trauma Unit) and the pre-hospital care service. I aimed to investigate the effects of Haemaccel on haemostasis. The study compared the effects on haemostasis of Haemaccel (colloid) with crystalloid in a randomised study. Johannesburg Hospital was the site chosen for the study as it had a large number of patients with both blunt and penetrating trauma. In addition, both the Trauma Unit and the pre-hospital care service followed the same ATLS guidelines in the management of trauma patients.

SUMMARY:

This study showed that the bleeding time was increased by almost 100% in patients who received Haemaccel compared with the crystalloid group. There was also a slight increase in blood loss in the Haemaccel group, which was consistent but not statistically significant. As there was very little change in the clotting parameters, it was hypothesised that this increased bleeding could be due to the effect of the gelatin on platelet function. This suggestion was supported by the limited platelet aggregation studies available, which showed that there was inhibition of platelet aggregation to certain agonists with Haemaccel. The study also showed that the blood level of ionised calcium rose to significantly high levels. A further hypothesis was made that it was possible that the ionised calcium in Haemaccel in itself may contribute to this platelet dysfunction and increased bleeding. This work was subsequently published, and presented at the Royal Society of Medicine where it won the Royal Society of Medicine Prize in 1995. These changes in ionised calcium, which were observed with the administration of Haemaccel, were investigated further and this led to the next stage of research.

2. CHANGES IN PLASMA IONISED CALCIUM WITHIN 24 HOURS OF TRAUMA IN PATIENTS INFUSED WITH THE CALCIUM CONTAINING COLLOID HAEMACCEL DURING FLUID RESUSCITATION.

(Journal of Accident & Emergency Medicine 1997; **14**: 73-75)

INTRODUCTION:

On returning to the UK, I set out to investigate the magnitude of change in ionised calcium when different volumes of Haemaccel were administered and to determine how long it would take the ionised calcium levels to return to their normal physiological levels.

Prior to this study, an extensive literature search was carried out, which showed that there had been no previous research to determine the level of calcium attained when Haemaccel was infused into the trauma patient.

SUMMARY:

This study showed that when Haemaccel was infused to correct blood loss, ionised calcium rose significantly. Indeed, in some cases it rose to 2.2 mmol/L (normal range 1.1-1.3 mmol/L). This study also showed that not only was there a significant rise in the level of ionised calcium, which was proportional to the volume of Haemaccel infused, but that this rise was often sustained, before it returned to normal, for 12 to 24 hours. This contrasted with the crystalloid group in which there was a consistent fall in ionised calcium levels to an average of 0.7 mmol/L. This fall in ionised calcium levels in the crystalloid group is a well-recognised physiological response to trauma, and is an independent finding.

I aimed to investigate the contribution, if any, of either gelatin or calcium on increased bleeding time and platelet function. In the next study, I carried out in vitro investigations to assess the independent inhibitory effects of calcium and/or gelatin on platelet dysfunction.

3. EFFECTS OF GELATIN-BASED RESUSCITATION FLUIDS ON PLATELET AGGREGATION.

(British Journal of Anaesthesia 1998; **81**: 198-202)

INTRODUCTION:

In this study the aim was to determine what effect calcium in Haemaccel had on platelet function and to compare the finding with those of the crystalloid control, normal saline. We also studied the effects of a non-calcium containing iso-oncotic gelatin (Gelofusine) on platelet aggregation.

A 40% dilution of intravenous fluid was used in whole blood (2:3 dilution), which was consistent with ATLS guidelines of giving 2 litres of intravenous fluid to correct fluid loss in trauma patients. As the previous surveys showed that the colloid albumin was mainly used for burns and in children, and only occasionally in haemorrhage, this fluid was not included in the trial at this stage.

SUMMARY:

Haemaccel proved to be an important and marked inhibitor of agonists leading to marked platelet inhibition. Although Gelofusine inhibited certain agonists, the inhibition was not as marked as by Haemaccel. This was due, in part, to the gelatin content binding to ristocetin as had been observed for Haemaccel.

When calcium was added to both Gelofusine and normal saline to bring it up to the same ionic concentrations as Haemaccel, this also led to a marked increase in inhibition for all agonists. This led to a decrease in platelet aggregation which was greater than that which had been observed with Gelofusine or saline alone.

In summary this study showed that Haemaccel was by far the greater inhibitor of platelet aggregation when compared with Gelofusine or normal saline. Although the high calcium content in Haemaccel had a marked effect in inhibiting platelet aggregation, it was not the only factor which led to increased platelet dysfunction.

Following this study, it was decided to undertake a prospective double blind randomised study of the effects of four intravenous fluids on platelet function and haemostasis in elective hip surgery. This was to determine whether Haemaccel and its calcium content caused platelet dysfunction in vivo.

However, before proceeding to this study further in vitro investigations on the effects of resuscitation fluids on platelet aggregation were carried out.

ACCIDENT AND EMERGENCY DEPARTMENTS INTERIM EPIDEMIOLOGICAL QUESTIONNAIRES:

4. FLUID RESUSCITATION IN A & E DEPARTMENTS – WHO USES WHAT AND WHY?

(Presented at the BAEM Meeting in Belfast April 1999)

INTRODUCTION:

As several years had elapsed since the initial research projects, I decided that before carrying out the next phase of laboratory investigation I would need to determine not only current fluid strategies but also what type of fluid was still being administered in Accident and Emergency Departments. All departments and their consultants throughout the UK were identified through the BAEM Handbook (a national registry of departments and consultants throughout the UK) and questionnaires were sent out to determine the type and volume of fluids used in haemorrhagic shock. This study was essential to ensure that the research focused on the resuscitation fluids in current use.

SUMMARY:

The survey response was nearly 90% following two consecutive captures. The results confirmed that the main fluids used were crystalloids (74%), the two gelatins, Haemaccel (12%) and Gelofusine (8%), and albumin (6%).

Many of the respondents showed a variation in the type, volume and reason for choice of these fluids. However, following the initial questionnaire, a study by Roberts et al appeared in the BMJ in 1997 and 1998. They showed that both colloids and albumin increased mortality of between 4-6% when administered in critically ill patients.

This new information, with its high media impact, could alter current practice of fluid management. Therefore, I sent out a further questionnaire to determine the effect of these publications before moving on to in vitro studies.

5. **INTRAVENOUS FLUID USE IN ACCIDENT AND EMERGENCY DEPARTMENTS. THE EFFECTS OF PUBLISHED STUDIES IN THE MEDICAL LITERATURE.**

(Journal of Accident & Emergency Medicine 2000; 17: 450)

INTRODUCTION:

In view of this continuing controversy, it was decided to reassess the prescribing practice by means of a poster questionnaire. These were sent to all A & E consultants in the UK and Republic of Ireland (459). Similar questionnaires were sent to Burns Units (205) and ITU's (1444).

SUMMARY:

The results of this questionnaire showed that there had been some change in the preference as regards the administration of the four groups of intravenous fluids. There had been a reduction in the use of colloids (Haemaccel and Gelofusine) by 12% and albumin by 5%. Therefore, the percentage of clinicians who used colloids and albumin showed a slight decrease, and this was mirrored by an increase in the preference for crystalloid use.

On the basis of the above findings, it was decided to continue using Haemaccel, Gelofusine, albumin and normal saline in the research as these were still the fluids of choice as confirmed by the questionnaire.

6. **WHOLE BLOOD PLATELET AGGREGATION DETERMINED USING A PLATELET COUNTING TECHNIQUE: A UNIVERSAL TOOL FOR STUDIES IN MAN.**

(Platelets 2000; 11: 347)

OVERVIEW:

Previously most in vitro studies on platelet aggregation had been carried out using either platelet rich or platelet poor plasma, both of which were not as "physiological" when compared to the whole blood platelet counting technique. The use of Heparin, as an anticoagulant in whole blood causes a certain amount of platelet activation whereas the use of citrate removes the divalent cations such as calcium and magnesium.

Furthermore, if our in vitro studies were to mimic the ex vivo system, then further research would have to be carried out to determine a different method of anticoagulation for whole blood aggregometry.

Following a literature review and my own laboratory assessment of the aggregometry method, it was decided to use Hirudin as our anticoagulant which neutralises thrombin and therefore has no direct effect on the platelets themselves. Previous studies had also shown that Hirudin did not alter the divalent cations concentration. Following the validation of this technique, the above method was used in the research as it enabled the use of physiological whole blood and closely mimicked our ex vivo work. Review of previous studies into this technique in conjunction with our own laboratory work led to the validation of this method.

7. STUDIES ON THE EFFECTS OF RESUSCITATION FLUIDS ON PLATELET AGGREGATION IN VITRO

(Platelets 2000; **11**: 348)

INTRODUCTION:

Platelet aggregation is a complex process involving several constituents in whole blood. Divalent cations such as calcium have been shown previously to influence platelet aggregation. Dilution of whole blood in vitro with certain resuscitation fluids will lower calcium, and also cause cellular dilution, both of which will have a separate effect on the aggregatory response.

Therefore a study was set up to investigate these two separate effects which occur when different resuscitation fluids are added to whole blood (normal saline, Gelofusine, Haemaccel and albumin) on platelet aggregation. To do this each patient's own autologous plasma was used as a diluent to firstly enable us to limit the change in divalent cation concentration which could occur with other controls, and secondly to study the effect that these fluids would have on cellular dilution while maintaining the cation concentration and plasma matrix. A literature review of this technique did not show any previous studies where this method of the use of autologous plasma as either a diluent or a control had been used.

SUMMARY:

Adding autologous plasma to whole blood, and thereby reducing the cell count, reduced the aggregation responses to collagen and adrenaline, but not ADP. Saline and Gelofusine enhanced the aggregation responses to ADP, collagen and adrenaline, possibly as a consequence of lower ionised calcium. Haemaccel always inhibited the aggregation further probably through increased ionised calcium and, like Gelofusine, abolished responses to ristocetin. Compared with saline and Gelofusine, albumin usually limited the enhancement of the aggregation response consequent to creating a low ionised calcium environment (by binding to ionised calcium).

As a result of these findings we anticipated that the effects of these agents on platelet aggregation in man would largely depend on the extent of the changes in blood cell counts and the rapidity with which homeostatic mechanisms corrected the changes in plasma ionised calcium that would accompany the various infusions.

Our two in vitro studies showed that ionised calcium played a significant part in inhibiting platelet aggregation in an above cellular dilution. In our next study, (The prospective double blind randomised study of the effects of four intravenous fluids on platelet function and haemostasis in elective hip surgery), we aimed to determine the in vivo and ex vivo effects of these various resuscitation fluids including Haemaccel. If this inhibition of platelet function did occur it was also vital that we should try and determine what effect this would have on the bleeding time and blood loss.

8. PROSPECTIVE DOUBLE-BLIND RANDOMISED STUDY OF THE EFFECTS OF FOUR INTRAVENOUS FLUIDS ON PLATELET FUNCTION AND HAEMOSTASIS IN ELECTIVE HIP SURGERY

(Journal of Thrombosis & Haemostasis 2003; 1: 1-9)

INTRODUCTION:

Fifty-five patients undergoing elective hip replacement were studied. Elective hip surgery is accompanied by blood and fluid loss of approximately 2 litres with significant soft tissue and bony injury. This causes enough injury to stimulate the systemic homeostatic processes and intravenous fluid replacement, is required routinely to maintain tissue perfusion.

The reason for studying patients undergoing elective hip replacements, as opposed to patients with polytrauma was to limit confounding variables which may also affect platelet aggregation. The patients in this study had a full set of baseline biochemical/haematological measurements including platelet aggregation and bleeding times. After the baseline bloods were collected all patients were infused with either crystalloid or colloid fluids consistent with ATLS guidelines. Repeat blood samples were then taken during and after surgery.

SUMMARY:

The results showed that all four fluids had a direct effect on platelet aggregation. Generally, the platelet aggregation appeared to result in platelet desensitisation and resulted in a persistent reduction in platelet aggregation to ADP and adrenaline, irrespective of the fluid used. Additionally, Haemaccel and Gelofusine inhibited ristocetin-induced platelet agglutination and albumin inhibited collagen-induced platelet aggregation. The results showed that all bleeding times were significantly elevated, irrespective of the type of fluid used but this rise was more marked with colloids than with normal saline. However, within four hours the bleeding times had almost returned to normal.

The measurement of blood loss showed that there was slightly more blood loss with the colloids than with the crystalloids, although this never reached statistical significance.

The data clearly showed that all colloids exhibited a much greater effect on platelet aggregation with an increase in bleeding time and a slight increase in blood loss when compared to normal saline. All four intravenous fluids had varying intrinsic effects on platelet function. Even normal saline had an intrinsic effect by promoting aggregation with certain agonists thus, suggesting that future studies of dilution should use autologous blood plasma as a diluent control rather than normal saline. This study also showed that although these intravenous fluids may exert a similar volume and intrinsic effect, they may also affect the haemostatic mechanism independently.

Despite infusion of two litres of Haemaccel the calcium never rose to the levels which we achieved in our previous in vitro experiments or to that which had been observed previously in trauma patients. This could be explained by the corrective homeostatic mechanisms and/or by the lower volumes of Haemaccel administered in this study compared to the study on the polytrauma patients. Different resuscitation fluids have varying effects on platelet aggregation. A review of our results and the literature was carried out to determine whether the effects of these fluids on platelet aggregation in vitro could be predictive of the ex vivo changes, which were observed.

9. CAN THE EFFECTS OF CLINICAL REPERFUSION FLUIDS ON PLATELET AGGREGATION IN VITRO PREDICT THE EFFECTS OF ADMINISTRATION OF SUCH FLUIDS IN A CLINICAL STUDY EX VIVO?

(Platelets 2003; 14(4): 253-257)

OVERVIEW:

A comparison was made between in vitro and ex vivo studies on the effects of these resuscitation fluids on platelet aggregation. There was either promotion or inhibition of aggregation depending on the fluid type and on the platelet agonist that was used.

Perhaps the most interesting finding was that overall there was a marked inhibition in the aggregation induced by ADP and by adrenaline ex vivo, irrespective of the fluid used. This was not predicted from the in vitro experiments. It is unlikely that this was an effect of the anaesthetic agents because all blood samples were obtained following the same anaesthetic administration. It is more likely that the surgery itself led to the reduced response observed. Possibly increases in ADP and adrenaline in blood plasma following surgery led to some down regulation of platelet responses to these agonists. However, platelet responses to collagen were not altered as a consequence of surgery as it is possible that not all platelets remaining in the circulation post surgery would have been exposed to collagen during the operation. Overall, the results showed that a combination of homeostatic and trauma response mechanisms complicate the ability to extrapolate from the findings in vitro to ex vivo.

Another example of the difficulty in extrapolating in vitro data to ex vivo was seen in the effects of Haemaccel on aggregation responses. The marked inhibition of aggregation by ADP, adrenaline and collagen in vitro is at least in part, a consequence of the large amounts of calcium in Haemaccel. In vivo homeostatic mechanisms served to moderate the changes in calcium concentration and overall, the effects of Haemaccel infusion proved to be very similar to those of the other fluids used.

In the in vitro studies, Haemaccel and Gelofusine proved to be potent inhibitors of ristocetin induced aggregation. This is thought to be a direct effect of the gelatin interfering with the interaction between platelets and von Willebrand factor. In this context the effects of Haemaccel and Gelofusine were reproduced in vivo.

EPIDEMIOLOGICAL PUBLICATIONS, REVIEWS AND META-ANALYSIS

Following initial research in 1993, there had been significant developments as regards fluid resuscitation. By now a considerable body of knowledge had been published which was focussed on the potential detrimental dilutionary haemodynamic and intrinsic effects of these fluids on haemostasis. Therefore many of the clinical guidelines into fluid resuscitation were brought into question.

Three systematic reviews were undertaken to try and determine what evidence there was for the justification of fluid administration protocols in practice. Reviews were undertaken to examine the factors which had influenced the drawing up of these guidelines.

The first systematic review was undertaken to determine the effect of blood pressure normalisation on bleeding in trauma patients. The timing and volume of fluid administration to patients with haemorrhagic hypovolaemia was specifically investigated.

The second review was a systematic analysis of randomised controlled trials of the timing or volume of fluid administration in animal models of uncontrolled haemorrhage. This was to try and determine whether information collected from animal experimentation on fluid resuscitation and haemorrhage could be extrapolated to human health care in terms of clinical management.

The third review systematically analysed randomised controlled trials of fluid resuscitation in animal models of uncontrolled haemorrhage and explored potential sources of heterogeneity. Unconfounded randomised controlled trials of fluid resuscitation in animal models of uncontrolled haemorrhage using mortality as our outcome measure were reviewed.

All three systematic reviews focused mainly on the effects that intravenous fluids had on haemostasis.

10. IS THE NORMALISATION OF BLOOD PRESSURE IN BLEEDING TRAUMA PATIENTS HARMFUL?

(The Lancet 2001; 357: 385-387)

OVERVIEW:

Previously the treatment of haemorrhage in the trauma patient focussed on controlling haemorrhage. Maintenance of blood pressure was aimed to prevent shock, but unfortunately this may also worsen bleeding. Restoring the blood pressure by either crystalloid or colloid may not only impair the formation of new blood clots but may dislodge the existing ones, and thereby contribute to on-going haemorrhage.

Review of the published clinical data showed that the evidence for normalising blood pressure in the face of on-going haemorrhage, far from having a beneficial effect may indeed lead to worsening of bleeding and shock. There was no conclusive evidence about the type and volume of fluid which should be given. On the other hand, many of the trials compared different colloids, or compared colloids with crystalloid solutions. Questions about the amount of fluid which should be administered had been eclipsed by the debate on the choice of fluids.

Vigorous fluid resuscitation in uncontrolled haemorrhage may be life-saving in some patients, and the results from the clinical trials are consistent with those from animal studies indicating that raising of blood pressure could worsen bleeding and increase mortality.

In the absence of evidence for the effectiveness of currently recommended resuscitation protocols, and the potential for harm, contemporary resuscitation practice can at best be regarded as experimental. Indeed it was unclear as to which criteria had been used to inform the clinical decisions as to the type and volume of fluid to be used.

11. **DOES ANIMAL EXPERIMENTATION INFORM HUMAN HEALTHCARE? OBSERVATIONS FROM A SYSTEMATIC REVIEW OF INTERNATIONAL ANIMAL EXPERIMENTS ON FLUID RESUSCITATION.**

(British Medical Journal 2002; **324**: 474-476)

OVERVIEW:

Previous systematic reviews of randomised trials of fluid resuscitation in bleeding trauma patients had provided no evidence that fluid resuscitation improved outcome. Indeed, much of the findings on fluid resuscitation protocols had utilised evidence from animal studies.

The review revealed that many of the studies used a range of animal species and models with different physiological endpoints. Outcomes could only be assessed accurately if the physiological response to intervention was consistent from species to species. However, some physiological systems may be different between animal species, which could lead to different outcomes. The studies showed considerable variation, not only from species to species, but also in terms of outcome measured. Many of the studies were under-powered and provided little information. Following the observations and conclusions from this systematic review a further systematic review of randomised controlled trials of fluid resuscitation in animal models of uncontrolled haemorrhage was undertaken to explore the potential sources of heterogeneity, which had been highlighted in this review.

12. FLUID RESUSCITATION STRATEGIES. A SYSTEMATIC REVIEW OF ANIMAL TRIALS

(Journal of Trauma 2003; **55**: 1-19)

OVERVIEW:

Much of the evidence for the type of resuscitation fluid came initially from research on animals. In the light of the relative lack of evidence in humans on the effects of delayed or reduced volume resuscitation, a review of animal trials was valuable. As previously argued the application of systematic review methodology to animal experiments would allow a more objective appraisal of the research evidence than narrative reviews, and by increasing the precision of estimates of treatment effects, would reduce the proportion of invalid results.

Thus, exploration of heterogeneity in meta-analyses of animal experiments has the potential to provide important insights into disease mechanisms. We used stratification and meta-regression, to identify sources of heterogeneity in a systematic review of 52 randomised controlled trials of fluid resuscitation in animal models of uncontrolled haemorrhage.

This review showed that the effect of fluid resuscitation on the risk of death in animal models of uncontrolled haemorrhage appears to be related to the severity of haemorrhage. When haemorrhage is severe (injury to the aorta or >50% of the rat tail removed), fluid resuscitation reduces the risk of death. However, when haemorrhage is less severe (injury to vessels other than the aorta or <50% of the rat tail removed) the risk of death is increased. This suggests that the risk versus benefits of fluid resuscitation are finely balanced. In animals with less severe injury, the potential risks of fluid resuscitation appear to outweigh the benefits of improved tissue perfusion and reduced tissue ischaemia. This may be due to the increasing hydrostatic pressure and impairment of clotting leading to increased blood loss and diluting the oxygen carrying capacity of the blood. However, in severe injuries, the effect of hypotensive resuscitation was to reduce the risk of death in all trials. This suggests that using a lower than normal blood pressure as a guide to fluid resuscitation consistently reduces the risk of death regardless of the severity of injury.

SUMMARY OF EPIDEMIOLOGICAL EVIDENCE:

These three reviews together make a case for hypotensive resuscitation. The review of clinical trials in humans showed that there was no evidence that normalising the blood pressure improved outcome, indeed the reverse seemed to occur.

Fluid resuscitation appears to reduce the risk of death in animal models of severe haemorrhage to a degree which would be considered massive in man, but increases the risk of death in those with less severe haemorrhage. Therefore, excessive fluid resuscitation could be harmful in some situations. An evaluation of the potential impact of hypotensive resuscitation in humans is now warranted.

Following the epidemiological reviews considerable interest arose into the effects of fluid resuscitation on clot quality. This was highlighted by the development of new therapeutic options such as factor VIIa and thus, led to the following studies on haemostasis.

13. ASSESSMENT OF rVIIa AS A UNIVERSAL HAEMOSTATIC AGENT IN A MODEL OF HAEMODILUTION:

1. Critical Care 2001; 5(1): 107
2. Critical Care 2002; 6(1): 127
3. Summary of Abstract

INTRODUCTION:

Recent research has shown that gelatins and starches get incorporated into clots and have a direct effect on reducing clot quality. If fluid administration has a detrimental volume and intrinsic effect on clot quality then it is appropriate to investigate therapeutic options which reduce these detrimental effects and also improve clot quality. This could also help reduce the need for volume replacement with its adverse dilutional, haemodynamic and intrinsic effect.

It would be beneficial if clot quality could be improved early on following trauma in the pre-hospital phase, to reduce the necessity for large volume replacement whilst maintaining clot quality. Factor VIIa is a new and potentially exciting therapeutic option, which could fulfil this role. It is a naturally occurring factor produced from the combination of factor VII with tissue factor from the vascular endothelium in trauma. It binds to tissue factor at the site of injury and both accelerates coagulation and improves clot quality resulting in reduced haemorrhage. It has been successfully used in haemophiliacs and there have been recent reports of its use in life-threatening cases of trauma.

Therefore, an in vitro study was carried out using the thromboelastogram (TEG) to investigate whether factor VIIa could overcome both the intrinsic and dilutionary effect of colloids and crystalloids. TEG is a device which gives a global picture of the haemostatic mechanism by measuring global haemostasis covering thrombogenesis as well as fibrinolysis. TEG allows us to repeated study whole blood coagulation, which helps to determine the effect of both volume and fluid type on haemostasis. It also determines the clot quality by measuring the durability and tensile strength of the clot.

Whole blood was diluted to 50% and 80% with Saline or Haemaccel. Each time the maximum amplitude was reached on the TEG, it corresponded to maximum clot strength. Clot structure was also studied by electron microscopy.

SUMMARY:

These results clearly showed that factor VIIa globally improved major parameters of haemostasis as assessed on the TEG machine, reversing both the intrinsic and dilutionary effects of saline and Haemaccel. Electron microscopy showed that clot structure had a greater number of fibrin strands and cross-linkages and showed increased clot quality. The improvement in clot quality occurred despite the high dilutions which were used.

If these results can be extrapolated and confirmed in clinical trials, then a patient with haemorrhagic shock could receive factor VIIa in the pre-hospital setting. 27

This could improve clot quality and further reduce haemorrhage and the need for fluid resuscitation. Consequently, this could enhance the time for definitive surgical intervention.

CONCLUSION OF THE THESIS:

Following my initial publication in 1995 into the increased bleeding time associated with Haemaccel, considerable interest has developed, which has stimulated debate and led to subsequent further publications by other investigators confirming these findings.

Haemaccel has been shown to affect haemostasis in three ways. Firstly, due to its volume expansion it has a dilutional and haemodynamic effect on haemostasis. Secondly, it has an intrinsic effect which has been clearly demonstrated both in the laboratory and clinically. Its gelatin content specifically inhibits platelet aggregation and increases bleeding. Thirdly, in addition, infusion of Haemaccel leads to a rise in systemic ionised calcium levels which inhibit platelet function and haemostasis.

Similarly Gelofusine affects haemostasis by having a dilutionary and intrinsic effect which is specifically related to its gelatin content. Albumin also has a volume and intrinsic effect on haemostasis. However, its intrinsic effect is mainly related to its interaction with collagen resulting in inhibition of platelet aggregation.

It has also been shown in this thesis that calcium, colloids and to a lesser extent crystalloids have a direct effect on platelet function and clot quality. The above research has shown that haemostatic dysfunction does lead to an increase in blood loss. Normal saline, which in previous studies has been used as a non-colloid control, has also been shown in these studies to have some intrinsic effect. However, compared to the colloids its intrinsic effect is more pro-thrombotic than fibrinolytic.

It could be suggested that previous studies which have used normal saline as diluents were liable to have produced unreliable results and conclusions. In this thesis, this was overcome by using the novel way of diluting blood with plasma and it is suggested that future in-vitro experiments with resuscitation fluids should be carried out using autologous plasma as a control.

The epidemiological studies showed that when intravenous fluids are given early, colloids are administered and blood pressure maintained, this results in a poorer outcome. Therefore, there is a clear case for low volume replacement and hypotensive resuscitation.

Finally, if in vitro work on factor VIIa can be established in a clinical situation this could lead to a new way of treating haemorrhage in trauma. This new therapeutic development could improve clot quality and reduce bleeding, leading to a method in which blood pressure is maintained whilst reducing the need for intravenous fluid itself. If future clinical trials into the use of factor VIIa in trauma, support our observations in this thesis, then it could be administered in a pre-hospital setting to reduce bleeding and expedite the patient's passage to surgery for definitive care.

The clinical and laboratory work in addition to the epidemiological reviews clearly complement each other and support our hypothesis that giving large volumes of fluids has a detrimental effect on haemostasis with a poorer outcome. In the future this new concept of hypotensive resuscitation with the administration of factor VIIa could overcome the complications associated with fluid resuscitation.

I believe that the research carried out in this thesis has made some contributions to our understanding of fluid resuscitation in haemorrhagic trauma.

INTRODUCTION

Haemaccel is widely used throughout Europe and South Africa in the resuscitation of trauma patients¹. Although it is very effective when used as the sole volume expander, it is usually used in conjunction with a crystalloid which has been shown both experimentally and clinically to increase its benefits². The colloid within Haemaccel is chemically modified bovine bone gelatine preparation. The gelatine is subjected to thermal degradation to produce a gelatine hydrolysate in the form of small polypeptides of molecular weight 12 000 to 15 000. These are then cross linked to form larger molecules which have an average molecular weight of 35 000 (range 25 000–50 000). In addition, the solution contains a number of other solutes including Ca^{2+} (6.2 mM), and exerts an oncotic pressure of 3.4–3.8 kPa at 37°C. It is isoncotic with plasma and it has a half life in the body of approximately 5 h.

Recent anecdotal reports from the Department of Surgery at the University Hospital Bloemfontein (Orange Free State, South Africa) suggested that trauma patients who had received Haemaccel for shock exhibited an increased bleeding tendency in the form of wound oozing, both intra- and post-operatively. The aim of the present study, therefore, was to perform a controlled investigation of the effects of Haemaccel on bleeding time *in vivo* in trauma victims at the Trauma Unit of Johannesburg Hospital.

PATIENTS AND METHODS

Protocol

Twenty-five patients who presented at the Trauma Unit of the Johannesburg Hospital were recruited over a 35 day period. All patients in our study had suffered either blunt or penetrating trauma, and all required fluid resuscitation due to their injuries. All patients were seen by the On call Trauma Team which consisted of a consultant, a registrar,

two senior house officers and a house officer. The Trauma Nursing Team in the resuscitation unit. All patients were scored independently by two members of the trauma unit using the Injury Severity Score and the Revised Trauma Score systems^{5,6}. For inclusion in the study the patients had to satisfy the following predetermined criteria

- 1 Age > 16 years
- 2 Blunt or penetrating trauma
- 3 Required intravenous fluid resuscitation
- 4 Arrival at trauma unit within 2 h of injury
- 5 Crystalloid (Ringer's lactate) as the volume expander
- 6 No underlying illness or medication which might affect the patient's coagulating system

All the patients described in this study were seen by the Trauma Unit within 2 h of the injury. They were scored according to the Advanced Trauma Life Support guidelines relating to resuscitation in pre-hospital and hospital settings. Patients having received only crystalloid (Ringer's lactate) as the volume expander. Base line bleeding time was measured for each patient on arrival at the hospital using a modified type II method as well as a full coagulation screen (see below). The patients were then allocated randomly to one of two groups to receive either (i) crystalloid (group 1) or (ii) further crystalloid (Ringer's lactate) (group 2), until the patient was fully resuscitated and had stable vital signs. The volume of fluid administered was documented and a further bleeding time and coagulation screen was carried out. A list of patients included in the study, group, and the injuries can be obtained from the author.

Ethical approval was obtained from the Research Committee of the University of the Witwatersrand.

Coagulation screen

Approximately 20 ml of blood withdrawn from the antecubital vein by direct venepuncture. Initial normal values for prothrombin, partial thromboplastin and activated partial thromboplastin were determined using a 1:10 standard of sodium citrate (31.3 g/l). Platelet count was determined on a separate aliquot of the venous blood collected.

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Table 1 Number of patients (*N*), males/females (*m/f*) and associated ages, suffering blunt or penetrating (pene) injuries, injury severity scores (ISS) and revised trauma scores (RTS). Following an initial resuscitation with crystalloid (Init-resusc), the patients were randomized into two groups and resuscitated further (Secondary-resusc) with either colloid (group 1) or crystalloid (group 2). All variables except *N* and injuries presented as medians (interquartile ranges).

	Group 1 (Colloid)	Group 2 (Crystalloid)
<i>N</i> (<i>m/f</i>)	11 (9/2)	14 (12/2)
Age (year)	30 (29–38)	30 (25–39)
Injuries (blunt/pene)	7/4	9/5
ISS	25 (16–34)	25 (14–36)
RTS	7.5 (4.9–7.8)	6.3 (4.4–7.8)
Init-resusc crystalloid vol (Ref 3)	1.0 (0.2–1.5)	0.8 (0.4–1.0)
Secondary-resusc vol (Ref 3)	1.0 (1.0–1.8)	2.2* (2.0–2.6)

* $P < 0.05$ group 1 versus group 2, Mann-Whitney *U* test

Statistical analysis

Within group comparisons were made using a Willcoxon match-paired signed rank test, while comparisons between crystalloid and colloid groups were made using a Mann-Whitney *U*-test. A value of $P < 0.05$ was considered statistically significant. All values are presented as median (interquartile range) unless indicated otherwise.

Results

The patients' ages, ISS and volumes of crystalloid used for initial resuscitation in the two groups are summarized in Table 1. These values did not differ significantly between the two groups. Furthermore, bleeding times, prothrombin, thrombin, partial thromboplastin times and platelet counts did not differ significantly between the two groups at the end of the initial resuscitation.

Effects of secondary resuscitation with either crystalloid or colloid solutions

The volume of fluid used for secondary resuscitation was significantly greater in the crystalloid treated group compared to that treated with colloid (Table 1). Following secondary resuscitation there was statistically significant increase in bleeding time in both groups (Figure 1). However, the increase in bleeding time was significantly greater in the colloid than in the crystalloid treated group, amounting to an increase of 5.5 min (2.8–7.3) or 93.6% (42.3–157.1) in the colloid group compared to 1.4 min (1.0–2.3) or 25.1% (14.1–46.9) in the crystalloid treated group. Prothrombin time showed a small increase in both groups (Figure 2), which failed to attain statistical significance. The thrombin time was unchanged in either group following secondary resuscitation from 28.8 s (26.7–33.0) and 30.7 s (25.2–32.4), respectively, in groups 1

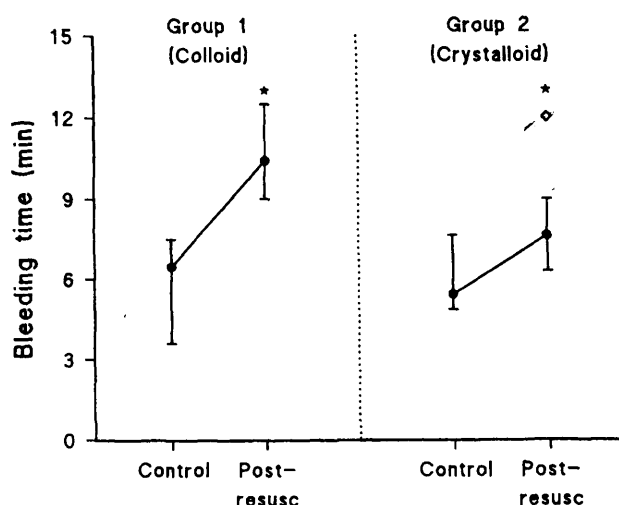


Figure 1 Bleeding times in two groups of patients recorded after the initial resuscitation with crystalloid (control) and again after secondary resuscitation with either colloid (group 1) or crystalloid (group 2). * $P < 0.05$ versus respective control, Willcoxon matched-pairs signed ranks test. $P < 0.05$ versus group 1, Mann-Whitney *U*-test

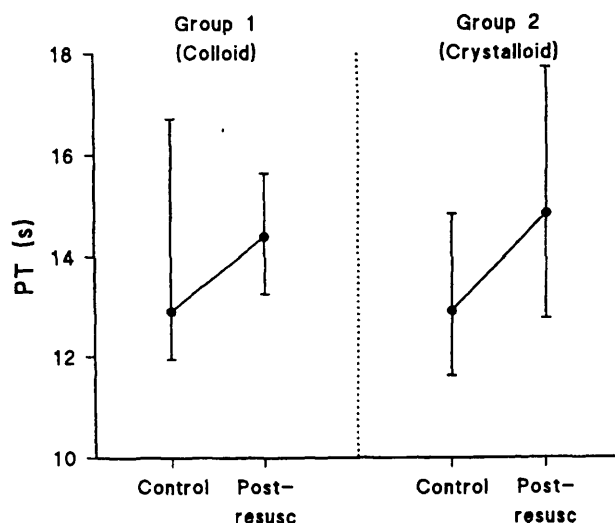


Figure 2 Prothrombin times (PT) in two groups of patients recorded after the initial resuscitation with crystalloid (control) and again after secondary resuscitation with either colloid (group 1) or crystalloid (group 2)

and 2. Partial thromboplastin time was increased significantly in the colloid treated group. However, the increase in the crystalloid group failed to achieve statistical significance ($P = 0.068$, Figure 3). There was a decrease in platelet count in both groups (Figure 4). These changes were not statistically significant. Although there was a marked increase in bleeding time in the colloid group, there was no direct relationship between the volume of colloid given and prolongation of bleeding times. The volumes of whole blood subsequently required by the crystalloid group was less than that required by the colloid group (3.5 units; (range 0–11) versus 4.6 units; (range 0–26), respectively).

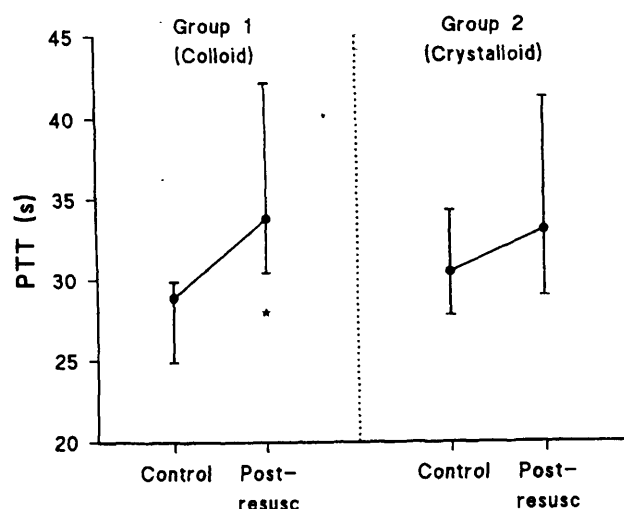


Figure 3 Partial thromboplastin times (PTT) in two groups of patients recorded after the initial resuscitation with crystalloid (control) and again after secondary resuscitation with either colloid (group 1) or crystalloid (group 2) * $P < 0.05$ versus respective control, Wilcoxon matched-pairs signed ranks test

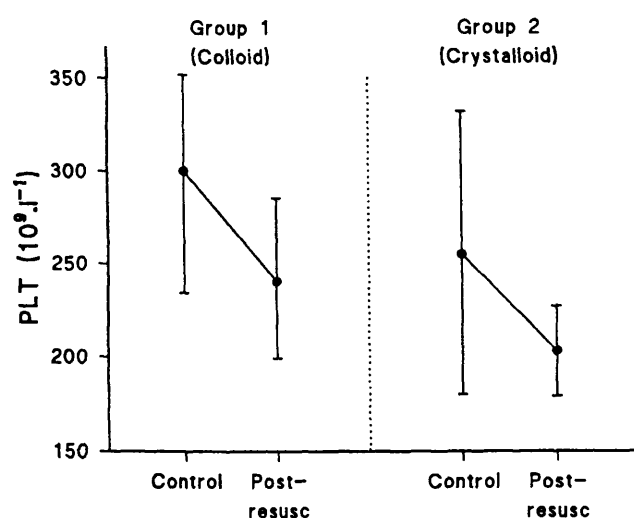


Figure 4 Platelet counts (PLT) in two groups of patients recorded after the initial resuscitation with crystalloid (control) and again after secondary resuscitation with either colloid (group 1) or crystalloid (group 2)

DISCUSSION

The present study has shown that there is an increase in bleeding time following resuscitation with asanguineous fluids, and that this increase is markedly greater when the fluid used is Haemaccel rather than crystalloid solution. It is difficult to assess the clinical significance of this finding; however, it was noted that the requirement for whole blood was somewhat higher in the group of patients treated with Haemaccel, when compared to those treated with crystalloid solution. While we accept that this finding may be artefactual because the discharge haemoglobins were not

measured and that there were a relatively small number of patients in our study, it remains a worrying finding. Obviously, a larger study is required to validate this particular result.

There are a number of potential mechanisms which could explain the increase in the bleeding time.

Haemostasis refers to the spontaneous arrest of bleeding from an injured blood vessel. Primary haemostasis in the trauma patient is initiated by the adhesion of platelets to the site of injury and local vasoconstriction with subsequent formation of a haemostatic plug brought about initially by the aggregation of platelets on their exposure to collagen. The bleeding time is thought to be one of the few global tests of haemostatic competency and reflects both platelet function as well as number⁸. It has been recognized and used in the surgical patient as an indicator of bleeding tendencies both pre-and post-operatively⁹. The primary haemostasis is followed by secondary haemostasis in which the coagulation system is activated resulting in stabilization of the initial platelet plug by fibrin.

The prolongation of bleeding time in the present study may, thus, be due to a deficiency in the primary haemostatic mechanism. Although in our study the platelet count dropped in both groups, they did not fall below physiological levels, nor was the decline statistically significant. Thus, the increased bleeding cannot be explained simply on the basis of a reduced platelet count. White *et al.*⁸ feel that the bleeding time evaluates platelet function. There is little evidence that Haemaccel may affect platelet function *in vivo* following trauma. In the rat bleeding time and blood loss were increased following liver resection and plasma replacement with Haemaccel, while ADP-induced platelet aggregation was significantly decreased after the infusion of Haemaccel¹⁰. Furthermore, *in vitro* studies have suggested that Haemaccel can inhibit von Willebrand factor and thereby reduce platelet aggregation^{11,12}, thus simulating von Willebrand's disease.

This inhibition was found to be dose dependent, and could be overcome by increasing the amount of von Willebrand factor, but was not found to prolong *in vitro* bleeding time significantly. These workers found that collagen, ADP, adrenaline induced platelet aggregation and serotonin release were inhibited markedly by Haemaccel. Thus, the increase in bleeding time following Haemaccel reported in the present study could be due to inhibition of platelet function.

Haemaccel may modify platelet function by coating of the platelet membrane with the colloid macromolecule causing a steric blockade of the surface receptors as occurs with other macromolecules^{13,14} thus interfering with the binding of ADP, collagen and adrenaline, resulting in a reduction in platelet aggregation and adhesion. This has been shown to occur *in vitro* but has not been shown to be of clinical significance. Platelet function can also be modified by ionized calcium.

Heptinstalls¹⁵ showed that free calcium ions, even at normal physiological levels, can reduce platelet aggregation *in vitro*, partly by promoting platelet disaggregation. Since Haemaccel contains free calcium ions (6.25mM), it might increase plasma calcium levels and hence reduce platelet aggregation *in vivo*.

Finally, a slight prolongation of prothrombin and partial thromboplastin times was also noted (in both groups) but these remained within the normal limits of the normal range and reflect a degree of haemodilution of the coagulation factors which occurred during volume replacement. A transient dilutional coagulopathy is consistent with earlier reports¹⁶, though other studies suggest that this is rapidly corrected when perfusion is good¹⁷.

In summary, the present study has shown that volume replacement with Haemaccel in the trauma patient produces a markedly greater increase in bleeding time compared to resuscitation with a crystalloid solution. Further studies are now required to elucidate the exact mechanisms of this action.

Acknowledgment I would like to thank Hoechst SA for their independent grant and sponsorship for this research project

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Changes in plasma ionised calcium within 24 hours of trauma in patients infused with the calcium containing colloid Haemaccel during fluid resuscitation

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Abstract

Objective—To determine the changes in ionised plasma calcium levels over a 24 h period in patients sustaining blunt trauma injuries and infused with the calcium containing colloid Haemaccel® (6.25 mmol/litre Ca²⁺).

Methods—The study was carried out on 24 trauma patients who attended the accident and emergency (A&E) department of the Leicester Royal Infirmary and required fluid resuscitation. Nineteen patients, with a mean injury severity score (ISS) of 14 (range 6 to 36), were given an infusion of Haemaccel; five patients in the control group with an ISS of 12 (range 6 to 19) were infused non-calcium-containing crystalloid. All types of fluids were recorded and serial plasma ionised calcium values were measured over a 24 h period.

Results—The mean pre-Haemaccel ionised calcium value fell to 0.71 mmol/litre following trauma. The mean values (mmol/litre) obtained in patients infused with Haemaccel were measured at 2, 4, 8, and 24 h. In the Haemaccel group these values were 1.38 (SD 0.34), 1.40 (0.44), 1.23 (0.27), and 1.18 (0.31) (at least $P < 0.001$ v baseline). The rise in calcium at 2 h was proportional to the volume of Haemaccel infused ($r = 0.917$; $P < 0.001$).

Conclusions—In all patients the plasma ionised calcium rose on infusion of Haemaccel and in a least one measurement 50% of patients developed hypercalcaemia (Ca²⁺ < 1.30 mmol/litre). The clinical significance of this is at present unclear.

(J Accid Emerg Med 1997;14:73-75)

Keywords: colloid; fluid replacement; ionised calcium; hypercalcaemia.

A fall in plasma ionised calcium is a recognised metabolic response to traumatic and non-traumatic muscle damage,^{1,4} and intracellular calcium mobilisation following injury has been demonstrated in vitro in human endothelial monolayers.⁵ Nevertheless the role of calcium supplementation during fluid resuscitation in trauma patients remains controversial, as reflected by the fact that certain intravenous fluids used contain calcium whereas others do not. Haemaccel® is a calcium containing

colloid which has a standard ionised calcium content of 6.25 mmol/litre and is commonly used, either alone or in combination with crystalloid,⁶ during resuscitation of shocked trauma patients in order to counteract the fall in plasma calcium in these patients.

It has, however, been observed that trauma patients who have received intravenous fluids containing calcium have significant prolongation of the bleeding time.⁷ This may be the result of inhibition of platelet aggregation by a direct effect of raised plasma calcium, as suggested previously.^{7,8}

In the present study we have determined the level of plasma ionised calcium over a 24 hour period following resuscitation of trauma patients with Haemaccel.

Methods

Twenty four consecutive trauma patients admitted through Leicester Royal Infirmary accident and emergency (A&E) department and requiring fluid resuscitation were included in the study. Nineteen patients (mean age 36 years, age range 19 to 77) with a mean injury severity score (ISS) of 14 (range 6 to 36) were given an infusion of Haemaccel, and a control group of five patients (mean age 42 years, age range 18 to 85) with an ISS of 12 (range 6 to 19) were given non-calcium-containing crystalloids; all agreed to supply blood samples for the duration of the study. The ISS was assessed as previously described.⁹ None of the patients were on any medication, or had any underlying illness known to affect plasma calcium concentrations. All patients were resuscitated following the advanced trauma life support guidelines.¹⁰ None of the patients had an arterial pH outside the normal range, as determined by blood gas analyser.

The fluid replacements were given in the usual way, with no modifications to patient management because of the study. The volumes and types of fluids infused and the infusion rates were recorded. Blood for the determination of ionised calcium was collected following the recommendations of Boink *et al.*¹¹ Mixed venous blood (collected without tourniquet or stasis) or arterial blood samples for the immediate measurement of ionised calcium and pH (by an Instrumentation Laboratory 1640 BGE analyser) were collected into dry heparin tubes on admission and at 2, 4, 8, and 24 hours. The pH adjusted calcium values

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Table 1 Effect of infusion of Haemaccel on plasma ionised calcium

	Pre-Haemaccel basal value	Time since trauma			
		2 h	4 h	8 h	24 h
Haemaccel group					
Mean ionised calcium (mmol/l) (SD)	0.71 (0.15)	1.38 (0.34)	1.40 (0.44)	1.23 (0.27)	1.18 (0.31)
Range of ionised calcium (mmol/l) of pre-Haemaccel value	0.49 to 0.90	0.91 to 2.02	0.97 to 1.94	0.88 to 1.61	0.79 to 1.7
Mean volume Haemaccel infused per patient (ml)	NA	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Range Haemaccel volume infused	None	1230	1500	1580	1640
	None	500-2500	500-3000	500-3500	500-3000
Non-Haemaccel group					
Mean ionised calcium (mmol/l) (SD)	0.71 (0.15)	0.78 (0.08)	0.75 (0.08)	0.90 (0.24)	0.90 (0.11)

The mean ionised calcium levels in five trauma patients who did not receive Haemaccel did not vary significantly from 0.71 mmol/l over the 24 hour period.

Reference range for ionised calcium in plasma is 1.18 to 1.30 mmol/l.

were then determined (normal range 1.18 to 1.30 mmol/litre). The results were compared using the Student *t* test and regression analysis.

Results

Table 1 shows that the mean volume of Haemaccel infused per patient over the 24 hour study period was 1640 ml (10.25 mmol Ca^{2+}), range 500 to 3500 ml (3.13 to 21.88 mmol Ca^{2+}). At all time intervals studied the mean ionised calcium values while receiving Haemaccel were highly significantly different from control values. The mean ionised calcium concentration (mmol/litre) before infusion of Haemaccel was 0.71 (SD 0.15, range 0.49 to 0.90), and at 2, 4, 8, and 24 hours, during infusion of Haemaccel, the values were 1.38 (SD 0.34, range 0.91 to 2.02), 1.40 (0.44, 0.97 to 1.91), 1.23 (0.27, 0.88 to 1.61), and 1.18 (0.31, 0.79 to 1.87), respectively (at least $P < 0.001$ compared with the pre-Haemaccel value, table). The mean ionised calcium values (0.68 to 0.84 mmol/litre) in five trauma patients who did not receive Haemaccel did not vary significantly from the basal value of 0.71 mmol/litre over the 24 hour study period. In some patients infusion of Haemaccel caused significant hypercalcaemia, with ionised calcium levels as high as 2.07 mmol/litre.

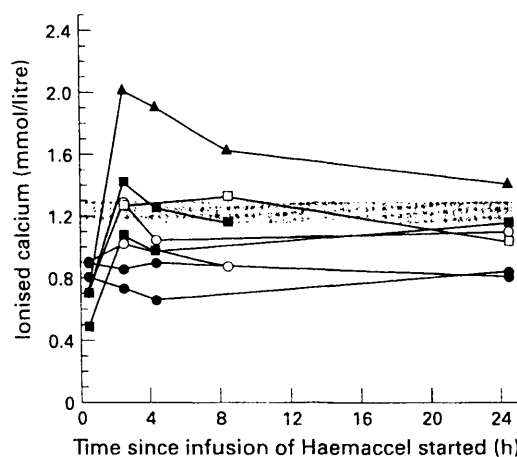


Figure 2 Ionised calcium was measured on admission in patients who required rapid fluid infusion during resuscitation. After the initial infusion no further Haemaccel was given and further ionised calcium measurements were repeated at 2, 4, 8, and 24 hours. Volumes of Haemaccel (calcium concentration 6.25 mmol/litre) initially infused were: none = ●; 500 ml; 1000 ml ■; 1500 ml □; 2000 ml ▲. The shaded area represents the normal range.

Figure 1 shows that the rise in ionised calcium two hours after infusion of Haemaccel was directly proportional to the volume of calcium containing fluid infused ($r = 0.917$, $P < 0.001$). Infusion of 1 litre (6.25 mmol Ca^{2+}) and 2 litres (12.5 mmol Ca^{2+}) of Haemaccel increased ionised calcium by about 0.5 and 1.0 mmol/litre respectively. Although the plasma ionised calcium concentrations rose rapidly in proportion to the volume of Haemaccel given, if the infusion rate was not maintained, the plasma calcium values tended to fall, usually to below the reference range, unless large volumes had been infused initially (fig 2).

Discussion

Our results confirmed previous observations¹² that following trauma, plasma ionised calcium levels fall within 24 hours in those patients not receiving calcium containing fluids. The mean pre-Haemaccel ionised calcium value was 0.71 mmol/litre (normal range 1.18 to 1.30) and did not change significantly over a 24 hour study period in control patients who did not receive Haemaccel infusions. We have also shown that infusion of Haemaccel causes a marked rise in the concentration of plasma ionised calcium, sometimes significantly above physiological levels. There was a direct relation between the amount of Haemaccel infused and

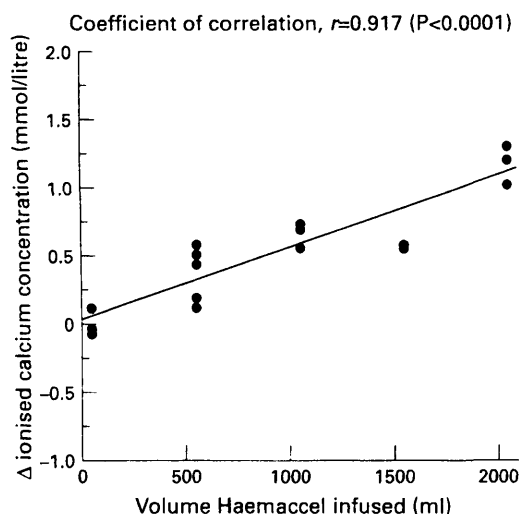


Figure 1 Ionised calcium was measured on admission in patients who required fluid resuscitation. The measurements were repeated two hours later following a rapid infusion with different volumes of Haemaccel at a calcium concentration of 6.25 mmol/litre. The change in ionised calcium over the 2 h period was calculated.

the increase in ionised calcium concentration. Hypercalcaemia ($\text{Ca}^{2+} > 1.30$ mmol/litre) was observed in at least one measurement in 50% of patients who were given Haemaccel infusions. In the trauma patients, following the infusion of Haemaccel the plasma ionised calcium concentration rose initially, but the levels then tended to fall, usually to below physiological levels, unless the infusion rate was maintained (fig 2), indicating a persistence of the normal response to trauma. Since this normal response is to lower the plasma ionised calcium, then raising the concentrations, especially above physiological values, may be deleterious for primary haemostatic mechanisms and cell function. In our study the calcium was raised, and quite markedly so, since the supranormal levels seen were superimposed on a low physiological level.

The high ionised calcium level may also reduce platelet aggregation by enhancing the rate of platelet disaggregation. There is an optimum concentration of extracellular plasma calcium required for platelet aggregation to be achieved.⁸ When the level of calcium goes above this optimum level the extent of platelet aggregation is reduced by enhancing the rate of disaggregation, and this could be one of the factors that leads to an increase in bleeding time seen in the trauma patient.

The inhibitory effect of calcium on platelet aggregation *in vitro*⁸ and the prolongation of the bleeding time in traumatised patients following infusion of Haemaccel⁷ has, however, to be contrasted with the potentially detrimental effects of low plasma ionised calcium concentrations on myocardial function and the circulation.¹³ However, the shocked trauma patient has a greater tendency to hypoxic cellular damage due to hypoperfusion and hypostatic flow in vascular capillary beds, and infusing calcium containing fluids into these patients may increase the likelihood of influx of calcium into the cells.¹⁴ Previous investigators have suggested that this intracellular accumulation of calcium may be one of the reasons for

a change from reversible to irreversible cell injury and death.¹⁵ The potential detrimental effects may therefore outweigh the benefits of the positive inotropic effects on the heart and the increase in vascular tone which may follow calcium infusion.

Further research is required to establish the optimal plasma calcium concentrations to be achieved following trauma.

We thank Mrs J Thomas for her secretarial help in constructing this manuscript.

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Effects of gelatin-based resuscitation fluids on platelet aggregation

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Summary

Fluid resuscitation aims to maintain intravenous volume without significant effects on haemostasis. Several different types of i.v. fluid are available for use in a patient who has suffered trauma, but there is evidence that some resuscitation fluids may affect primary haemostasis. We have compared the effects of two resuscitation fluids, Haemaccel and Gelofusine, on platelet aggregation *in vitro*. These resuscitation fluids are both based on gelatin but Haemaccel contains a high concentration of Ca^{2+} whereas Gelofusine does not. Their effects on platelet aggregation in whole blood, induced by a range of different agents, were determined using a platelet-counting technique. Both Haemaccel and Gelofusine prevented platelet aggregation induced by ristocetin ($P < 0.05$, Mann–Whitney). In addition, Haemaccel proved to be a potent inhibitor of the platelet aggregation that occurred in response to all of the other agonists investigated: adenosine diphosphate, platelet-activating factor, collagen, a thromboxane A_2 mimetic (U46619) and epinephrine. The additional inhibitory effects of Haemaccel were largely, but not completely, attributable to its high Ca^{2+} content. Inhibition of platelet aggregation by ristocetin may indicate a mechanism by which Haemaccel or Gelofusine may contribute to impaired haemostasis. The presence in Haemaccel of high concentrations of Ca^{2+} , which is largely responsible for inhibition of the aggregation induced by other agents, may provide an additional means by which haemostasis could be impaired. (*Br. J. Anaesth.* 1998; 81: 198–202)

Keywords: haemostasis; resuscitation fluids, Gelofusine; resuscitation fluids, Haemaccel; platelet aggregation; trauma

Treatment of hypovolaemic shock requires immediate restoration of the circulating volume by rapid infusion of i.v. fluid to restore and sustain blood volume, cardiac output, stroke volume, blood pressure, urinary output and oxygen delivery. Some resuscitation fluids contain the colloid gelatin; some also contain Ca^{2+} , which is intended to correct the decrease in ionized calcium that follows traumatic hypovolaemia.¹ The aim of adding calcium is to improve cardiac output by increasing and maintaining the force of contraction and also by increasing peripheral vascular tone.² The amount of calcium in the fluids is variable, and one gelatin-based solution, Haemaccel, contains a high concentration, 6.25 mM. Gelofusine is a gelatin-containing solution that does not contain Ca^{2+} .

There is concern that Haemaccel may interfere with primary haemostasis. In a previous study it was noticed that Haemaccel infusion to traumatized patients was associated with an increased bleeding time and pronounced blood loss.³ An increased bleeding time in animals receiving Haemaccel has been documented,⁴ and this was associated with significantly reduced adenosine-diphosphate-induced platelet aggregation in platelet-rich plasma derived from these animals. Haemaccel infusion to trauma patients can lead to hypercalcaemia, because of its high Ca^{2+} content.⁵ It is also known that increasing the concentration of Ca^{2+} in blood plasma *in vitro* can impair the platelet aggregation that is a determinant of primary haemostasis.^{6,7} It was therefore speculated that impaired haemostasis might be a consequence of the high concentration of Ca^{2+} in Haemaccel.⁵ In the present study we have investigated the effects of Haemaccel and Gelofusine on the platelet aggregation induced by several different agonists. The agonists chosen were adenosine diphosphate (ADP), platelet-activating factor (PAF), collagen, a thromboxane A_2 mimetic (U46619), epinephrine and ristocetin. The effects of Haemaccel and Gelofusine on platelet aggregation were determined in whole blood using a platelet-counting technique.⁸ The fluids were added to achieve a final concentration of 40% in blood, equivalent to an infusion of about 2 litres into a 70 kg person. As hypercalcaemia is also known to affect the platelet aggregation induced by some agents,^{6,7} we also compared the effects of Haemaccel and Gelofusine with those of Ca^{2+} alone. We also carried out experiments in which Ca^{2+} had been added to Gelofusine. The whole blood was prepared using hirudin as the anticoagulant rather than the conventional citrate. This is because hirudin, unlike citrate, does not reduce the physiological level of ionized calcium in plasma, and additional Ca^{2+} can be added without affecting its ability to act as an anticoagulant.

Materials and methods

BLOOD COLLECTION

Venous blood was obtained from normal subjects who denied taking any medication in the 2 weeks before sampling. Blood was taken into a polypropylene

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syringe using a 19-gauge needle, and dispensed into a polypropylene tube containing Revasc (recombinant hirudin, final concentration 50 g ml^{-1}). The sample was routinely kept for 30 min at 37°C before testing.

Haemaccel (3.5% w/v polygeline) was purchased from Behring and Gelofusine (4% w/v succinylated gelatin) from B. Fraun Medical. Calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$) was obtained from Fisons, and sodium chloride (0.9% w/v) from Baxter Health Care. All platelet agonists were obtained from Sigma Chemicals except collagen (Hormon-Chemie) and ristocetin A sulphate (AL). Revasc (recombinant hirudin) was a gift from Ciba Geigy. The platelet fixative solution contained 150 mM NaCl, 4.6 mM Na_2EDTA , 4.5 mM Na_2HPO_4 , 1.6 mM KH_2PO_4 and 0.16% w/v formaldehyde, pH 7.4.

PLATELET AGGREGATION

Platelet aggregation in whole blood was measured using the platelet counting technique described by Fox and colleagues.⁸ A blood sample ($292 \mu\text{l}$) was mixed with $200 \mu\text{l}$ of the test solution (Haemaccel, Gelofusine or isotonic saline, previously warmed to 37°C) in a small polystyrene tube. After removing a $12 \mu\text{l}$ aliquot to 30 litre of fixative solution, $20 \mu\text{l}$ of a solution of an aggregating agent was added, and the sample placed in a water bath (37°C) and stirred (1000 r.p.m.). At appropriate time-points, $12 \mu\text{l}$ aliquots were removed from the sample and fixed as described. The number of single platelets in the fixed samples was measured using an Ultra-Flo 100 Whole Blood Platelet Counter. Platelet aggregation was expressed as the percentage fall in the number of single platelets compared with the starting count. The results are expressed as medians and interquartile ranges; in each case $n=7$, using blood from different volunteers. When the effects on platelet aggregation of additional Ca^{2+} were investigated, $10 \mu\text{l}$ of an isotonic solution of 100 mM CaCl_2 was added to saline ($190 \mu\text{l}$) or Gelofusine ($190 \mu\text{l}$) before adding the blood and aggregating agent. In the case of saline and Gelofusine this resulted in a concentration of ionized calcium in the blood of 2.6 mM and 2.5 mM respectively. This compared with a concentration of 2.8 mM when Haemaccel was used alone, and with 0.7 mM and 0.6 mM respectively when saline and Gelofusine were used alone. These measurements were performed using an AVL 988-4 analyser.

Statistical analysis was performed using a Mann-Whitney test. $P \leq 0.05$ was considered statistically significant.

Results

The effects of the various fluids on the platelet aggregation induced by a range of aggregating agents are shown in figs 1–6. The agonists used were ristocetin (1 mg ml^{-1} , fig. 1) adenosine diphosphate (ADP, $0.3 \mu\text{M}$, fig. 2), collagen ($1 \mu\text{g ml}^{-1}$, fig. 3), platelet-activating factor (PAF, $0.3 \mu\text{M}$, fig. 4), the thromboxane A_2 mimetic U46619 ($0.3 \mu\text{M}$, fig. 5) and epinephrine ($100 \mu\text{M}$, fig. 6). The timepoint after stimulation at which aggregation was measured was dependent on the agonist used. With ADP and PAF, aggregation was determined at 30 s (figs 2A and 4A) and 2 min (fig. 2B and 4B), as aggregation to low con-

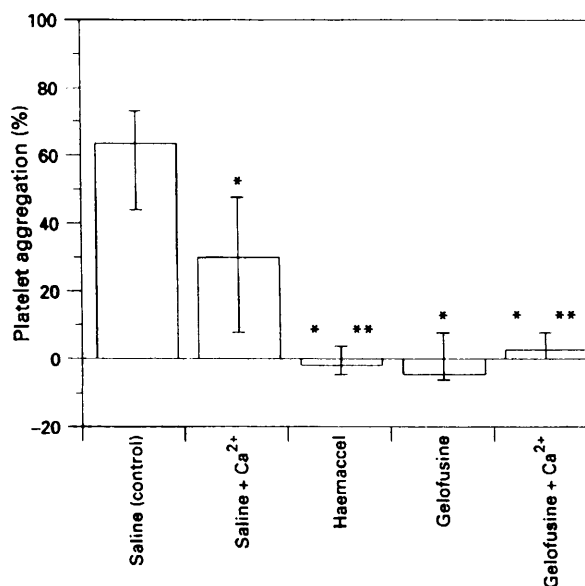


Figure 1 The effect of Haemaccel, Gelofusine and Ca^{2+} on the platelet agglutination induced by 1 mg ml^{-1} ristocetin at 4 min in hirudinized whole blood (* $P < 0.05$ compared with saline, ** $P < 0.05$ compared with saline and saline + Ca^{2+}).

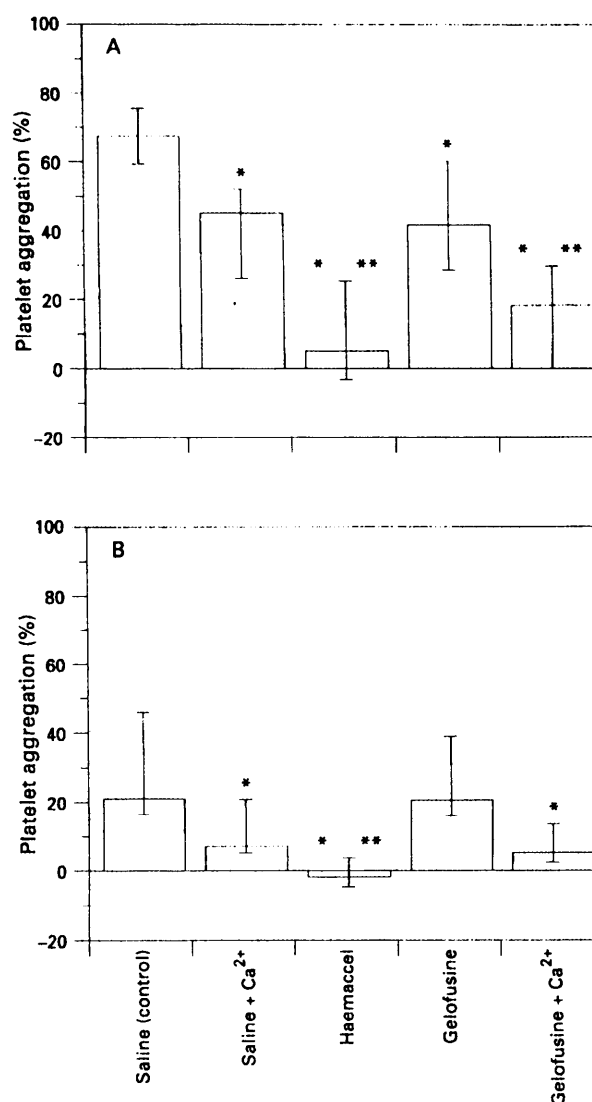


Figure 2 The effects of Haemaccel, Gelofusine and Ca^{2+} on the platelet aggregation induced by $0.3 \mu\text{M}$ ADP at 30 s (fig. 2A) and 2 min (fig. 2B) in hirudinized whole blood (* $P < 0.05$ compared with saline, ** $P < 0.05$ compared with saline + Ca^{2+}).

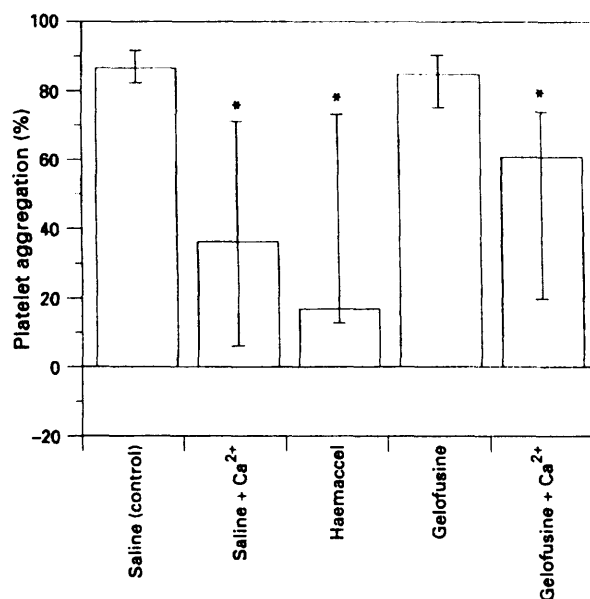


Figure 3 The effects of Haemaccel, Gelofusine and Ca²⁺ on the platelet aggregation induced by 1 g ml⁻¹ collagen at 2 min in hirudinized whole blood (**P* < 0.05 compared with saline).

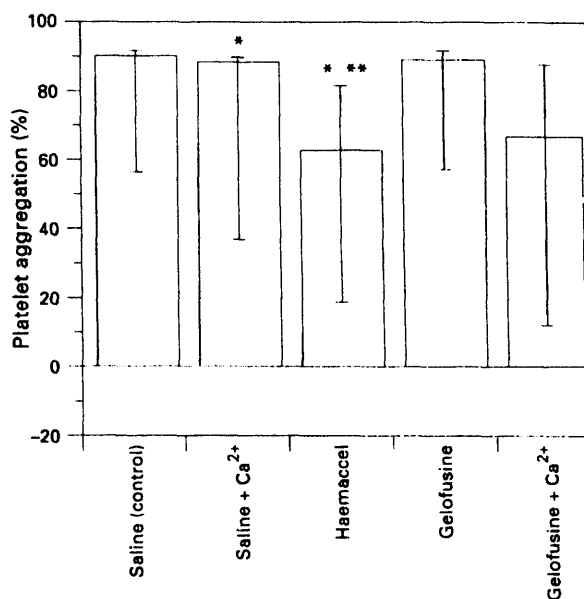


Figure 5 The effects of Haemaccel, Gelofusine and Ca²⁺ on the platelet aggregation induced by 0.3 μM U46619 at 2 min in hirudinized whole blood (**P* < 0.05 compared with saline, ***P* < 0.05 compared with saline + Ca²⁺).

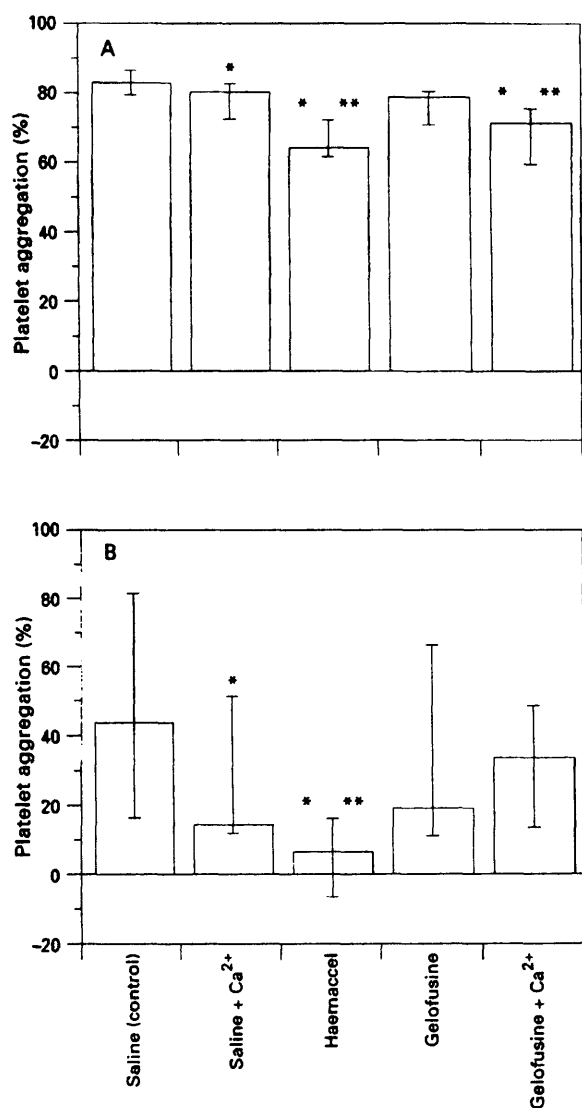


Figure 4 The effects of Haemaccel, Gelofusine and Ca²⁺ on the platelet aggregation induced by 0.3 μM PAF at 30 s (fig. 4A) and 2 min (fig. 4B) in hirudinized whole blood (**P* < 0.05 compared with saline, ***P* < 0.05 compared with + Ca²⁺).

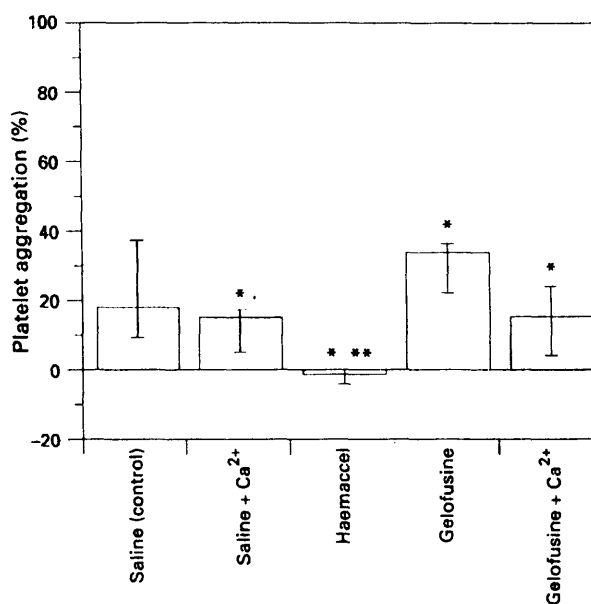


Figure 6 The effect of Haemaccel, Gelofusine and Ca²⁺ on the platelet aggregation induced by 100 μM epinephrine at 4 min in hirudinized whole blood (**P* < 0.05 compared with saline, ***P* < 0.05 compared with saline + Ca²⁺).

centrations of these agonists tends to be reversible. The response to the remaining agonists was measured when aggregation would normally be complete (collagen and U46619, 2 min; epinephrine and ristocetin, 4 min).

In the presence of Haemaccel or Gelofusine the platelet aggregation induced by ristocetin was totally prevented (fig. 1). Adding Ca²⁺ to the blood also inhibited the aggregation but this inhibition was only partial. The abolition of platelet aggregation in response to ristocetin by Gelofusine was not affected by increasing the Ca²⁺ concentration.

Haemaccel significantly inhibited the platelet aggregation that occurred in response to all of the other agonists (figs 2–6). Haemaccel also appeared to make the aggregation in response to ADP and PAF

more reversible (figs 2 and 4). Adding CaCl_2 to the whole blood also resulted in a significant inhibition of platelet aggregation in response to all of the agonists studied. The inhibition seen with Haemaccel was always significantly greater than that seen with CaCl_2 alone, the only exception being when collagen was used as the agonist (fig. 3).

Except when ristocetin was used as the agonist, Gelofusine had only a slight effect on platelet aggregation. Significant inhibition of aggregation was seen in response to ADP at 30 s (fig. 2A), but this effect was lost by 2 min (fig. 2B). Gelofusine appeared to slightly potentiate the aggregation brought about by epinephrine (fig. 6).

Generally, there was less platelet aggregation with Gelofusine containing added CaCl_2 than with Gelofusine alone. This was the case in response to ADP (fig. 2), collagen (fig. 3), PAF (at 30 s, fig. 4A) and epinephrine (fig. 6). The results obtained for U44619 (fig. 5), and PAF (2 min, fig. 3B) show a trend towards inhibition but this did not reach statistical significance. The degree of inhibition seen with Gelofusine containing CaCl_2 was significantly greater than that observed with CaCl_2 alone for ADP (30 s, fig. 2A) and PAF (30 s, fig. 4B). Overall, the effect of Ca^{2+} on platelet aggregation was similar whether it was used alone or in combination with Gelofusine.

Discussion

One of the platelet agonists chosen for this investigation was ristocetin, which induces platelet aggregation through a mechanism involving von Willebrand factor and its receptor glycoprotein Ib on the platelet surface. Von Willebrand factor is also important in mediating adhesion of platelets to damaged vascular tissue, by forming a link between glycoprotein Ib and the damaged tissue. The aggregation induced in whole blood by this agent was prevented by Haemaccel and Gelofusine. Inhibition of ristocetin-induced aggregation by Haemaccel confirms earlier observations,^{9 10} and extends the finding to the aggregation that occurs in whole blood as well as that in platelet-rich plasma. Platelets from patients with von Willebrand disease do not respond to ristocetin and this lack of response is associated with markedly reduced platelet adhesion to damaged vascular tissue and increased bleeding. There are indications that other, non- Ca^{2+} -containing, gelatin resuscitation fluids, as well as Haemaccel, may impair haemostasis. In a retrospective analysis of patients who had received oxypolygelatin before cardiopulmonary bypass, use of high doses of the fluid was associated with increased postoperative blood loss.¹¹ A recent prospective study, in which Gelofusine was infused into healthy volunteers, reported a significant increase in bleeding time.¹² Interference with haemostatic mechanisms involving von Willebrand factor could therefore be one way in which Haemaccel and Gelofusine, and presumably other gelatin-containing fluids, could increase bleeding and blood loss. An *in vitro* test of the effects of Haemaccel or Gelofusine on "clot quality" revealed that the presence of either agent led to production of clots that were more friable than normal clots.¹³

The other platelet agonists used in this investigation were ADP, PAF, collagen, U44619 and epinephrine. All these agents activate glycoprotein IIb/IIIa

receptors on the platelet surface, leading to fibrinogen binding and platelet aggregation—another mechanism through which platelets contribute to primary haemostasis. Haemaccel inhibited the aggregation that occurred in response to all these agonists and increased the reversibility of the response. Gelofusine did not produce the same effect. The effect of Gelofusine on the aggregation induced by all agents other than ristocetin was slight, and although there was significant inhibition of the early aggregation induced by ADP this was matched by a slight potentiation of the aggregation induced by epinephrine. The different results obtained for the two gelatin preparations appeared to be largely, but not entirely, due to the presence of a high concentration of Ca^{2+} in Haemaccel.

There are reports that gelatin-containing resuscitation fluids can inhibit platelet aggregation^{9 10} but, until now, there have been no direct comparative evaluations of the antiplatelet effects of Ca^{2+} -containing *vs* non- Ca^{2+} -containing preparations. Infusion of Gelofusine during cardiopulmonary bypass in man can lead to a reduced platelet response to Gelofusine¹⁴, but it is not known how the fluids affect the different pathways leading to platelet aggregation.

Stibbe and colleagues studied the effects of several gelatin plasma substitutes on the platelet aggregation in platelet-rich plasma induced by ADP, collagen and epinephrine.¹⁰ Haemaccel always inhibited the aggregation induced by all these agents whereas aggregation was enhanced by some other gelatin preparations. Our own experiments extend these observations to whole blood, emphasize that different results may be obtained with different types of gelatin colloids, and demonstrate that the presence of high concentrations of Ca^{2+} in Haemaccel increases the inhibition of aggregation seen using a range of platelet agonists. The inhibitory effects of Haemaccel on platelet aggregation induced by agents that act through glycoprotein IIb/IIIa and fibrinogen seem largely to be attributable to its high Ca^{2+} content, but some of the inhibitory effect may lie with its gelatin component, polygeline, which differs from succinylated gelatin, the gelatin component of Gelofusine.¹⁵

As Haemaccel inhibits the platelet aggregation induced by a range of agonists in addition to ristocetin, its administration could have an additional detrimental effect on primary haemostasis. On the other hand, agents that inhibit platelet aggregation can reduce the tendency to thrombosis, and in an animal model, platelet thrombus formation was significantly impaired after Haemaccel infusion.¹⁶ Agents that specifically block platelet aggregation by interfering with the ability of fibrinogen to bind to glycoprotein IIb/IIIa receptors are being developed for use as antithrombotic agents in man.

We believe that further studies of gelatin-containing colloids are warranted to determine their potential for increasing bleeding after large-volume blood replacement. It might be important to balance this effect against the perceived need to restore calcium lost as a result of hypovolaemic traumatic shock. There may also be a case for further study of circumstances in which the inhibitory effects of such fluids on platelet aggregation could be beneficial. For example, an i.v. fluid with antiaggregatory properties might benefit trauma patients who have underperfused hypostatic

capillary beds, by promoting blood flow and enhancing organ perfusion and tissue oxygenation. Similarly, inhibition of platelet aggregation could provide a degree of protection from thrombosis. However, whether these possible benefits would outweigh the potential for an increase in bleeding in the trauma patient remains to be proven.

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Fluid Resuscitation in A&E Departments - Who uses What and Why?

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INTRODUCTION

The choice of fluid for resuscitation for victims of trauma remains controversial. Research suggests that some solutions are associated with poorer outcomes^{1,2}. Both crystalloids and colloids are regularly used to treat these patients in Accident and Emergency departments throughout the U.K. and Ireland. We sought to

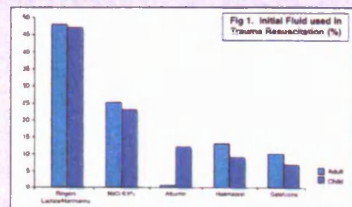
determine the factors that lead senior Accident and Emergency clinicians to choose a particular resuscitation fluid, the volumes they use and the complications they have experienced using these fluids.

METHODS

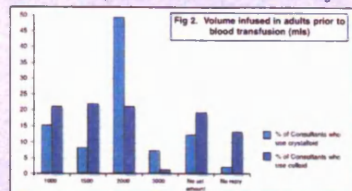
An anonymous questionnaire was sent to all clinicians who were, in 1996, Accident and Emergency Consultants in the U.K. or Ireland. Using the 1996 BAEM directory and the Department of Health as reference sources, a total of 459 consultants were identified.

RESULTS

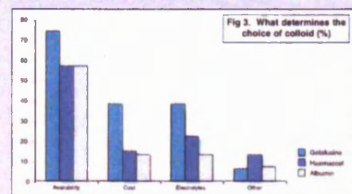
The response rate was 81.9% (378/459), and of these 70% work in a district general hospital, 84% of teaching hospital respondents and 77% of district general hospital respondents have departmental guidelines on fluid resuscitation. However clinical practice varies considerably. Crystalloids are used as the primary resuscitation fluid in adults by 74.3% and colloids by 23.9%. The primary resuscitation fluid used in trauma resuscitation in adults and children is shown in Fig 1.



The volumes of fluid used prior to commencing blood transfusion in adults ranged from 500-4000mls. Although the volumes infused will depend on local factors, including use of rapid fluid infusion sets and the time for blood to become available, the results show significant variations in clinical practice. The results are shown in Fig 2.

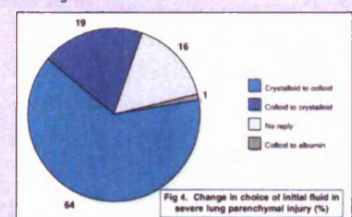


Consultants who use colloid were asked what factors influenced their decision to choose a particular colloid (See Fig 3).

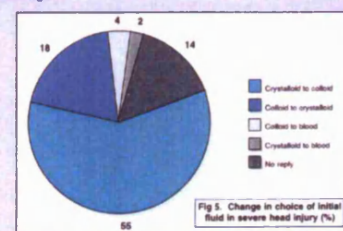


It is interesting to note that the same reasons were given for choosing colloids with significantly different electrolyte constituents. Both Gelofusine and Haemaccel were selected on the basis of "Hospital policy" by some respondents.

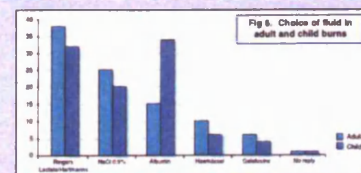
When faced with a patient with a severe lung parenchymal injury, 28% of consultants would change their choice of initial fluid. These changes are shown in Fig 4.



Similarly, when faced with a patient with a severe head injury, 13% of consultants would change their choice of initial fluid. These changes are shown in Fig 5.



The variations in fluid use noted in trauma patients are also seen in both adult and child burns patients. Interestingly, albumin is the number one choice of fluid in children but not in adults. The questionnaire was answered prior to the publication of the review on the use of albumin³ (see Fig 6).

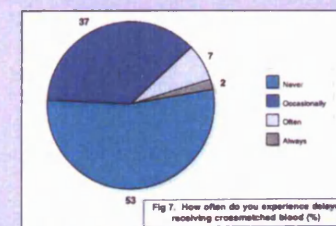


ALLERGIC REACTIONS

Although 8.8% of consultants noted the development of an allergic reaction to a colloid in a patient they were treating, only 39.3% of incidents were reported to the Committee on Safety of Medicines. Similarly, only 20% of the reactions to blood noted by 11% of consultants were reported. The severity of these reactions was not requested in the questionnaire, and these episodes may vary from the very mild to life-threatening incidents.

ACCESS TO BLOOD

All Accident and Emergency departments receiving major trauma must have ready access to blood. Some departments have significant problems when cross-matched blood is urgently required (see Fig 7).



CONCLUSION

Despite the majority of departments having guidelines for fluid resuscitation, clinical practice shows significant variations both in terms of the quantity and type of solutions used to resuscitate trauma and burns patients.

Although crystalloid is the preferred type of fluid for trauma resuscitation by A&E Consultants, it is clear there is no consensus on the most appropriate fluid for resuscitation in either trauma or burns. This applies equally to the resuscitation of both adults and children. The recent Cochrane report on the use of Albumin may result in changes in clinical practice in Accident and Emergency departments. Further

research is indicated to determine the ideal resuscitation fluid in trauma and burns in the immediate post-injury phase.

The quoted rates of allergic reaction to colloid may not reflect the true incidence due to under-reporting. All incidents should be reported in order to obtain a true figure for allergic reactions to colloids.

Some departments have serious recurring problems obtaining cross-matched blood in an appropriate timeframe. Group O -ve and cross-matched blood must be readily available in all departments receiving multi-trauma patients.

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Designed and produced by The Medical Illustration Department, The Leicester Royal Infirmary NHS Trust

fashion from which a list of all the injuries sustained was prepared, enabling them to be scored using the Abbreviated Injury Scale (1990 revision).

One hundred and fifty nine deaths (122 male, 37 female) occurred during the five year period. Various forms of "human error" were implicated in causing the majority of deaths, which included combinations of the following: excessively fast driving, driving under the influence of alcohol, attempting dangerous overtaking manoeuvres and failure to wear an available seat belt. Most of those who died were found dead at scene. There were 72 people with unsurvivable injuries (AIS = 6, Injury Severity Score = 75) causing immediate death at scene, which mainly involved injury to the brain, brainstem, thoracic aorta and upper spinal cord.

The results of this study confirm the continuing role of road traffic collisions in causing premature death in south east Scotland. The predominance of prehospital deaths and the frequency of unsurvivable injury underlines the importance of injury prevention measures in the future prevention of such deaths. Given the background to many of the collisions it is clear that efforts need to be aimed at changing the behaviour of both car drivers and their passengers.

Intravenous fluid use in accident and emergency departments: effects of published studies in medical literature

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Background and methods—In view of the continuing controversy over the safety of colloids and albumin since the meta-analyses published in the *British Medical Journal* in 1998 we sought to assess prescribing practice by means of postal questionnaires. These were sent to all accident and emergency (A&E) consultants (459) in the UK and Republic of Ireland around the time of the first meta-analysis with a follow up in 1999. Similar questionnaires were then sent to all consult-

Table 14

	ADP	Collagen	Adrenaline	Ristocetin	Ca ²⁺	Mg ²⁺
Whole blood	58 (9)	91 (1)	38 (5)	28 (9)	1.16	0.46
Plasma	55 (9)	57 (12)*	19 (3)*	13 (5)	1.16	0.50
Saline	69 (4)+	80 (7)	35 (6)+	65 (11)+	0.77+	0.30+
Haemaccel	32 (7)+	42 (11)	11 (2)+	-8 (3)+	2.88+	0.36+
Gelofusine	82 (3)+	88 (2)+	45 (8)+	4 (3)	0.70+	0.28+
Albumin	70 (4)+	74 (5)	26 (5)+	38 (11)+	0.67+	0.26+

*p<0.05 cf whole blood. +p<0.05 cf dilution with autologous plasma.

ants in burns units (205) and intensive therapy units (ITUs) (1444). In the A&E survey consultants chose the one fluid they would use: for burns and ITU, they ranked fluids (1=first choice, etc).

Results—Overall response rates: 88% initial and 64% follow up A&E surveys, 55% burns and ITU surveys. For trauma: A&E consultants preferred crystalloids: Ringer's lactate (46% adults, 43% children), normal saline (25% adults, 23% children). In ITUs the first choice was Gelofusine (mean rank 4.6) in adults and normal saline (4.5) in children. For burns: In adults: A&E consultants preferred crystalloids: Ringer's lactate (37%), normal saline (25%). For children and all ages at the burns unit: albumin is the preferred fluid (34% for children at A&E, rank orders 4.0 adult, 4.5 children at burns unit).

Conclusions—Since the meta-analyses consultants have changed their practice. Those who have stopped using colloids: in A&E for trauma: 11% adults, 13% children. In ITU practice: 1.5% adults and 1.7% children. For burns in A&E: 13% adults, 14% children. At burns units: 7% changed their practice in view of the colloid meta-analysis. Those who have stopped using albumin: in A&E for trauma: 1.7% adults, 7.2% children. In ITU practice: 31% adults, 18% children. For burns in A&E: 12% adults, 16% children. At burns units: 14% changed their practice in view of the albumin meta-analysis. Thus the published meta-analyses have had a marked impact on intravenous fluid use resulting in a significant reduction in the use of these fluids in clinical practice.

Studies on the effects of resuscitation fluids on platelet aggregation in vitro

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Continuing controversy exists over fluids used for trauma and burns and their haemostatic effects. In this study, effects on platelet aggregation were determined in whole blood and compared with results obtained after diluting blood with autologous plasma. This acted as a non-crystalloid, non-synthetic colloid control.

The fluids or plasma were added to hirudinised whole blood from volunteers (n=10) in the ratio 2:3 (to model the ATLS protocol). Aggregation was measured in response to ADP, collagen, adrenaline and ristocetin. Results were compared with those for undiluted blood and expressed as mean (SEM) percentage aggregation. Ionised Mg²⁺ and Ca²⁺ were measured using an AVL Analyser (table 14).

Reducing the cell count by adding autologous plasma to whole blood, reduced aggregation in response to collagen and adrenaline, but not ADP. Saline and Gelofusine enhanced aggregation in response to ADP, collagen and adrenaline, possibly as a consequence of lowered Ca²⁺. Haemaccel always inhibited aggregation further, probably through increased Ca²⁺ and, like Gelofusine, abolished ristocetin induced responses. Compared with saline and Gelofusine, albumin usually limited enhancement of aggregation consequent to creating a low Ca²⁺ environment. Therefore we anticipate that the effects of these fluids in clinical practice will depend largely on the extent of changes in blood cell counts and the rapidity with which homeostatic mechanisms correct accompanying changes in plasma Ca²⁺.

WHOLE BLOOD PLATELET AGGREGATION DETERMINED USING A PLATELET COUNTING TECHNIQUE: A UNIVERSAL TOOL FOR STUDIES IN MAN

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Measurement of platelet aggregation in whole blood using a platelet counting technique has been performed routinely in the Universities of Nottingham, Leicester and Jena and the Fachhochschule Jena for almost two decades. Despite this, the approach is not used universally. The purpose of this presentation is to remind those involved in platelet research that platelet aggregation in whole blood using a platelet counting technique (1) provides a highly sensitive and close to physiological means of studying the aggregation process, (2) is easy to perform, and (3) sometimes provides information that cannot be obtained using other approaches. Further, the technique can be used in conjunction with flow cytometric analyses of platelet and leucocyte activation.

Highly sensitive: Platelet aggregation is measured by determining the fall in the number of single platelets in stirred whole blood. Samples are fixed and counted using an appropriate whole blood platelet counter. Even formation of small microaggregates results in an extensive fall in platelet count, unlike, for example, studies in PRP (light absorbance) or whole blood (impedance) which are only sensitive to macroaggregation.

Physiological: The effects of red cells and white cells on platelet aggregation should not be under-estimated. Studies in whole blood allow the influence of these cell types to be assessed, which is not the case in PRP. Appropriate choice of anticoagulant (or none) can ensure maintenance of near-physiological conditions.

Easy to perform: Using a standard stirring device and a common fixing solution, the blood can be treated in the same way in different centres. It is hoped that whole blood cell analysers will also be available quite soon.

New information obtained: A search through the published literature reveals numerous examples of new information obtained using this approach. Examples include: pharmacological studies of serotonin-antagonists; effects of SH-blocking reagents on platelet function; studies of 'spontaneous' platelet aggregation in physiological and pathological conditions; inhibitory effects of dipyridamole; easy detection of heterozygous Glanzmann's thrombasthenia; detection of marked platelet hyperactivity in a patient studied just prior to having a stroke; effects of glucose on platelet aggregation; enhanced platelet aggregation in the presence of streptokinase or some radiographic contrast media; and the inhibitory effects of aspirin, dipyridamole, ticlopidine, clopidogrel, direct acting P_{2T} antagonists, GPIIb/IIIa antagonists, GTN and certain infusion fluids.

Studies of platelet and leucocyte activation: Flow cytometric analyses of the same samples used for studies of platelet aggregation reveal further quantitative information on platelet-leucocyte conjugate formation and the level of activation of individual cell types.

COMPARATIVE STUDIES ON PLATELET FUNCTION AND HAEMOSTASIS IN PATIENTS UNDERGOING SURGICAL TRAUMA: EFFECTS OF FOUR INTRAVENOUS FLUIDS IN PATIENTS UNDERGOING HIP REPLACEMENT: A PROSPECTIVE DOUBLE-BLIND RANDOMISED STUDY

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Investigations were performed on the effects of four different intravenous fluids on platelet aggregation, platelet expression of surface glycoproteins and measures of coagulation as well as bleeding time and post-operative blood loss. We studied 55 patients undergoing primary total hip arthroplasty. Two litres of 4.5% human albumin, Gelofusine, Haemaccel or saline were given during the operation. Blood was taken and bleeding time performed after induction of anaesthetic before start of surgery (PRE), at the end of surgery (POST) and 2 h after the end of surgery (LAST). Hirudinised whole blood was stimulated with varying concentrations of adenosine diphosphate (ADP), collagen, adrenaline and ristocetin for appropriate times before fixation. Platelet aggregation was then determined by counting fixed single platelets.

Gelofusine and Haemaccel completely abolished aggregation in response to ristocetin at the POST ($p<0.001$ and $p=0.001$) and LAST ($p=0.001$ and $p=0.006$) time points. Albumin inhibited collagen-induced aggregation ($p=0.004$ at POST and $p=0.006$ at LAST time points). All fluids slightly but significantly inhibited ADP- and adrenaline-induced aggregation at both POST and LAST time points ($p=0.042$ or below). The effects on adrenaline-induced aggregation may be due to downregulation of catecholamine receptors in the face of elevated plasma catecholamines due to the operative trauma. We found no significant alteration in platelet surface glycoprotein expression.

There were persistent elevations in prothrombin F1+2 complex and thrombin/antithrombin III complexes ($p<0.0017$ and $p<0.0013$ for all fluids) indicating that the operative trauma had activated the coagulation system. However there was a reduction in fibrinogen and elevation of INR for all fluids ($p<0.0146$ and $p<0.0125$), and persistent reduction of factor XIII for the colloid fluids ($p<0.0077$). APTT and factor VIII activity only fell at the POST time point when colloid fluids were used ($p<0.0105$ and $p<0.0166$).

All the colloid fluids increased bleeding time ($p<0.0324$) but this was only at the POST time point, returning back to baseline by the LAST time point. It is difficult to explain this transient effect in terms of alteration in platelet function, as those changes were persistent. We failed to find any significant difference in post-operative blood loss between the groups.

Overall colloid fluids inhibit certain aspects of platelet function and the coagulation system. This may be clinically useful as operative trauma is associated with a pro-thrombotic state.

STUDIES ON THE EFFECTS OF RESUSCITATION FLUIDS ON PLATELET AGGREGATION *IN VITRO*

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These studies were performed as a prelude to a clinical study in which four different fluids were administered to patients undergoing total hip replacement and in which the effects of the fluids on platelet aggregation and haemostasis were to be investigated. The fluids were saline, Haemaccel, Gelofusine and albumin. For these *in vitro* studies the effects on platelet aggregation were determined in whole blood and compared with results obtained after diluting the blood with autologous plasma. The eventual aim was to obtain data *in vitro* that might help explain results that would be obtained *ex vivo* after administering the agents to man.

Blood was collected into hirudin as anticoagulant. The fluids or plasma were added to whole blood in the ratio 2:3 and aggregation responses were measured in response to ADP, collagen, adrenaline and ristocetin by whole blood platelet aggregometry using a platelet counting technique. Percentage aggregation was expressed as percentage fall in platelet count. The results were compared with those for undiluted whole blood. A full set of data was obtained using blood from 10 different volunteers and expressed as mean (SEM). Plasma Mg^{2+} and Ca^{2+} were measured using an AVL Analyser.

	ADP	Collagen	Adrenaline	Ristocetin	Ca^{2+}	Mg^{2+}
Whole blood	58 (9)	91 (1)	38 (5)	28 (9)	1.16	0.46
Plasma	55 (9)	57 (12)*	19 (3)*	13 (5)	1.16	0.50
Saline	69 (4)+	80 (7)	35 (6)+	65 (11)+	0.77+	0.30+
Haemaccel	32 (7)+	42 (11)	11 (2)+	-8 (3)+	2.88+	0.36+
Gelofusine	82 (3)+	88 (2)+	45 (8)+	4 (3)	0.70+	0.28+
Albumin	70 (4)+	74 (5)	26 (5)+	38 (11)+	0.67+	0.26+

* $p < 0.05$ cf. whole blood; + $p < 0.05$ cf. dilution with autologous plasma.

Adding autologous plasma to whole blood, and thereby reducing the cell count, reduced the aggregation responses to collagen and adrenaline, but not ADP. Saline and Gelofusine enhanced the aggregation responses to ADP, collagen and adrenaline, possibly as a consequence of lowered Ca^{2+} . Haemaccel always inhibited the aggregation further probably through increased Ca^{2+} and, like Gelofusine, abolished responses to ristocetin. Compared with saline and Gelofusine, albumin usually limited the enhancement of the aggregation response consequent to creating a low Ca^{2+} environment. As a result of these findings we anticipated that the effects of these agents on platelet aggregation in man would largely depend on the extent of the changes in blood cell counts and the rapidity with which homeostatic mechanisms corrected the changes in plasma Ca^{2+} that would accompany the various infusions.

MECHANISM FOR ENHANCED PHOSPHORYLATION OF THE PLATELET PLASMA MEMBRANE Ca^{2+} -ATPase IN HYPERTENSION

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Intracellular Ca^{2+} is increased in the platelets of hypertensive individuals. Previously, we demonstrated that platelet plasma membrane Ca^{2+} -ATPase (PMCA) activity inversely correlates with diastolic blood pressure and that inhibition of this Ca^{2+} -pump could explain the elevation of cytosolic Ca^{2+} in hypertension. More recently, we discovered that PMCA is phosphorylated on tyrosine residues during thrombin-stimulated platelet aggregation and this phosphorylation causes inhibition of PMCA activity. In the present work, we tested the hypothesis that tyrosine phosphorylation of PMCA in hypertensive patients accounts for the observed inhibition of the Ca^{2+} -pump. Platelets were obtained from untreated hypertensive and normotensive volunteers. PMCA was immunoprecipitated from solubilized platelets and tyrosine phosphorylation was quantified by chemiluminescence of immunoblots treated with anti-phosphotyrosine. PMCA content was measured on the same immunoblots by stripping and reprobing with anti-PMCA. The average PMCA tyrosine phosphorylation for 15 normotensive subjects (reported as normalized phosphotyrosine chemiluminescence/ng PMCA) was 0.53 ± 0.09 , while the average for eight hypertensive individuals was 1.82 ± 0.25 ($P < 0.0005$, Mann-Whitney U test). Age, gender, and systolic blood pressure did not correlate with PMCA phosphorylation. These results suggest that PMCA in platelets of hypertensive individuals is inhibited due to tyrosine phosphorylation resulting in increased platelet intracellular Ca^{2+} , hyperactive platelets, and increased risk for heart attack and stroke.

We next investigated the mechanism of enhanced tyrosine phosphorylation of PMCA in hypertension. Addition of plasma obtained from untreated hypertensives to platelets isolated from normotensive volunteers resulted in an increase in intracellular Ca^{2+} within 1 min and an increase in PMCA tyrosine phosphorylation when compared to platelets mixed with plasma obtained from a normotensive individual. PMCA phosphorylation after addition of plasma reached a maximum at 30 min, decreased at 60 min, but did not return to baseline levels. Co-immunoprecipitation experiments indicate that the non-receptor tyrosine kinases Src and Yes are associated with PMCA in resting and activated platelets. These results suggest that a factor present in the plasma of hypertensives interacts with platelets causing enhanced tyrosine phosphorylation of PMCA resulting in inhibition of PMCA and increased cytosolic Ca^{2+} . PMCA tyrosine phosphorylation may be catalysed by closely associated Src or Yes.

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ORIGINAL ARTICLE

Prospective double-blind randomized study of the effects of four intravenous fluids on platelet function and hemostasis in elective hip surgery

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Summary A prospective randomized double-blind study was performed to determine the effects of three colloids, Haemaccel, Gelofusine and albumin, and also saline on platelet activation, platelet aggregation (induced by adenosine diphosphate (ADP), epinephrine, collagen) platelet agglutination by ristocetin and other hemostatic variables in 55 patients undergoing primary unilateral total hip replacement. The fluids were administered according to normal clinical practice and assessments were made immediately before, at the end, and 2 h after the end of surgery. Surgery was accompanied by thrombin generation (increases in thrombin/antithrombin III complex, prothrombin 2 fragment) platelet activation (TG) and compromised coagulation. Generally, the platelet activation appeared to result in platelet desensitization and brought about a persistent reduction in platelet aggregation to ADP and epinephrine, irrespective of the fluid used. Additionally, Haemaccel and Gelofusine inhibited ristocetin-induced platelet agglutination and albumin inhibited collagen-induced platelet aggregation. Gross inhibitory effects of Haemaccel that had been predicted from an earlier *in vitro* study did not occur. Particular fluids had selective additional effects on the hemostatic system. Albumin infusion served to maintain plasma albumin at normal concentrations postsurgery. The two gelatin preparations, Haemaccel and Gelofusine, maintained plasma viscosity. All three colloids led to a transient increase in activated partial thromboplastin time postsurgery and also a transient fall in the concentration of factor VIII, which were accompanied by a transient increase in bleeding time, but there was no measurable increase in blood loss. Inhibition of platelet

aggregation by certain colloids may provide additional protection against the increased thrombotic risk in patients following major surgery.

Keyword: bleeding time, coagulation, hemostasis, hip arthroplasty, intravenous fluids, platelet aggregation.

Introduction

Major surgery is always associated with administration of intravenous fluids to maintain blood volume, blood pressure and tissue perfusion. Several different types of fluid are used including crystalloids such as saline, or colloids such as Haemaccel, Gelofusine and albumin. There is continuing debate as to which fluids should be used in terms of their potential for enhancing blood loss and/or protection against postsurgical thrombosis [1–9]. The results of a previous study [8] performed wholly *in vitro* suggested that Haemaccel, in particular, might affect hemostasis and thrombosis through pronounced inhibition of platelet aggregation as a consequence of the high levels of ionized calcium present in the preparation (6.25 mmol L⁻¹) [10]. We conducted a prospective randomized double-blind study to determine the effects of four different fluids administered during hip surgery on platelet aggregation and other hemostatic parameters. The effects of the fluids on bleeding time (BT) and blood loss were also monitored. Assessments were made immediately before surgery (Pre), at the end of surgery (Post) and 2 hours after the end of surgery (Last).

Patients and methods

Patients and procedures

After obtaining local ethics committee approval and informed consent, we randomized consecutive eligible patients

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undergoing primary unilateral cemented total hip replacement in a prospective randomized double-blind study to receive either albumin (4.5% w/v human serum albumin; BPL, Elstree, UK; $n = 13$), Gelofusine (4% w/v succinylated gelatin; B Braun Medical, Sheffield, UK; $n = 14$), Haemaccel (3.5% w/v polygelatin with 6.25 mmol L^{-1} calcium; Beacon Pharmaceuticals, Tunbridge Wells, UK; $n = 14$), or saline (0.9% w/v sodium chloride; Baxter Health Care, Thetford, UK; $n = 14$). Randomization was achieved by the use of numbered envelopes. Data for one patient (randomized to albumin) were incomplete and excluded from the analysis.

Following randomization, the patient groups were as follows: albumin group (3M, 10F; median age 69.2 years; median bodyweight 66 kg); Gelofusine group (7M, 7F; median age 73.9 years; median bodyweight 75.8 kg); Haemaccel group (4M, 10F; median age 73.8 years; median bodyweight 77.8 kg); and saline group (11M, 3F; median age 69.1 years; median bodyweight 82.3 kg). There were no significant differences in ASA grade, initial ionized calcium and magnesium concentrations, serum albumin concentrations or hematocrit when classified by fluid group. Crosstabulation of sex, ABO blood group and Rhesus blood group showed no significant bias for either of the blood groups but a significant bias for sex.

Patients were not included in the study if there was a pre-existing defect in platelet function or coagulation, or if they were on warfarin or heparin therapy or on aspirin that could not be stopped for 2 weeks prior to the operation. No nonsteroidal agents were given on the morning of surgery, and low molecular weight heparin prophylaxis was given on the evening after surgery as was the normal practice in the Orthopaedic Unit. This was after all blood samples had been taken for the study reported here.

All patients received a standard general anesthetic comprising temazepam (10–20 mg) premedication and propofol ($2.0\text{--}2.5 \text{ mg kg}^{-1}$), and were maintained with isoflurane and nitrous oxide (1.0–2.5% isoflurane in nitrous oxide with 20–30% oxygen), to avoid effects of the anesthetic on BT and platelet function [11].

Immediately prior to surgery a dedicated 16G venous cannula was inserted and 56 mL blood taken into a polypropylene syringe and distributed as follows: 15 mL into a tube (Bibby Sterilin Ltd, Stone, UK) containing 150 U recombinant hirudin (RevasTM, a gift from Novartis, Farnborough, UK, final concentration 50 U mL^{-1}) for platelet aggregometry; 2.7 mL into a tube containing EDTA (Sarstedt, Leicester, UK) for a full blood count (FBC); 4 mL into a tube (Sarstedt) containing 0.106 mol L^{-1} citrate (achieving a final dilution ratio 1 part citrate: 4 parts blood) for plasma viscosity (PV); 11 mL into tubes (Sarstedt) containing lithium–heparin; 9.4 mL into serum gel tubes (Sarstedt) containing kaolin-coated beads for urea and electrolytes, calcium, and albumin; 9 mL into tubes (Sarstedt) containing 0.106 mol L^{-1} citrate for coagulation tests; 4.5 mL into a CTAD tube containing a mixture of sodium citrate/citric acid supplemented with the platelet aggregation inhibitors theophylline, adenosine and dipyridamole (Becton Dickinson,

Cowley, UK) for measurement of platelet factor 4 (PF4) and beta-thromboglobulin (BTG).

The citrate tubes and CTAD tubes were centrifuged at $250 \times g$ for 30 min at 4°C . Plasma was then aspirated and frozen. These were then stored at -80°C . Ionized calcium and magnesium were measured from the lithium–heparin sample on an AVL 988–4 analyzer. The hirudinized sample was placed in a flask at 37°C for transport and then in a water bath at the same temperature. Those for FBC and PV were kept at room temperature whilst the others were placed on ice.

The patient was transferred to the operating room and one arm placed on an arm board, with continuous monitoring of skin-surface temperature. Under the influence of an arm cuff inflated to 40 mmHg, a BT determination was performed using Simplate IIR device (Organon-Teknica, Cambridge, UK) [12], with the cuts arranged longitudinally on the forearm. This determination and blood sample constituted the Pre timepoint. The determination of BT was always performed by the same investigator.

Surgery then commenced and 2 L fluid infused during the operative period, the fluid choice of either saline, Haemaccel, Gelofusine or 4.5% human albumin being determined by the contents of the sealed envelope, and set up by an independent operator and covered with an opaque black bag. The amount of fluid used (2 L) corresponds to ATLS guidelines for fluid administration [13].

Repeat BT was performed and a further 56-mL blood removed from the cannula following skin closure (approximately 1 h after initiation of surgery). These determinations were the Post timepoint.

Two hours later, BT and blood sampling were repeated this being the Last timepoint. Following this final sampling, nonsteroidal analgesics and/or blood were given as required. Transfusion requirements were noted, as were the effects of these upon hemoglobin and hematocrit as assessed by the usual standard postoperative checks on full blood count.

Blood loss in theatre was determined by volume of blood and fluids in suction containers, blood on swabs, and volume of washing solutions used. The nursing staff on the wards, using a dedicated protocol sheet with set timepoints, noted postoperative blood loss into the drains. This was part of the usual recording of wound drainage, which had been ongoing for the previous year.

Platelet function

Platelet function tests began 30 min after sampling from the patient. Platelet aggregation/agglutination in whole blood was measured using the platelet-counting technique described by Fox *et al.* [14] and the fixation technique described by Bevan and Heptinstall [15]. Blood (480 L) was added to LP3 polystyrene tubes (LIP Equipment and Services Ltd, Shipley, UK) containing 20 L of varying concentrations of agonist, and the sample placed in a multisample agitator operating at 37°C . At appropriate timepoints, 1 mL fixative solution was added. Platelet counting was performed using an Ultra-Flo100 Whole

Blood Platelet Counter within 24 h. Percentage aggregation/agglutination was expressed as the percentage fall in the number of single platelets compared with the starting count. Results were expressed as response curves to increasing agonist dose. Mean values were plotted for each agonist concentration. The following agonist concentrations and timepoints were used: adenosine 5'-diphosphate (ADP) 0.3, 1.0, 3.0, 10 $\mu\text{mol L}^{-1}$, reaction stopped at 2 min; collagen 0.25, 0.5, 1.0, 2.0 $\mu\text{g mL}^{-1}$, reaction stopped at 2 min; epinephrine 0.1, 0.3, 1.0, 3.0 $\mu\text{mol L}^{-1}$, reaction stopped at 2 min; ADP, epinephrine 10 $\mu\text{mol L}^{-1}$, 0.1 $\mu\text{mol L}^{-1}$ ADP, and epinephrine 30 $\mu\text{mol L}^{-1}$, 0.1 $\mu\text{mol L}^{-1}$ ADP, reaction stopped at 4 min; ristocetin 0.6, 0.8, 1.0, 1.2, 1.4 mg mL^{-1} , reaction stopped at 4 min.

Collagen was obtained from Nycomed (Munich, Germany). Ristocetin was obtained from Forum Products Ltd (Redhill, Surrey, UK), and ADP (as sodium salt) and epinephrine were obtained from the Sigma Chemical Co (Poole, Dorset, UK). The platelet fixative solution contained 150 mmol L^{-1} NaCl, 4.6 mmol L^{-1} N. EDTA, 4.5 mmol L^{-1} N. HPCl, 1.6 mmol L^{-1} K_2PCl_4 and 0.16% w/v formaldehyde, pH 7.4.

Platelet activation

Platelet factor 4 (PF4) and beta-thromboglobulin (TG) were measured using, respectively, Asserachrom PF-4 and Asserachrom TG enzyme immunoassay kits (Diagnostica Stago, Asnières, France). Absorbance for the enzyme immunoassays was measured using an Argus 300 microtitre plate reader.

Other hemostatic parameters

The activated partial thromboplastin time (APTT), prothrombin time (PT), factor (F)VIII and fibrinogen were measured on an MDA 180 analyzer (bioMérieux, Basingstoke, UK). Total

von Willebrand antigen was measured using a monoclonal antibody from Dako as part of an ELISA assay. FXIII was measured using an activity assay (Berichrom Dade Behring, Milton Keynes, UK) on a Cobas-Mira analyzer (ABX Diagnostics, Bedfordshire, UK). Thrombin/antithrombin III complex (TAT) and prothrombin 1-2 fragment were measured using Enzygnost TAT micro and Enzygnost 1-2 micro enzyme ELISA immunoassay kits (Dade Behring, Milton Keynes, UK).

Other analyses (albumin, full blood count and plasma viscosity) were measured routinely at the Leicester Royal Infirmary.

Statistical analysis

Platelet aggregation dose-response curves were compared by analysis of variance (ANOVA) using SPSS. Other data were sometimes non-normally distributed and for consistency were always expressed as medians and analyzed by the non-parametric Kruskal-Wallis test using UniStat 4 and S-plus 2000.

Results

For all three colloid fluids there was a significant prolongation of BT at the Post timepoint compared with the Pre timepoint, but no difference between Pre and Last (Table 1). No significant change in BT was seen in those patients receiving saline, and no significant differences were identified between the prolongation for albumin, Haemaccel or Gelofusine. A sex-based difference in BT has been reported [16] (females tending to have a longer BT than males), but although there was significant sex bias between the fluid groups in this study there was no significant difference in the baseline BTs.

There were no significant differences in median blood loss between the fluid groups (median total blood losses: albumin

Fluid group				
Time	Albumin	Gelofusine	Haemaccel	Saline
Median bleeding time (BT) (min)				
Pre	6.5 (5.2–7.8)	5.78 (5.0–6.9)	7.2 (5.4–8.8)	5.9 (4.8–6.5)
Post	10 (8.5–11.0)	11.8 (6.8–15.9)	10.1 (8.0–12.7)	7.0 (5.6–8.9)*
Last	7 (5.2–7.8)††	8 (5.0–10.0)†	6.5 (5.0–8.5)†	6 (4.9–6.6)
Median β-thromboglobulin (TG) (IU mL⁻¹)				
Pre	47.5 (27.7–54.5)	58.6 (49.9–66.0)	39.3 (24.6–63.9)	43.8 (32.7–57.6)
Post	92.0 (70.5–117.5)	75.5 (67.6–109.2)	154.4 (83.0–202.3)	76.1 (60.8–97.7)
Last	64.4 (51.5–183.2)†	81.7 (68.7–155.6)†	123.4 (84.4–193.7)†	51.8 (51.2–100.0)
Median platelet factor 4 (PF4) (IU mL⁻¹)				
Pre	13.0 (9.0–36.7)	21.2 (10.5–29.9)	10.1 (6.7–25.8)	11.5 (8.9–18.3)
Post	17.5 (12.9–31.7)	20.0 (10.2–35.5)	37.2 (26.2–110.9)	14.8 (12.7–18.1)
Last	18.4 (9.5–32.6)	44.0 (10.7–77.7)	25.9 (19.6–43.4)	17.0 (10.2–22.0)
Median VWF (U dL⁻¹)				
Pre	75.1 (52.6–150.1)	100.8 (74.7–118.7)	90.8 (56.9–119.9)	70.4 (52.5–86.8)
Post	62.7 (49.2–129.9)	79.6 (72.9–104.2)	86.4 (70.8–107.7)	54.8 (45.4–78.6)
Last	65.9 (57.0–193.4)	105.4 (84.0–138.6)	94.4 (76.2–101.8)	71.0 (62.8–93.4)

Longitudinal comparison is of the time-points Pre (immediately prior to surgery), Post (immediately after surgery) and Last (2 h after Post sample); significant differences $P < 0.05$; † $P < 0.005$. Cross-sectional comparison is of the four resuscitation fluids used; significant differences $P < 0.05$; †† $P < 0.005$. Bracketed figures are first and third quartiles.

Table 1 Platelet factors: cross-sectional and longitudinal median effects of surgical trauma and fluid therapy, median differences tested by Kruskal-Wallis (a non-parametric comparison)

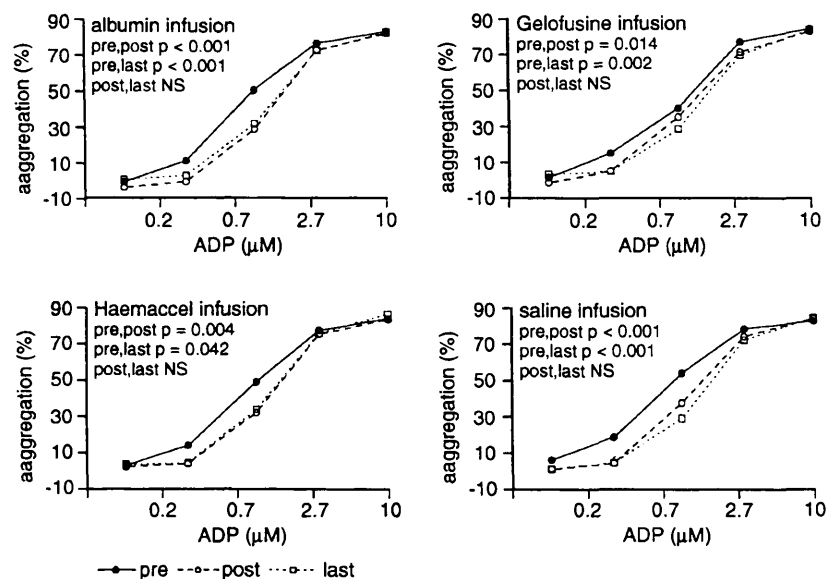


Fig. 1. Effect of surgical trauma on platelet aggregation induced by ADP (at 0, 0.3, 1.0, 3.0 and 10 $\mu\text{mol L}^{-1}$) in blood from patients receiving albumin, Gelofusine, Haemaccel and saline.

group 840 mL, Gelofusine group 945 mL, Haemaccel group 932.5 mL, saline group 885 mL = 0.5587).

The effects of the different fluid therapies on platelet aggregation varied with both type of fluid and stimulating agonist. With aggregation in response to ADP, the effect seen in all fluid groups was a shift of the dose-response curve to the right, i.e. a reduced response of platelets to agonist at the low to mid range of agonist concentration. However, maximal aggregation was unaffected (Fig. 1). This reduced response to ADP was evident at both the Post and Last time-points.

Aggregation in response to the combination of epinephrine and ADP was qualitatively different to that obtained with ADP alone. There was a significant reduction in aggregation in all fluid groups at all agonist concentrations, with a tendency to a

greater reduction at higher agonist concentrations, particularly so for the albumin-treated group (Fig. 2).

Only one of the fluid therapies, albumin, significantly and markedly affected aggregation in response to collagen. In the Gelofusine group there appeared to be a small and transient inhibition of aggregation. In the group treated with albumin there was a much greater inhibition of aggregation at all but the highest concentration of agonist, and this effect persisted (Fig. 3).

Platelet agglutination in response to ristocetin was completely and persistently suppressed in the Gelofusine and Haemaccel groups (Fig. 4). In contrast, there was a small but significant increase in this parameter in the groups treated with albumin and saline at both Post and Last timepoints.

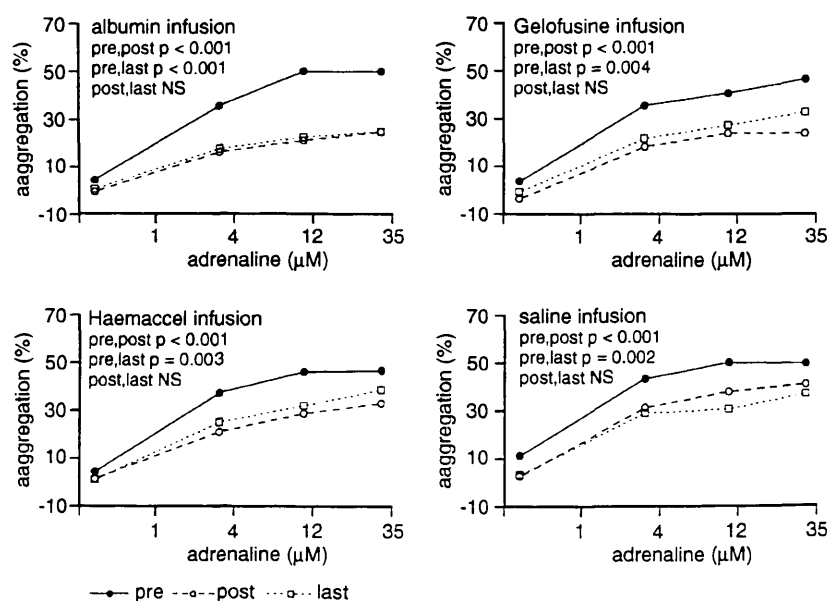


Fig. 2. Effect of surgical trauma on platelet aggregation induced by epinephrine (at 0.3, 1.0, 3.0 and 30 $\mu\text{mol L}^{-1}$, each with 0.1 $\mu\text{mol L}^{-1}$ ADP) in blood from patients receiving albumin, Gelofusine, Haemaccel and saline.

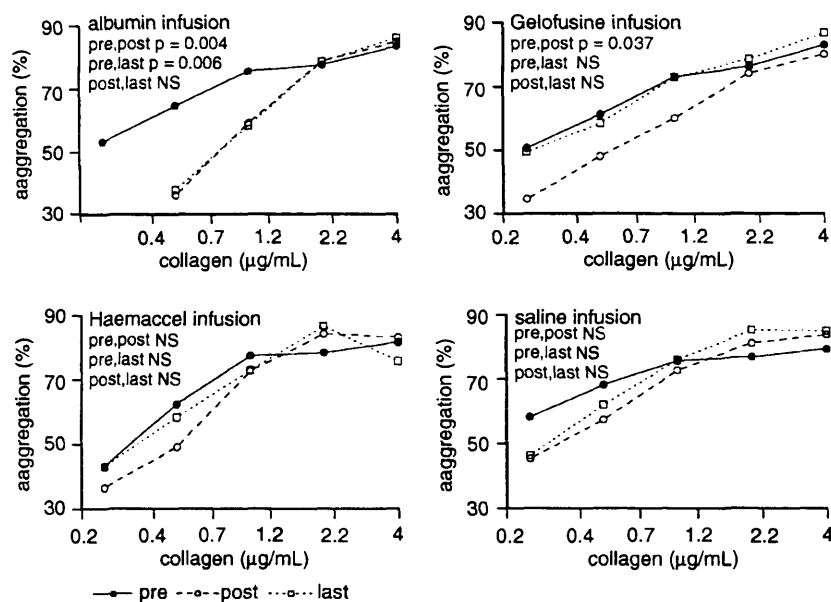


Fig. 3. Effect of surgical trauma on platelet aggregation induced by collagen (at 0.25, 0.5, 1.0, 2.0 and 4.0 $\mu\text{g mL}^{-1}$) in blood from patients receiving albumin, Gelofusine, Haemacel and saline.

In the colloid groups, albumin, Gelofusine and Haemacel, there were significant increases in TG at the Post timepoint, which tended to remain high at Last. In addition, the increase in TG with saline was almost significant. There were no significant changes in PF4, nor in von Willebrand factor (VWF) (Table 1).

There was a significant increase in APTT for the three colloid-treated groups at the Post timepoint, but this was only transient, returning to normal at the Last timepoint. FVIII activity decreased significantly, and also transiently. No significant changes in APTT or FVIII activity were seen in the group receiving saline (Table 2).

There were significant increases in prothrombin 1-2 fragment and in TAT in all the fluid groups at the Post timepoint, which were sustained (Table 2).

Effects on fibrinogen and PT showed that for all fluid groups there was a significant change at the Post timepoint, a decrease for fibrinogen and an increase for PT, and this was maintained through to the Last timepoint. However the magnitude of this change was lower for the saline group with respect to fibrinogen, and for the saline and albumin groups with respect to PT (Table 2).

There was a significant decrease in FXIII at the Post timepoint for the colloid fluids, albumin, Gelofusine and Haema-

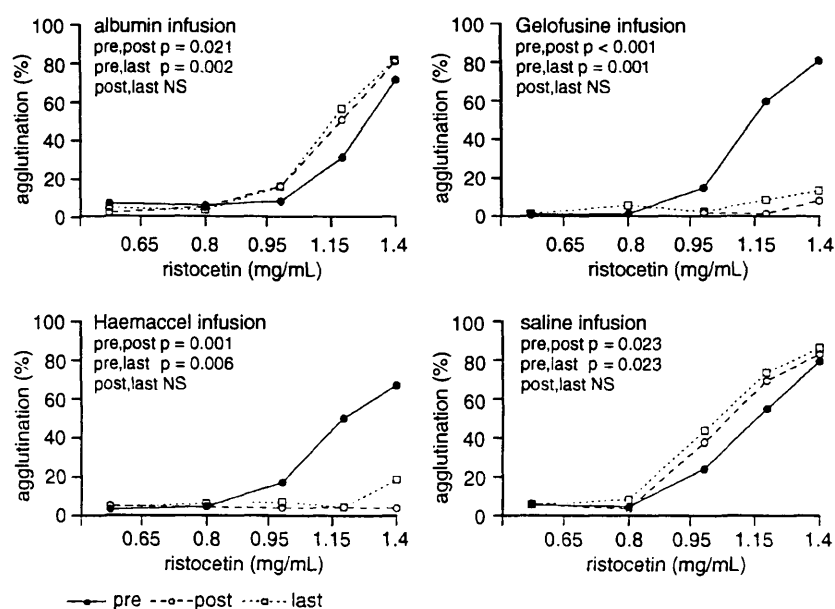


Fig. 4. Effect of surgical trauma on platelet agglutination induced by ristocetin (at 0.6, 0.8, 1.0, 1.2 and 1.4 mg mL^{-1}) in blood from patients receiving albumin, Gelofusine, Haemacel and saline.

Table 2 Clotting parameters: cross-sectional and longitudinal median effects of surgical trauma and fluid therapy, median differences tested by Kruskal-Wallis (a non-parametric comparison)

Time	Fluid groupw			
	Albumin	Gelofusine	Haemaccel	Saline
Median APTT (s)				
Pre	28.2 (26.6–30.6)	29.7 (25.6–31.6)	28.4 (27.4–30.9)	28.9 (27.5–32.2)
Post	34.7 (33.0–38.6)	37.2 (31.5–39.9)	35.6 (31.0–41.3)	32 (29.2–34.7)
Last	31.2 (29.4–35.0) †	31.1 (27.9–32.8) †	30.7 (28.7–32.2) †	28.3 (26.0–37.8)
Median FVIII (IU dL⁻¹)				
Pre	154.1 (118.2–164.3)	151 (104.7–166.2)	141.6 (124.1–187.4)	139.6 (114.9–146.7)
Post	94 (77.8–102.9)	87.8 (82.4–103.2)	91.4 (70.4–102.5)	116 (87.6–132.4)
Last	108.6 (93.5–117.6) ††	118.4 (100.2–142.3) †	123.8 (111.0–148.1) ††	143.3 (99.7–159.6)
Median prothrombin time + 2 fragment (mmol L⁻¹)				
Pre	2.44 (2.26–2.57)	2.57 (2.40–2.76)	2.24 (2.09–2.83)	2.45 (2.22–2.67)
Post	3.13 (2.73–3.52)	2.93 (2.69–3.46)	3.65 (3.15–4.78)	3.09 (2.63–3.30)
Last	3.43 (2.98–5.07) ††	3.4 (3.01–3.76) ††	4.02 (3.34–5.78) ††	3.02 (2.85–3.51) ††
Median TAT complex (TAT) (g L⁻¹)				
Pre	10.6 (2.9–15.7)	112 (4.0–17.3)	19.7 (5.2–23.0)	4.84 (3.2–24.1)
Post	56.5 (37.4–64.1)	44.1 (33.6–55.6)	58.3 (48.3–78.6)	41.1 (35.2–56.6)
Last	61.8 (40.5–90.0) ††	47.8 (25.9–62.0) ††	54.7 (39.3–67.4) ††	27.3 (21.9–60.1) ††
Median fibrinogen (g L⁻¹)				
Pre	2.9 (2.6–3.1)	3.1 (2.6–3.5)	2.95 (2.1–3.1)	3.05 (2.5–3.5)
Post	1.65 (1.5–1.8)	1.55 (1.4–1.9)	1.3 (1.3–1.9)	2.3 (2.0–2.6,**)
Last	1.65 (1.4–1.7) ††	1.5 (1.1–1.7) ††	1.3 (1.2–1.4) ††	2.3 (1.9–2.5,* ††)
Median prothrombin time (PT) (s)				
Pre	13.6 (13.5–13.9)	14 (13.7–14.6)	14.6 (14.0–14.8)	14.2 (13.6–14.6)
Post	15.8 (15.1–16.5)	16.3 (15.8–16.8)	17 (15.9–17.3)	15.2 (14.8–15.6,*
Last	15.4 (15.3–16.8) ††	16.3 (15.8–16.9) ††	16.3 (15.8–17.2) ††	15 (14.0–15.3, †
Median factor XIII (%)				
Pre	130 (120–176)	159 (137–174)	157 (152–174)	135.5 (117–150)
Post	92.5 (78–110)	101 (85–115)	101.5 (92–114)	105 (99–132)
Last	87.5 (69–107) ††	105.5 (84–118) ††	113 (91–114) †	118.5 (98–143)

Longitudinal comparison is of the time-points Pre (immediately prior to surgery), Post (immediately after surgery) and Last (2 h after Post sample); significant differences < 0.05 ; † < 0.005 . Cross-sectional comparison is of the four resuscitation fluids used: significant differences < 0.05 ; * < 0.005 . Bracketed figures are first and third quartiles.

cel, but no significant difference between these fluid groups at any of the timepoints. This reduction in FXIII persisted through to the Last timepoint. The trend with saline was similar but not significant (Table 2).

There was a significant decrease in ionized calcium concentration in the albumin, Gelofusine and saline groups at both Post and Last timepoints. However there was a significant increase in ionized calcium at these timepoints in the Haemaccel group. The magnitude of the changes was about 10% in either direction. There were no significant changes in ionized magnesium concentrations (Table 3).

Whilst there was a significant decrease in serum albumin between Pre and Post, and Pre and Last timepoints in the groups treated with Gelofusine, Haemaccel and saline, there was no such decrease in the albumin-treated group (Table 3).

Both hematocrit and hemoglobin decreased significantly between Pre and Post, and Pre and Last timepoints in all the fluid groups, however, this fall was less in the saline group. Similar changes occurred with respect to the effects on platelet count. Plasma viscosity decreased significantly at the Post timepoint in groups receiving albumin and saline. This was maintained through to the Last timepoint. However there was no

significant change in groups receiving the gelatin based colloids, Gelofusine and Haemaccel (Table 4).

Discussion

The first aim of this present investigation was to establish the extent to which effects of fluids on platelet aggregation observed in previous studies *in vitro* were reproduced *ex vivo* after administration to patients undergoing hip replacement. At the same time we sought to obtain additional information on the effects of various fluids on other hemostatic variables. These included measures of platelet activation, thrombin generation and several other hematological indices. The effects of the fluids on BT and on blood loss were also determined. Studies were performed immediately before the start of surgery, at the end of surgery and 2 hours after the end of surgery. The fluid groups used were saline, Haemaccel, Gelofusine and 4.5% albumin.

Based on our previous *in vitro* findings [8] of the effects of Haemaccel and Gelofusine compared with saline on platelet aggregation, we had expected to see pronounced inhibition by Haemaccel of the platelet aggregation induced by agents such as ADP, epinephrine and collagen, and also pronounced

Time	Fluid group			
	Albumin	Gelofusine	Haemaccel	Saline
Median ionized calcium (mmol L⁻¹)				
Pre	1.21 (1.08–1.22)	1.17 (1.11–1.21)	1.17 (1.12–1.24)	1.21 (1.09–1.24)
Post	1.04 (0.98–1.08)	1.05 (0.99–1.09)	1.28 (1.23–1.39)	1.09 (1.05–1.14,**)
Last	1.035 (0.98–1.11 †)	1.07 (1.03–1.08 †)	1.215 (1.11–1.28 †)	1.09 (1.01–1.15, †)
Median ionized magnesium (mmol L⁻¹)				
Pre	0.64 (0.59–0.66)	0.66 (0.61–0.71)	0.59 (0.56–0.76)	0.60 (0.59–0.66)
Post	0.59 (0.55–0.63)	0.62 (0.54–0.63)	0.62 (0.58–0.74)	0.55 (0.54–0.60)
Last	0.61 (0.60–0.65)	0.62 (0.56–0.66)	0.62 (0.60–0.68)	0.57 (0.56–0.65)
Median albumin (g L⁻¹)				
Pre	39 (37–40)	40 (38–40)	39 (38–41)	39 (36–41)
Post	40 (39–43)	22 (21–24)	25 (25–27)	30 (26–33,**)
Last	42 (39–44 †)	24 (22–26 ††)	28 (26–29 ††)	34 (31–34,* ††)

Longitudinal comparison is of the time-points Pre (immediately prior to surgery), Post (immediately after surgery) and Last (2 h after Post sample): significant differences < 0.05; † < 0.005. Cross-sectional comparison is of the four resuscitation fluids used: significant differences < 0.05; * < 0.005. Bracketed figures are first and third quartiles.

Table 3 Blood biochemistry: cross-sectional and longitudinal median effects of surgical trauma and fluid therapy, median differences tested by Kruskal–Wallis (a non-parametric comparison)

Table 4 Hematological parameters: cross-sectional and longitudinal median effects of surgical trauma and fluid therapy, median differences tested by Kruskal–Wallis (a non-parametric comparison)

Time	Fluid group			
	Albumin	Gelofusine	Haemaccel	Saline
Median hematocrit				
Pre	0.379 (0.370–0.392)	0.395 (0.356–0.418)	0.368 (0.346–0.387)	0.392 (0.365–0.420)
Post	0.280 (0.258–0.291)	0.278 (0.244–0.316)	0.291 (0.285–0.323)	0.330 (0.312–0.364,**)
Last	0.276 (0.255–0.283 ††)	0.292 (0.267–0.319 ††)	0.299 (0.283–0.312 ††)	0.347 (0.319–0.372,* ††)
Median hemoglobin (g dL⁻¹)				
Pre	13.0 (12.4–13.5)	13.8 (11.7–14.3)	12.5 (12.0–13.3)	13.2 (12.5–14.5)
Post	9.4 (8.9–10.0)	9.6 (8.2–10.4)	9.9 (9.5–11.2)	11.4 (10.7–12.3,**)
Last	9.2 (8.7–9.6 ††)	9.9 (8.9–11.0 ††)	10.4 (9.8–10.6 ††)	11.4 (11.0–12.6,* ††)
Median platelets (10⁹ L⁻¹)				
Pre	236 (206–252)	215 (182–266)	196 (176–246)	221.5 (184–262)
Post	170.5 (153–202)	150 (131–217)	188 (162–201)	204 (180–216)
Last	172 (137–197 †)	160 (151–213 †)	187 (160–222)	208 (178–238)
Median plasma viscosity (centipoise)				
Pre	1.63 (1.58–1.72)	1.68 (1.61–1.73)	1.59 (1.56–1.64)	1.64 (1.59–1.71)
Post	1.46 (1.40–1.49)	1.69 (1.65–1.70)	1.60 (1.55–1.62)	1.42 (1.40–1.47,**)
Last	1.46 (1.42–1.49 ††)	1.67 (1.60–1.72)	1.59 (1.51–1.67)	1.47 (1.42–1.53,* ††)

Longitudinal comparison is of the time-points Pre (immediately prior to surgery), Post (immediately after surgery) and Last (2 h after Post sample): significant differences < 0.05; † < 0.005. Cross-sectional comparison is of the four resuscitation fluids used: significant differences < 0.05; * < 0.005. Bracketed figures are first and third quartiles.

inhibition by Haemaccel and Gelofusine of the platelet agglutination induced by ristocetin. In fact we did see profound effects of both Haemaccel and Gelofusine on ristocetin-induced responses at both Post and Last timepoints. Inhibition by gelatin colloids of platelet agglutination by ristocetin is a well-established observation [2,4], and confirms our previous *in vitro* work [8]. It is thought this may be due to an interaction of the gelatins with VWF [2,7].

However, the expected effects of Haemaccel on the aggregation induced by other agents were not evident. There was significant inhibition of the aggregation induced by ADP and by epinephrine, but no more than was seen with saline, Gelofusine or albumin. The explanation for the different results obtained in our previous experiments conducted wholly *in vitro*

and these experiments *ex vivo* is that the increases in plasma calcium ions obtained *in vitro* that resulted from the high concentrations of calcium in Haemaccel were not evident *ex vivo*. In our *in vitro* system Haemaccel caused a rise in ionized calcium to 2.8 mmol L⁻¹, whereas in the *ex vivo* system, despite a similar dilutional load, median ionized calcium rose to only 1.28 mmol L⁻¹ (maximum concentration 1.63 mmol L⁻¹). In a previous *in vivo* study of trauma patients resuscitated with the calcium-containing fluid Haemaccel ionized calcium concentrations in excess of 2 mmol L⁻¹ were obtained [17]; however, these patients were critically injured and received greater volumes of Haemaccel than those in our current study. Although our patients had significant surgical trauma, this was not severe enough to affect their homeostatic compensatory

mechanisms. Clearly, homeostatic mechanisms had resulted in avoidance of large increases in plasma calcium, despite large amounts (2 L) of Haemaccel being infused. This resulted in less inhibition of aggregation by Haemaccel in the blood from the patients receiving this particular fluid.

There was persistent inhibition of platelet aggregation induced by ADP and by epinephrine in all fluid groups. We believe the most likely explanation of this reduced response is that desensitization to the aggregatory effects of these agents occurred consequent to prior platelet activation. There was clear evidence of platelet activation during surgery as judged by release of PGI_2 , even though PF4 did not increase. This was probably because of the short half-life of PF4 in plasma due to its binding to glycosaminoglycans on the vascular endothelium. It is certainly possible that ADP had been generated both through release from damaged tissue during surgery and via secretory mechanisms following platelet aggregation, resulting in desensitization of ADP receptors. Additionally, epinephrine receptors may have been desensitized consequent to the surgical trauma.

Collagen-induced aggregation was markedly inhibited following albumin infusion, which suggests that maintenance of plasma albumin concentrations results in a further decrease in platelet function. This may be related to the ability of albumin to bind and neutralize platelet agonists such as thromboxane A_2 [18,19].

Although there was a pronounced inhibitory effect on ristocetin-induced agglutination of platelets by the gelatin colloids, in contrast there was some potentiation of the effects of ristocetin following infusion of saline and albumin. This is likely to be a consequence of hemodilution.

Overall in this study, platelet activation as a consequence of surgery was seen to be accompanied by a general downregulation of platelet aggregation, together with selective additional inhibition of collagen-induced aggregation by albumin and of the ristocetin-induced platelet agglutination by the gelatin colloids. Such inhibition of platelet function might be interpreted as providing less risk of untoward thrombotic events, but with the potential to increase bleeding (but see below).

In addition to changes in platelet aggregation, several other changes were recorded that might also be expected to influence risk of thrombosis and/or bleeding. Overall there was significant and prolonged hemodilution, particularly with the three colloids, which was reflected in decreases in fibrinogen, FXIII, albumin (except during albumin infusion) and platelet count, and an increase in prothrombin time. Decreases in fibrinogen and albumin appeared to be somewhat larger than could be accounted for by simple hemodilution. Changes in FVIII and APTT also occurred but here there was some recovery between Post and Last, which could not be associated with hemodilution. Consumption of coagulation factors also occurred as reflected by increases in TAT complex and prothrombin 1-2 fragment. Generally there was a small reduction in ionized calcium (except with Haemaccel), and infusion of saline or albumin produced a reduction in plasma viscosity. There were no significant changes in ionized magnesium concentrations. It

was thus interesting to determine the effects of these various changes on BT and on blood loss.

We found that BT was significantly prolonged in the groups that received colloid fluids, but not in the group receiving saline. However, this was only a transient phenomenon, with BT returning to baseline by 2 h after the end of surgery. Between colloid fluids there was very little difference in degree of prolongation. The fact that BT was not increased in the saline group confirms that the effect is due to colloid infusion and is in accord with earlier data that indicated that BT can increase in patients receiving colloids in both traumatic and non-traumatic situations [5,7].

It is tempting to speculate that the increases in BT following colloid infusion relates to the various inhibitory effects of the colloids on platelet aggregation and the effects on the various coagulation parameters referred to above. However, while the effects on most of the parameters that were measured were seen at both the Post and Last timepoints, the increases in BT had returned to Pre values at the Last timepoint. This was mirrored by the changes in FVIII and the APTT measurements, which also showed some recovery at Last. Also, although not statistically significant, the changes in VWF followed the same trend.

Although we saw a transient increased BT in the colloid groups, this was not mirrored by increased blood loss in these groups compared with the group receiving saline. This concurs with results found by others. Boldt *et al.* [20] found that there was no significant increase in blood loss when either gelatin or albumin were infused immediately prior to cardiopulmonary bypass in patients undergoing elective aortocoronary bypass grafting. Furthermore, Scott *et al.* [21] found no significant difference in blood transfusion requirement following coronary bypass grafting between patients in whom the coronary artery bypass pump was primed with crystalloid, Haemaccel or albumin.

In summary, despite considerable changes in platelet, coagulation and other parameters brought about by colloid infusion, the effects on BT were only transient and there was no measurable effect on blood loss in this controlled study of hip arthroplasty. Surgery alone resulted in some reduction in platelet aggregation to agents such as ADP and epinephrine, probably as a consequence of platelet desensitization. Albumin had an additional inhibitory effect on collagen-induced platelet aggregation, and Haemaccel and Gelofusine inhibited ristocetin-induced platelet agglutination. This further inhibition of platelet function by the colloids may have the potential to provide additional protection against the increased thrombotic risk in patients following major surgery, and should be taken into account when assessing the need for and level of heparin prophylaxis following surgery.

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Contributions by authors. This study was devised and supervised by P.A. Evans and S. Heptinstall who also co-ordinated

the writing of this manuscript. E.C. Crowhurst entered the patients into the study, allocated them to groups, took all blood samples, performed BTs, recorded blood loss and collated the experimental data. The surgical operations were performed under the supervision of J. Hoskinson and C.M. Stray. Specialist assays that required particular expertise were conducted and/or supervised by J.R. Glenn, W. Madira, S.J. Davidson and J. Burman. T. Davies performed the statistical analyses.

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SHORT COMMUNICATION

Can the effects of clinical reperfusion fluids on platelet aggregation *in vitro* predict the effects of administration of such fluids in a clinical study *ex vivo*?

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Reperfusion fluids are administered routinely during surgical operations and following trauma. Studies performed wholly *in vitro* have indicated effects of some fluids on haemostasis through inhibition of platelet aggregation. Recently we performed a study to further evaluate the effects of reperfusion studies *in vitro* and also a study in a clinical setting to determine the extent to which the results of *in vitro* experiments can be extrapolated to the clinical situation. The results indicate that a combination of homeostatic and trauma response mechanisms complicate the ability to extrapolate from the findings *in vitro*.

Introduction

Platelet aggregation is one of the factors that contributes to normal haemostasis and also to the thrombosis that can occur following surgical operations, therefore it is of interest to observe any changes in platelet aggregation that might accompany surgery. It is also of interest to assess the effects of any additional influences that might affect the way in which platelets behave as a consequence of surgery. For example, as well as the trauma associated with the surgery itself, resuscitation fluids are administered as a routine part of the procedure to maintain blood volume.

Previously we performed an *in vitro* study to assess the effects of resuscitation fluids with a view to extrapolating the effects to the clinical setting.¹ In this study we added three different fluids to hirudinized

whole blood and assessed aggregation in response to ADP, collagen, adrenaline and ristocetin as well as some other agents. The fluids were saline, Haemaccel and Gelofusine and we compared the results obtained for Haemaccel with saline and for Gelofusine with saline. We found that Haemaccel and Gelofusine abolished aggregation to ristocetin, thus confirming the effects of gelatins observed previously by others.^{2,3} Haemaccel also proved to be a potent inhibitor of aggregation to all other agonists. Haemaccel, unlike Gelofusine, contains a high concentration of calcium, and control experiments in which calcium alone was added to whole blood indicated that it was the presence of calcium that was largely responsible for inhibition of the aggregation induced by ADP, collagen and adrenaline.

In a further study we sought to evaluate the effects of several factors associated with fluid administration on platelet aggregation in addition to the effects of the specific fluids themselves. These included the effects of reducing the number of blood cells as a consequence of introducing the fluid, and the effects of diluting plasma components. We also included albumin as an additional fluid which has also been shown to inhibit platelet aggregation *in vitro*.⁴ Later we went on to perform an *ex vivo* study following administration of the same fluids in a surgical setting, and thus were able to compare the two sets of data obtained.

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Here we present both sets of data and comment on the value of the experiments performed wholly *in vitro* in predicting the results obtained *ex vivo*.

Materials and methods

Materials

Haemaccel (3.5%, w/v, polygeline with 6.25 mM calcium) was from Beacon Pharmaceuticals (Tunbridge Wells, UK), Gelofusine (4%, w/v, succinylated gelatin) was from B Braun Medical, Sheffield, UK, albumin (4.5%, w/v, human serum albumin) was a gift from BPL (Elstree, UK), and the saline (0.9%, w/v, sodium chloride) was from Baxter Health Care (Thetford, UK). Collagen was obtained from Nycomed (Munich, Germany), ristocetin was from Forum Products Ltd (Redhill, Surrey, UK), and adenosine 5'-diphosphate (as sodium salt) and adrenaline were from Sigma Chemical Co (Poole, Dorset, UK). Recombinant hirudin (RevascTM) was a gift from Novartis (Farnborough, UK). The platelet fixative solution contained 150 mM NaCl, 4.6 mM Na₂EDTA, 4.5 mM Na₂HPO₄, 1.6 mM KH₂PO₄ and 0.16% (w/v) formaldehyde, pH 7.4.

In vitro study

The *in vitro* study involved measurement of platelet aggregation in whole blood induced by ADP, adrenaline, collagen and ristocetin. The whole blood contained hirudin as anticoagulant at a final concentration 50 µg/ml. It was studied undiluted and following dilution with Haemaccel, Gelofusine, 4% human albumin or saline at a blood-fluid ratio of 3:2. The results were also compared with those for blood diluted with autologous hirudinized plasma. The aim was to determine the effects of dilution, as well as the intrinsic effects of the different fluids. Median results were obtained using blood from 10 different volunteers.

Ex vivo study

After obtaining local ethical committee approval and informed consent, we randomised consecutive eligible patients undergoing primary unilateral cemented total hip replacement in a prospective randomised double-blind study to receive either Haemaccel ($n=14$), Gelofusine ($n=14$), albumin ($n=13$) or saline ($n=14$). All patients received a standard general anaesthetic comprising Temazepam (10–20 mg) pre-medication and propofol (2.0–2.5 mg/kg) and maintained with isoflurane and nitrous oxide (1.0–2.5% isoflurane in nitrous oxide with 20–30% oxygen). Immediately prior to surgery blood (Pre-sample) was taken for platelet aggregometry and anticoagulated with hirudin, exactly as for the *in vitro* studies. Surgery then commenced and 2 l fluid were infused during the operative period. Further blood

was taken at skin closure (Post-sample, about 1 h after initiation of surgery) and another (Last sample) 2 h later. Ionised calcium and magnesium were measured from lithium heparin sample taken concurrently on an AVL 988-4 analyser.

Platelet aggregation

Platelet aggregation was always measured exactly 30 min after blood sampling. Measurements were performed in whole blood using a platelet counting technique.⁵ A total of 480 µl of blood was added to polystyrene tubes containing 20 µl of varying concentrations of agonist, and the sample placed in a Multi-Sample Agitator (University of Nottingham, UK), operating at 37°C and an agitation setting of 8 (1000 rpm). Fixative solution (1 ml) was added to terminate the aggregation. Platelet counting was performed using an Ultra-Flo 100 Whole Blood Counter. Percentage aggregation was expressed as the percentage fall in the number of single platelets compared with the starting count. Median aggregation values were plotted for each agonist concentration. Data are presented (Figures 1 and 2) for the aggregation induced by 1 µM ADP, 1 µg/ml collagen, 10 µM adrenaline (with 0.1 µM ADP) and 1.2 mg/ml ristocetin. Differences between the medians were tested using Kruskal-Wallis with $P < 0.05$ taken as significant.

Results and discussion

In vitro data following dilution of blood with plasma or saline

The *in vitro* experiments first involved comparison of the aggregation induced by ADP, collagen, adrenaline or ristocetin in whole blood, in whole blood diluted with plasma and in whole blood diluted with saline (Figure 1). For ADP there was no significant effect of either of these additions. For collagen, dilution with plasma (but not saline) slightly but significantly reduced the aggregation. For adrenaline, the same picture emerged. For ristocetin, plasma had no effect but aggregation was promoted by saline.

It can be seen then, that for collagen and adrenaline only, dilution of the blood with plasma significantly reduced the aggregation response. Addition of plasma resulted in a reduction in the concentration of blood cells but not in the concentration of any components of the blood plasma. Thus the blood cell concentration must influence the degree of aggregation that occurs. It may be then that, for collagen and for adrenaline, co-factors for aggregation that are released from the platelets themselves (the concentration of which will depend on the number of platelets present) are contributing to the aggregation response. Plasma did not reduce the degree of aggregation

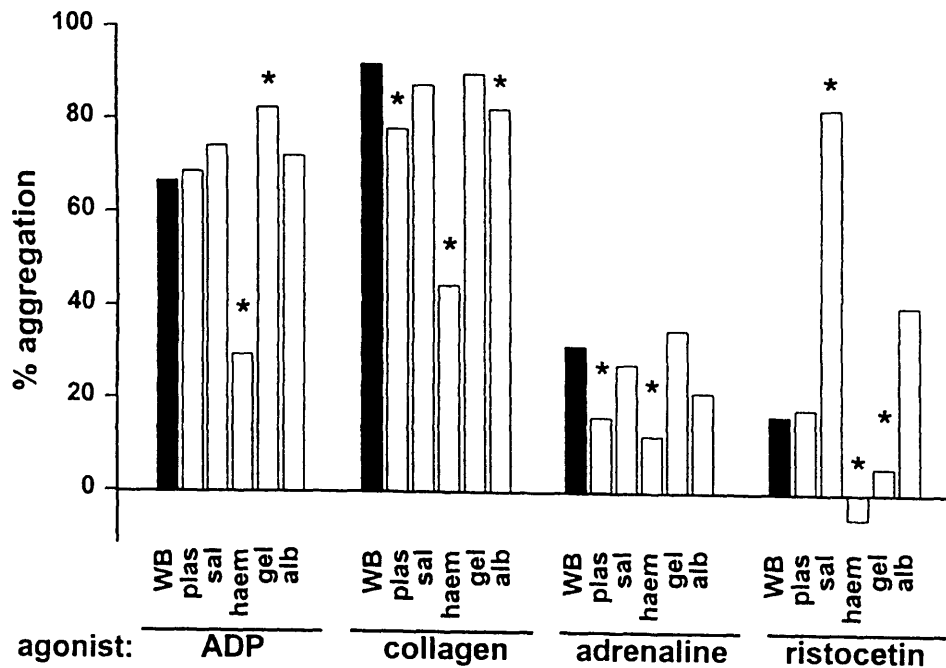


Figure 1. Effects of various fluids added *in vitro* to platelet aggregation in whole blood. * $P < 0.05$ cf whole blood (WB).

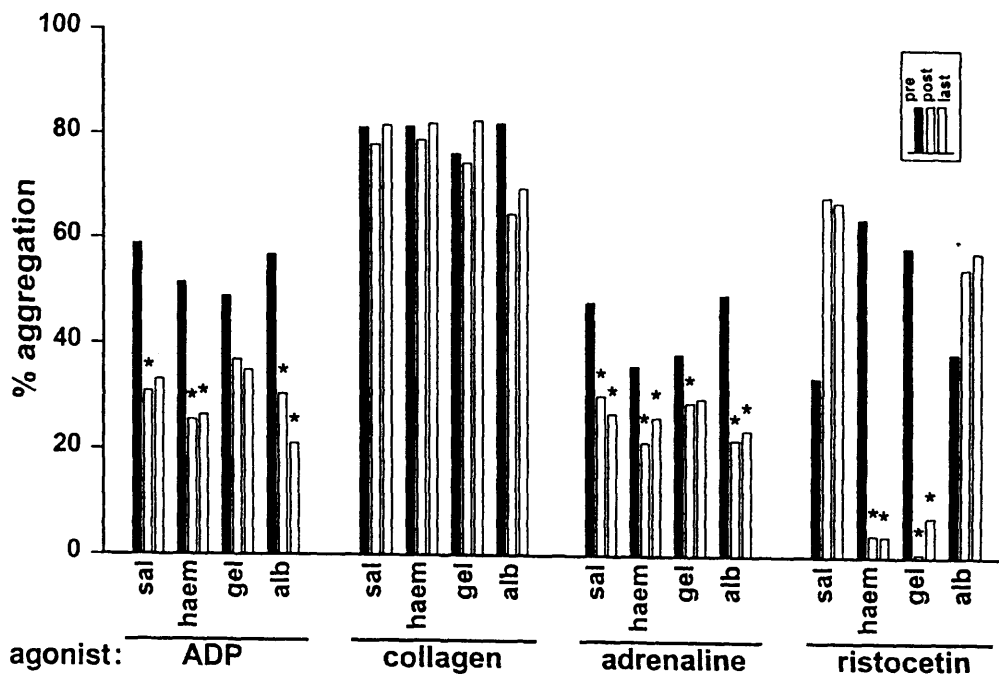


Figure 2. Effects of various fluids studied *ex vivo* on platelet aggregation in whole blood. * $P < 0.05$ cf pre-sample.

produced by ADP or ristocetin. It may be that in these cases there is less reliance on platelet-derived co-factors to bring about the aggregation response.

For collagen and adrenaline, although addition of plasma to whole blood reduced the aggregation response, addition of saline did not. In the case of saline addition, plasma components are being diluted as well as the blood cells. Presumably, therefore, there are plasma components which serve to attenuate the degree of aggregation that occurs in response to these two agents. This also seemed to be the case for ristocetin. For ADP, the results obtained with plasma and saline were not significantly different

but there was still a trend to enhanced aggregation following dilution with saline.

In conclusion, the number of blood cells present influences the degree of aggregation induced by collagen and adrenaline, and plasma components attenuate the degree of aggregation induced by collagen, adrenaline and ristocetin.

In vitro data following dilution of blood with Haemaccel

For ADP, Haemaccel markedly reduced aggregation against a background of no change on addition of

plasma or saline (Figure 1). This has been observed before¹ and was attributed to the high levels of Ca in Haemaccel, with raised Ca levels inhibiting the aggregation response. For collagen, Haemaccel also reduced aggregation. A reduction would have been expected as a consequence of reducing the number of blood cells. On the other hand, saline, by reducing the plasma components, had enhanced aggregation compared with the situation with plasma. Presumably the reduction seen with Haemaccel was a consequence of a combination of the reduced number of blood cells, the added calcium and the (opposing) effect of the dilution of plasma constituents. For adrenaline, the result was very similar to that for collagen. For ristocetin, aggregation was abolished. This is thought to be due the gelatin in the Haemaccel.^{2,3} Overall, then, Haemaccel had marked inhibitory effects on aggregation in WB *in vitro*.

In vitro data following dilution of blood with Gelofusine

For ADP, Gelofusine enhanced aggregation against a background of no change with plasma (Figure 1). There had also been a trend towards increased aggregation with saline, although this had not proved significant. Again, this might have resulted from a dilution of plasma components. The main difference in the composition of Gelofusine and Haemaccel appears to be the high level of calcium in the latter. For collagen and for adrenaline, results with Gelofusine were very similar to those with saline. For ristocetin aggregation was abolished by Gelofusine. Again, this is thought to be due the gelatin in the fluid.^{2,3} Overall, then, Gelofusine differed from Haemaccel except for its effects on ristocetin-induced aggregation, and more closely resembled the results obtained with saline.

In vitro data following dilution of blood with albumin

For all the agonists albumin produced a response intermediate between that of plasma and saline (Figure 1). Only in one case (collagen) was the effect of albumin to change aggregation significantly relative to whole blood. These results are consistent, with albumin being one of the plasma components that attenuates platelet aggregation induced by collagen, adrenaline and ristocetin.

Comparison of in vitro data with ex vivo data: saline

In the *in vitro* experiments, addition of saline to whole blood had not significantly changed the aggregation induced by ADP, collagen or adrenaline and had potentiated the aggregation induced by ristocetin. *Ex vivo*, ADP aggregation was significantly reduced

following saline infusion compared with the situation in the same patients prior to infusion and this was also the case for adrenaline (Figure 2). For collagen, saline infusion had produced no effect, and for ristocetin, the aggregation was increased although not significantly. Thus there were some similarities when comparing the *in vitro* and *ex vivo* data (collagen and ristocetin) but there were also discrepancies (ADP and adrenaline).

Comparison of in vitro data with ex vivo data: Haemaccel

In the *in vitro* experiments Haemaccel had significantly and markedly reduced the aggregation induced by ADP, collagen and adrenaline, and this was attributed to the high levels of Ca in the Haemaccel preparation, with raised Ca levels inhibiting the aggregation response. Haemaccel abolished ristocetin-induced aggregation and this was attributed to the gelatin component. In the *ex vivo* experiments there was also some inhibition of the aggregation induced by ADP and by adrenaline, but not collagen, and again there was profound inhibition of the ristocetin-induced response (Figure 2). For ADP, collagen and adrenaline the results were very similar to those obtained following saline infusion. We believe that the gelatin in Haemaccel is affecting ristocetin-induced aggregation both *in vitro* and *ex vivo*. However, measurements of ionised calcium in the two groups (Haemaccel *in vitro* and *ex vivo*) demonstrated marked differences in the calcium levels attained. This is a consequence of the operation of homeostatic mechanisms *in vivo*. Following addition of Haemaccel *in vitro* the median plasma ionised calcium increased from 1.16 to 2.88 mM, whereas following infusion the levels only changed from 1.17 to 1.28 mM. Thus, whatever changed the responses following saline infusion were probably the same as following Haemaccel infusion.

Comparison of in vitro data with ex vivo data: Gelofusine

In the *in vitro* experiments Gelofusine enhanced the aggregation induced by ADP, had no effect with collagen and adrenaline and abolished ristocetin-induced aggregation. In the *ex vivo* experiments there was inhibition of the aggregation induced by ADP and by adrenaline, but not collagen, and profound inhibition of the ristocetin-induced response (Figure 2). Thus the results were very similar to those obtained following Haemaccel infusion. Again, for ADP, collagen and adrenaline the results were very similar to those obtained following saline infusion.

Comparison of *in vitro* data with *ex vivo* data: albumin

In the *in vitro* experiments albumin we had seen slightly increased aggregation with ADP and ristocetin, and reduced aggregation with collagen and adrenaline. In the *ex vivo* experiments there was inhibition of the aggregation induced by ADP and adrenaline and a slight increase in ristocetin-induced aggregation (Figure 2). For collagen there was a non-significant trend to reduced aggregation. Except for the trend to reduced aggregation with collagen, the results seem very similar to those obtained following saline infusion.

Conclusions

The *in vitro* studies described above did show some significant effects of various fluids on platelet aggregation. There was either promotion or inhibition of aggregation depending on the nature of the fluid and on the platelet agonist that was used. Some of these effects were reproduced *ex vivo*, however overall there were other influences that were more powerful in determining the actual effects on aggregation that ensued.

Perhaps the most interesting finding was that overall there was a marked reduction in the aggregation induced by ADP and by adrenaline *ex vivo* irrespective of the fluid that was used, which was not predicted from the *in vitro* experiments. It is unlikely that this was an effect of the anaesthetic because all blood samples were obtained following anaesthetic administration. It is more likely that the surgery itself led to the reduced response that was observed. Possibly increases in ADP and adrenaline in blood plasma following surgery led to some down-regulation of platelet responses to these agonists. Platelet responses to collagen were not altered as a consequence of surgery possibly because platelets remaining in the circulation post-surgery had had no contact with any collagen exposed during the operation.

Another instance of a failure to predict an *in vivo* effect from data obtained *in vitro* was in the effects of Haemaccel on aggregation responses. The marked inhibition of aggregation by ADP, adrenaline and

collagen *in vitro* is a consequence of the large amounts of calcium in this fluid. *In vivo* homeostatic mechanisms served to moderate the changes in calcium concentration and overall, the effects of Haemaccel infusion proved to be very similar to those of the other fluids that were used.

In vitro Haemaccel and Gelofusine had proved to be potent inhibitors of ristocetin-induced aggregation, believed to be a direct effect of the gelatin in interfering with the interaction between platelets and von Willebrand factor.^{2,3} In this case the effects of Haemaccel and Gelofusine were reproduced *ex vivo*.

The fact that a combination of homeostatic and trauma response mechanisms was found to complicate the ability to extrapolate from findings obtained wholly *in vitro* underlines the importance of performing tests of platelet aggregation in the clinical situation as a means of determining the true effects of particular procedures. From the results obtained in the actual clinical setting some anticipated effects of some reperfusion fluids were not realised.

The overall effects of all the fluids that were administered within the setting of acute surgery was to decrease platelet responses to most agonists. Theoretically, reduced platelet aggregation might contribute to impaired haemostasis but it could also help reduce any tendency to thrombosis, a well-known complication of acute surgical interventions.

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Viewpoint

Is the normalisation of blood pressure in bleeding trauma patients harmful?

Ian Roberts, Phillip Evans, Frances Bunn, Irene Kwan, Edward Crowhurst

In 1990, about 5 million people died worldwide as a result of injury.¹ For people younger than 35 years, injury is now the leading cause of death. Nevertheless, the global epidemic of injury is only beginning. By 2020, deaths from injury will probably increase to 8·4 million.² About a third of these deaths are from haemorrhagic shock.³ Acute blood loss following injury leads to a reduction in tissue perfusion and tissue oxygen delivery, that, if prolonged, causes lactic acidosis and organ failure.

Current treatment of haemorrhagic shock involves maintaining blood pressure and tissue perfusion until bleeding is controlled. However, although maintenance of blood pressure might prevent shock, it could worsen bleeding.⁴ Raising of blood pressure can increase tissue perfusion and tissue oxygenation, but the increased pressure might impair the formation of new blood clots or dislodge existing ones.⁴ Over the past 50 years several resuscitation strategies have been used to raise the blood pressure in trauma patients until bleeding is controlled. Assessing the evidence for the effectiveness of these approaches has been the aim of a number of systematic reviews by the Cochrane Injuries Group and others. We draw on the results of these reviews to formulate a hypothesis about the management of bleeding trauma patients.

Evidence from systematic reviews

Medical anti-shock trousers (MAST) provides external pneumatic compression of the legs and was first used in the Vietnam war to stabilise patients with haemorrhagic shock during transportation. After the war, MAST was widely used in the care of bleeding trauma patients. MAST increases blood pressure by compressing blood vessels in the legs thus raising systemic vascular resistance, and by shunting blood from the lower body to the brain, heart, and lungs.⁵ The hope was that by increasing venous return to the heart, MAST would maintain blood flow to vital organs until definitive care was given. In 1977, the committee on trauma of the American College of Surgeons included antishock trousers on the list of essential equipment for ambulances.⁶

There have been two randomised-controlled trials of MAST use in the prehospital care of bleeding trauma patients. In the first, 911 trauma patients with systolic blood pressure (SBP) less than 90 mm Hg were

randomised to MAST or no MAST groups, before being taken to a level-one trauma centre.⁷ Risk of death in the MAST group (31%) was 6% higher than in the no MAST group. In the second trial, 291 hypotensive trauma patients (SBP <90 mm Hg) were randomised to MAST or no MAST groups.⁸ Risk of death was similar in the two groups, although subgroup analyses showed that 47% of MAST-treated patients were dead on arrival compared with 40% in the no MAST group. Taken together, the two trials provide no evidence that MAST improves recovery in bleeding trauma patients, but do suggest that it is harmful since the pooled relative risk of death with MAST was 1·13 (95% CI 0·97–1·32).⁹

In developed countries, an increasing number of ambulance crews include a paramedic trained in advanced life support. Paramedic ambulance crews receive extra training in intubation, intravenous cannulation, and administration of intravenous fluids. Only a small proportion of trauma patients who are attended by paramedics is intubated (1%), but a larger proportion (18%) receives intravenous fluids.¹⁰ Because of the strong conviction among the public and medical profession that paramedic intervention is beneficial, randomised controlled trials comparing paramedic and non-paramedic care have been difficult to do. UK workers¹⁰ attempted to randomise ambulance dispatch calls to paramedic crews or to emergency medical technicians (EMT) with only basic life support skills. Unfortunately, only 16 patients were randomised. However, the investigators followed cohorts of patients attended by paramedics and EMT crews, and adjusted for injury severity and other potential confounding factors in the analyses. The results were set in the context of a systematic review of three previous contemporary cohort studies^{11–13} of trauma patients. A meta-analysis of all four studies showed an increased ($p=0\cdot03$) risk of death in patients attended by paramedics (relative risk 1·26). The UK study was the largest of the four, and some subgroup analyses were undertaken. The risk of death after paramedic care was especially high in patients with bleeding injuries (relative risk 4·60, 95% CI 1·07–20·0).¹⁰ Because of the potential for confounding by injury severity, results from cohort studies are of doubtful validity. Nevertheless, the results are consistent with the hypothesis that efforts to return blood pressure to normal in bleeding trauma patients can be counterproductive.

Intravenous fluid administration, with colloid or crystalloid solutions, is the mainstay of the non-surgical management of bleeding trauma patients. Colloids are better than crystalloid solutions in expanding the circulation because they are retained in the blood vessels to a greater extent. Crystalloid solutions leak out of the blood vessels into the interstitial spaces. After a colloid infusion, the circulating volume increases by the volume of colloid infused, whereas only a quarter of the volume of a crystalloid infusion remains in the circulation.¹⁴

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Although colloids are effective in expanding the circulation, there is no evidence that this improves survival after trauma.

In a systematic review of randomised-controlled trials comparing colloid and crystalloid resuscitation in critically-ill trauma patients, the relative risk of death with colloid was 1.3 (95% CI 0.95–1.77).¹⁵ The methods of this review, however, have been criticised on several grounds: the inclusion of trials of hypertonic crystalloid, use of allocation concealment as the measure of trial quality, and use of a fixed effect model in analysis. Nevertheless, the results of a second systematic review, with similar inclusion criteria, that addressed these concerns were even more discouraging, with a relative risk of death with colloid of 2.6 (1.1–5.9).¹⁶ Choi and colleagues¹⁶ argue that colloid use should not be curtailed on the basis of their results, but that the findings should be viewed as hypothesis generating. Several hypotheses might be invoked to explain the study outcomes but they are consistent with the hypothesis that expansion of the circulating volume in bleeding trauma patients is harmful.

The reviews considered so far provide only indirect support for the hypothesis that blood pressure maintenance in bleeding trauma patients might be harmful. In view of the concerns raised by these reviews, a systematic review of the effect on mortality of early versus delayed fluid resuscitation, and of larger versus smaller fluid volumes was undertaken.¹⁷ We investigated the timing or volume of fluid administration among patients with haemorrhagic hypovolaemia. Because the impact of fluid resuscitation is likely to be closely similar among patients with internal bleeding (eg, bleeding peptic ulcer) and those with external bleeding (eg, penetrating trauma), we included trials with both types of participants.

We identified six trials. Bickell and co-workers¹⁸ compared immediate versus delayed fluid resuscitation with isotonic crystalloid in 598 hypotensive patients with penetrating injuries. Among the 289 patients with delayed fluid resuscitation, 203 (70%) survived to discharge, whereas of the 309 patients who received immediate resuscitation only 193 (62%) lived. The relative risk for death with early fluid administration was 1.26 (1.00–1.58). Prothrombin and partial thromboplastin times were significantly raised in the immediate resuscitation group, although the clinical significance of these changes is uncertain. Unfortunately, allocation in this trial was by alternation, and the possibility of bias cannot be excluded. Blair and colleagues¹⁹ randomised 50 patients with acute severe upper gastrointestinal haemorrhage to receive, during the 24 h after admission, either at least two units of blood, or no blood transfusion unless the haemoglobin fell below 8 g/dL or they were shocked. Two patients in the early transfusion group died (8%) and nine patients re-bled, but there were no deaths in the non-transfused group and only one patient re-bled. Partial thromboplastin time was raised in the transfused group. Other workers²⁰ randomised 25 bleeding trauma patients to receive smaller or larger volume blood transfusions to maintain the haematocrit at 30% or 40%, respectively. There were no deaths in either group and data on coagulation were not reported. Dunham and colleagues²¹ compared rapid fluid administration with conventional fluid administration in a randomised trial of 36 hypovolaemic trauma patients. Five of the 16 (31%) patients administered fluid by rapid infusion, and five of the 20 (25%) patients who received fluid in the conventional way, died. The relative risk of death with rapid fluid administration was 1.25 (0.44–3.58). Finally, in the last of the published trials²² paramedics were

randomised to one of two protocols for prehospital intravenous fluids. One protocol required intravenous fluids to be given at the incident scene, and the other required fluids to be withheld until arrival at hospital, unless time to hospital was over 1 h. Half way through the trial the paramedics were crossed over to the alternative protocol. Of the 699 patients treated by paramedics with fluids, 73 (10.4%) died, and of the 610 patients treated by paramedics operating the no fluids protocol, 60 (9.8%) died. In a subgroup analysis, the relative risk of death in the fluids group for patients with severe bleeding injuries was 1.19 (0.69–2.04). However, compliance with the protocol was poor and the difference in the proportion of patients receiving fluids before theatre was only 7%.

We also identified one unpublished, continuing randomised controlled trial comparing systolic blood pressure resuscitation targets of 100 mm Hg or 70 mm Hg in patients with blunt and penetrating trauma (Dutton RP, McKenzie CF, Scalea TM, unpublished results). In preliminary analyses of data from 99 patients, the risk of death was similar in both groups, although comparison of injury severity scores showed that patients in the low blood pressure target group were more severely injured.

Conclusions

The use of MAST, early fluid administration, and colloid resuscitation is based on the idea that raising blood pressure in bleeding trauma patients will maintain tissue perfusion and so prevent haemorrhagic shock and its consequences. However, there is no unbiased evidence that any of these strategies improve survival, and there is a suggestion that they are harmful. Taken together, evidence from systematic reviews calls into question resuscitation protocols, such as the advanced trauma life support (ATLS) protocol of the American College of Surgeons that recommends the liberal use of isotonic crystalloid to correct hypotension. Although vigorous fluid resuscitation might be lifesaving in some patients, results from clinical trials are consistent with results from animals with uncontrolled haemorrhage that raising of blood pressure could worsen bleeding and increase mortality.⁴

By what mechanisms could fluid resuscitation worsen bleeding? The process of haemostasis is a continuum starting from the activation of platelets and formation of a platelet plug. Binding of von Willebrand factor to glycoprotein Ib is important at this stage. Haemostasis continues, through activation of the coagulation cascade, to the formation and stabilisation of a definitive clot. This process can be affected at every stage by fluid resuscitation. Jørgensen and Stoffersen²³ showed that albumin inhibits thrombin induced platelet aggregation *in vitro*. Ristocetin induces platelet aggregation by binding glycoprotein Ib, and Stibbe and Kirby²⁴ showed *in vitro* that the gelatin colloid Haemaccel (Hoechst Marion Roussel, Middlesex, UK) completely abolishes this aggregation. Others²⁵ showed this reaction *ex-vivo* for the gelatin colloid Gelofusine (Braun, Sheffield, UK). Furthermore, Evans and co-workers²⁶ replicated this result, and showed that the additional effects of Haemaccel were largely attributable to the increase in ionised calcium that resulted from the high calcium content of this fluid.

Excessive fluid administration might also worsen bleeding by diluting clotting factors. In a trial of immediate versus delayed crystalloid resuscitation,¹⁸ prothrombin and partial thromboplastin times were both significantly raised in the immediate resuscitation group. Similarly, in a trial of early blood transfusion after

gastrointestinal haemorrhage¹⁰ clotting times were significantly raised in the transfused group. Dalrymple-Hay and colleagues²⁷ reported a case in which infusion of hydroxyethyl starch induced acquired von Willebrand's disease, and Treib and co-workers²⁸ showed that all multimers of von Willebrand factor were reduced by about the same amount as a result of this infusion.

Intravenous fluids could also have effects on the coagulation cascade that are in excess of dilutional effects. Stump et al²⁹ showed that infusion of hydroxyethyl starch resulted in a significant increase in partial thromboplastin time and decrease in factor VIII, in excess of that caused by haemodilution. Albumin infusion prolongs prothrombin time and reduces fibrinogen activity.³⁰ Albumin also has a heparin-like activity, enhancing the antifactor Xa activity of antithrombin III.³¹ Even when a clot is forming, intravenous fluids can have an effect. Mardel and co-workers³² showed a reduced clot elasticity, as measured by thromboelastography, and reduced clot weight after in-vitro dilution with gelatin colloids. Gelatin is known to bind with fibronectin, and it is thought that gelatin products might become incorporated into developing clots. Finally, the increased pulse pressure that results from crystalloid resuscitation could mechanically disrupt a formed clot. Stern³³ suggested this hypothesis and recorded that attempts to restore blood pressure with crystalloid in pigs with near-fatal haemorrhage resulted in increased haemorrhage volume and greatly raised mortality.

Every year, tens of thousands of patients receive intravenous fluids in the management of bleeding. Nevertheless, there is no reliable evidence about how much fluid should be given or what targets should guide resuscitation. On the other hand, there are many trials comparing different colloids, and colloids with crystalloid solutions.³⁴ Questions about the amount of fluid seem to have been eclipsed by questions about the choice of fluid. This deficit is likely to indicate the commercial interests of pharmaceutical companies in obtaining a share of the market for resuscitation fluids. In view of the absence of evidence for the effectiveness of currently recommended resuscitation protocols, and the potential for harm, contemporary resuscitation practice can at best be regarded as experimental.

Contributors

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Education and debate

Does animal experimentation inform human healthcare? Observations from a systematic review of international animal experiments on fluid resuscitation

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Animal models are often used to test the effectiveness of a drug or procedure before proceeding to clinical trials. One reason for use of animal models is that they allow researchers to focus on particular pathological processes without the confounding effects of other injuries and treatments. However, it is essential that their results are valid and precise. Biased or imprecise results from animal experiments may result in clinical trials of biologically inert or even harmful substances, thus exposing patients to unnecessary risk and wasting scarce research resources. Moreover, if animal experiments fail to inform medical research then the animals suffer unnecessarily.

The Italian pathologist Pietro Croce criticised vivisection on scientific grounds. He argued that results from animal experiments cannot be applied to humans because of the biological differences between animals and humans and because the results of animal experiments are too dependent on the type of animal model used.¹ Croce's arguments were based on insights into zoology and pathophysiology. In this paper, we make some methodological observations on animal experiments. Our observations were made in the context of a systematic review of all available randomised controlled trials of fluid resuscitation in animal models of uncontrolled bleeding. We conducted this review because we wanted to assess the scientific basis for fluid resuscitation. A previous systematic review of randomised trials of fluid resuscitation in bleeding trauma patients had provided no evidence that fluid resuscitation improved outcome.²

Systematic review of fluid resuscitation in uncontrolled haemorrhage

We did a systematic review of randomised controlled trials of the timing or volume of fluid administration in animal models of uncontrolled haemorrhage. Details of the review methods, search strategy, and included trials are available on bmj.com. The combined electronic search strategies identified 3193 potentially eligible reports. Two reviewers examined each of these records and 104 reports were retrieved in full. From these, we identified 44 randomised controlled trials meeting the inclusion criteria. The 44 trials included a total of 2039 experimental animals (1772 rats, 251 pigs, and 16 sheep). Mortality data were reported in 42

Summary points

New drugs and procedures are usually tested in animals before conducting clinical trials

Validity of animal experiments is essential for human health care and fundamental to animal welfare

A systematic review of animal experiments on fluid resuscitation found that most studies were underpowered and provided little information on possible bias

Systematic reviews of animal experiments allow a more objective appraisal of the evidence and reduce the chance of false negatives results

Systematic reviews across species would help determine whether the results could be generalised to humans

trials, of which 31 were in rats, 10 in pigs, and one in sheep. In most of the rat experiments uncontrolled bleeding was induced by resecting the tail. Three trials in large animals (pigs and sheep) could not be included in the meta-analysis because they did not include a no fluid resuscitation group: one compared early and late resuscitation and two compared different blood pressure resuscitation targets. Three trials in rats could not be included in the meta-analysis: one compared early and late fluid resuscitation, one compared different blood pressure resuscitation targets, and one presented time to death data only.

The pooled odds ratio (fixed effect) for death in large animals (pigs and sheep) with fluid resuscitation was 0.63 (95% confidence interval 0.15 to 2.61) but there was statistical heterogeneity ($\chi^2 = 16.84$, $df = 7$, $P = 0.018$). The pooled odds ratio (fixed effect) for death in small animals with fluid resuscitation was 1.14 (0.65 to 2.02). Again, there was substantial heterogeneity ($\chi^2 = 93.40$, $df = 27$, $P < 0.0001$). When the meta-analysis was stratified according to how uncontrolled bleeding was induced, a large amount of the heterogeneity was accounted for. Figure 1 shows the



The methods of the systematic review and details of included trials appear on bmj.com

results of meta-analysis of the 16 randomised controlled trials of fluid resuscitation in rats in which bleeding was induced by resecting the tail. The meta-analysis is stratified according to where the tail was cut. Fluid resuscitation seems to be harmful (odds ratio = 2.88, 95% confidence interval 1.72 to 4.80) with less than 50% tail resection ($\chi^2 = 5.57$, $df = 7$, $P = 0.59$) but beneficial (odds ratio = 0.25, 0.15 to 0.42) with greater than 50% tail resection ($\chi^2 = 6.14$, $df = 7$, $P = 0.52$).

Are the individual experiments valid?

In clinical trials, systematic error can arise from problems with the study design, especially if allocation of treatment is inadequately concealed.^{1b} Bias is avoided by ensuring strict randomisation with well concealed treatment allocation. The extent to which inadequate concealment of allocation might introduce bias in animal experiments is uncertain. However, it is easy to imagine how bias could arise. For example, weaker animals may be easier to catch than healthy animals, and this could result in systematic differences between the intervention and control groups on baseline prognostic factors. Of the 44 randomised controlled trials meeting the inclusion criteria, only two described how the animals were divided into treatment groups; both of these trials used alternation.

Random error in clinical trials is minimised by increasing the number of randomised participants.^{1b} However, animal researchers are encouraged to reduce the number of experimental animals to a minimum. Indeed, the need to use the minimum number of animals to obtain valid results is embodied in the Animals (Scientific Procedures) Act 1986 and European legislation.²⁰ As a result, some animal experiments are underpowered and provide little reliable information. All of the animal experiments in our systematic review were small (fig 2). The average number of animals per trial was 46 (2039/44), and the largest trial included only 207 animals (rats). None of the trials would have been large enough to detect reliably a 10% absolute difference (halving) in the risk of death between the intervention and comparison groups. Moreover, many of the trials included several different fluid resuscitation groups, which we combined for our analyses. The average number of experimental animals per treatment group was only 13 (160 groups). If, as was the case in most trials, the aim was to compare the effects of different fluid resuscitation regimens, the studies had little power.

Has all the evidence been assessed?

Although each individual animal experiment provides little reliable information on the effectiveness of fluid resuscitation, each contributes to the total body of evidence. Any inferences should be based on all the evidence.²¹ A 1996 narrative review of fluid resuscitation in animal experiments included only nine of the 24 trials (38%) that were available at that time.²²

Systematic reviews and meta-analyses of animal experiments are uncommon. About 1 in 1000 Medline records pertaining to human research is tagged as a meta-analysis compared with 1 in 10 000 records pertaining to animal research. In his book *The Principles of Humane Experimental Technique*, William Russell pro-

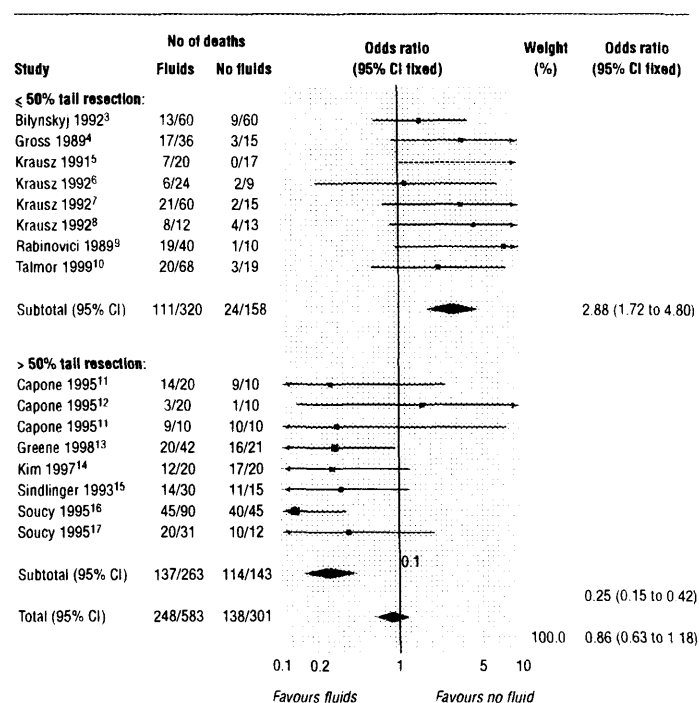


Fig 1 Meta-analysis of 16 randomised controlled trials of fluid resuscitation in rats with uncontrolled haemorrhage by tail resection. *Capone¹¹ reported two trials

posed the principle of reduction—that is, the use of methods to “reduce the number of animals needed to obtain information of a given amount and precision.”²³ Meta-analyses of the results of previous animal experiments would increase the precision of estimates of treatment effects and therefore reduce the number of animals needed in future experiments.

Publication bias may be as potent a threat to validity in systematic reviews of animal experiments as it is in systematic reviews of clinical trials. We contacted the authors of included trials to ask about unpublished studies but none were identified. However, it would be surprising if there were no unpublished trials meeting our inclusion criteria. Prospective registration of

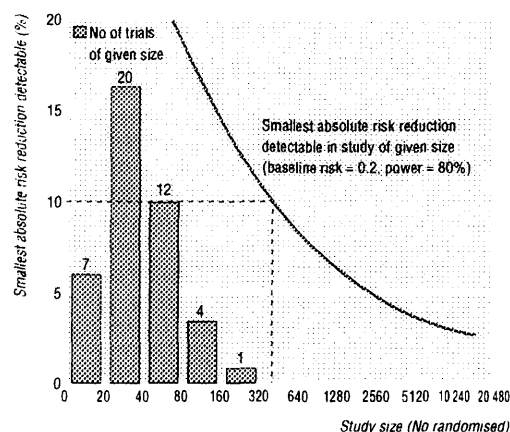


Fig 2 Trial size and smallest absolute risk reduction detectable

animal experiments at inception may help to avoid the problem of publication bias.²⁴ In the United Kingdom, the Animals (Scientific Procedures) Act 1986 regulates "any experimental or other scientific procedure applied to a protected animal which may have the effect of causing that animal pain, suffering, distress, or lasting harm." Researchers must have a project licence from the Home Office before conducting any animal research, and the licence application describes the experimental protocol. These data could be used for prospective registration of all animal experiments.

Systematic reviews of animal models could, like ours, include a range of animal species and models. If the results were consistent across species and models this would indicate that they might also apply in humans. Since the primary aim of animal experimentation is to inform human experimentation, this would be valuable information.

We found substantial statistical heterogeneity in our meta-analysis, making it impossible to interpret the odds ratios. Investigation of heterogeneity is essential and can increase the scientific and clinical relevance of their results. In our meta-analysis, stratification according to how uncontrolled bleeding was induced accounted for a large amount of the heterogeneity, but these results need to be interpreted with caution. Meta-analytic subgroup analyses are akin to subgroup analyses within trials and are prone to bias. Although we specified in our protocol that the analyses would be stratified according to the animal model used, we did not specify that we would stratify according to where the tail was cut. Nevertheless, the meta-analysis provides an insight into model dependency that could be taken into account in future animal experiments and when considering whether the results can be generalised to humans.

Implications for human health

Animal experiments can inform human health care only if their results are valid and can be generalised. However, little information is available on the methodological determinants of bias in animal experiments, and in our example the sample sizes were too small to obtain precise estimates of the effects of the interventions. Systematic reviews of animal experiments would help to ensure that animal experiments do not set out to answer questions that have already been answered, reduce bias and increase precision, and provide reassurance about whether the results can be generalised. Prospective registration of animal experiments would help to avoid publication bias. In a recent editorial, Smith promoted the three Rs of animal research first suggested by William Russell: replacement, reduction, and refinement.²⁵ On methodological grounds, animal experimentation would better contribute to human health care if we promoted registration, randomisation, and systematic reviews.

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contacted authors for further information and IK and IR conducted the analyses. IR drafted the paper that was revised on the basis of comments from IK, PE, SH. IR will act as guarantor.

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Endpiece

Self pity

I never saw a wild thing sorry for itself.
A small bird will drop frozen from a bough
without ever having felt sorry for itself.

D H Lawrence

Fluid Resuscitation Strategies: A Systematic Review of Animal Trials

James Mapstone, MB, BChir, Ian Roberts, PhD, FFPHM, and Phillip Evans, MB, BS

Objectives: Our objective was to systematically review randomized, controlled trials of fluid resuscitation in animal models of uncontrolled hemorrhage and to explore potential sources of heterogeneity.

Methods: We conducted an electronic bibliographic search of published research, reviewed reference lists of included trials, and contacted authors about unpublished studies. We included all unconfounded, randomized, controlled trials of fluid resuscitation (timing, volume, or resuscitation targets) in animal models of uncontrolled hemorrhage. The outcome measure was mortality at the end of the scheduled follow-up period of the trial. Two reviewers independently applied the selection criteria to the trial reports. A third reviewer resolved disagreements.

Results: Forty-four trials compared fluid versus no fluid resuscitation. There was marked heterogeneity in the effect of fluid resuscitation on the risk of death, much of which was explained by the hemorrhage model used. In aortic injury models, the adjusted relative risk of death with fluid resuscitation was 0.48 (95% confidence interval [CI], 0.33–0.71). In organ incision models, the adjusted relative risk of death was 0.76 (95% CI, 0.49–1.18). In tail resection models, the adjusted relative risk of death was 0.69 (95% CI, 0.38–1.25) if 50% or more was removed and 1.86 (95% CI, 1.13–3.07) if less than 50% was removed. In other vascular injury models, the adjusted relative risk of death with fluid resuscitation was 1.70 (95% CI, 1.01–2.85), respectively. Nine trials com-

pared hypotensive versus normotensive resuscitation. The relative risk of death with hypotensive resuscitation was 0.37 (95% CI, 0.27–0.50).

Conclusion: Fluid resuscitation appears to reduce the risk of death in animal models of severe hemorrhage but increases the risk of death in those with less severe hemorrhage. Excessive fluid resuscitation could therefore be harmful in some situations. Hypotensive resuscitation reduced the risk of death in all the trials investigating it. An evaluation of the potential impact of hypotensive resuscitation in humans could now be warranted.

Key Words: Fluid resuscitation, Systematic review, Hemorrhage, Animal, Critical care, Mortality.

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An intervention that improved survival from hemorrhagic shock would prevent many premature deaths. Globally, it is estimated that approximately 3 million people die each year from hemorrhagic shock. Close to 2 million of these deaths are from injury and the rest are from maternal hemorrhage, ruptured aortic aneurysms, or esophagogastrintestinal hemorrhage.^{1–3}

Fluid resuscitation is frequently used in the management of patients acutely bleeding. The Advanced Trauma Life Support course, from the American College of Surgeons, suggests boluses of crystalloid and blood and early definitive treatment.⁴ Maintaining an adequate perfusion pressure in this way is thought to assist tissue oxygenation and reduce the risk of short- and long-term organ failure and sepsis. Despite

these recommendations, there is very little evidence from randomized, controlled trials. A systematic review investigating the timing or volume of fluid administration identified only five trials⁵ and found no evidence to support early or larger volume fluid administration.

Much of the evidence for the type of resuscitation fluid came initially from research on animals. In light of the relative lack of human evidence of the effects of delayed or reduced volume resuscitation, a review of animal trials is valuable. We have previously argued that the application of systematic review methodology to animal experiments would allow a more objective appraisal of the research evidence than narrative reviews and, by increasing the precision of estimates of treatment effects, would reduce the risk of false-negative results.⁶ Exploration of heterogeneity in meta-analyses of animal experiments has the potential to provide important insights into disease mechanisms and the extent to which the results of animal experiments can be generalized to humans. In this article, we use stratification and meta-regression to examine sources of heterogeneity in a systematic review of 52 randomized, controlled trials of fluid resuscitation in animal models of uncontrolled hemorrhage.

MATERIALS AND METHODS

Study Identification

We conducted electronic searching of MEDLINE and EMBASE to identify all unconfounded, randomized, controlled trials of fluid resuscitation in animal models of uncontrolled hemorrhage. Reference lists of included studies

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were reviewed and authors contacted to identify unpublished trials. The search strategy identified 3,193 records of potentially eligible trials. Two reviewers examined the titles and abstracts and 104 reports were retrieved in full. From these, 52 trials meeting the inclusion criteria (randomized, controlled trial of the timing or volume of fluid resuscitation in an animal model of uncontrolled hemorrhage) were identified in 43 articles.⁷⁻⁴⁹

Study Selection

Two reviewers applied the selection criteria independently and disagreements were resolved by a third.

Data Extraction

Two reviewers independently extracted information on the methods of randomization and allocation concealment, the type of injury, the type of intervention, the number of animals in each arm of the trial, and the associated number of deaths.

Prior Hypotheses Regarding Heterogeneity

The potential sources of heterogeneity considered were the animal species used, the risk of death in the control arm, the type of hemorrhage model, the volume of blood lost in the no-fluid trial arm, the type and volume of fluid infused, and the time at which mortality was measured. The same sources of heterogeneity were considered for the studies of hypotensive versus normotensive resuscitation, with the addition of the difference in the amount of fluid infused between the treatment arms also being considered. These data were extracted by one reviewer. Normotensive was defined as resuscitation aiming for a mean arterial pressure of 80 mm Hg or higher.

Statistical Analysis

Data were analyzed as risk ratios with 95% confidence intervals calculated using Review Manager version 4.1 and Metaview version 4.1 (Update Software, Oxford, United Kingdom). Risk ratios were used because they are more readily interpretable than odds ratios. A risk ratio of 0.8 when comparing mortality in an intervention group compared with a control group means that the intervention group had 20% less risk of dying than the control group. For example, if the normal risk of dying from a particular injury was 10%, the observed risk in the intervention group would be 8%. A risk ratio > 1 suggests an increased risk with the intervention. A risk ratio < 1 suggests a decreased risk.

Where there was no evidence of a significant difference between the trial results (heterogeneity), a weighted regression using a random effects model was used.⁵⁰ Where heterogeneity existed, it was explored with stratification and meta-regression.⁵⁰ Stratification was used to observe the effects of variables and assist with categorization of the hemorrhage models. Meta-regression was used to objectively assess the relative significance of the different variables. The

aim of meta-regression is to relate the observed effect estimates to characteristics of the included trials. It is similar to other regression techniques where associations are sought between variables and the outcome of interest (e.g., socioeconomic status, smoking status, and life expectancy). This is performed for individual patients. Meta-regression does this, but at the level of the overall trial results. For example, it would be used to look at the association between the animal model used, the type of hemorrhage, and the observed risk ratio, taking into account the precision of the study. For this analysis, a random effects meta-analysis using an iteration method to provide restricted maximum likelihood measures was performed (STATA version 7, STATA Corp., College Station, TX; and the ado program by Stephen Sharp).

The numbers in each trial, the numbers dying, the unadjusted risk ratio, the 95% confidence interval, and the calculated weighting (based on the method described by DerSimonian and Laird⁵¹) associated with each trial are presented. This is commonly called a Forest plot and usually includes a pooled overall risk ratio and confidence interval. The column heading n/N in the plots describe the number of animals dying in the specific arm of the trial (n) and the total number of animals in the trial (N).

RESULTS

Study Characteristics

The 52 randomized, controlled trials included a total of 2,039 animals (1,772 rats, 251 pigs, and 16 sheep) (Table 1). Mortality data were available for 50 trials, of which there were 39 trials in rats, 10 trials in pigs, and 1 trial in sheep. Two trials did not use anesthetic at the time of hemorrhage,^{44,45} 17 trials used halothane,^{8,9,11,12,22,23,33,39,47,49} 2 used isoflurane,^{41,48} 3 used ketamine,^{21,35,38} 15 used neurolidol and ketamine,^{9,18-20,24-29,36,43,45} 3 used pentobarbital and ketamine,^{10,31,32} 5 used pentobarbital,^{9,13,34,42,45} 2 used chloralose,^{9,15} and 1 used ether.¹⁶ Only two trials described how the animals were divided into treatment groups (alternation).^{46,47} None described allocation concealment.

Fluid against No Fluid Resuscitation

There was a large amount of heterogeneity in the 44 trials comparing fluid and no fluid resuscitation (χ^2 , 126.99; *df*, 42; $p < 0.00001$) (Fig. 1). Stratification according to the type of hemorrhage model used explained some of the observed heterogeneity. The remaining heterogeneity was mainly in the tail resection models. Stratifying by where the tail was cut explained most of this. The observed heterogeneity was best reduced when considering the tail cutoff as $\geq 50\%$ or $< 50\%$, leaving five categories of hemorrhage model (Fig. 2). An alternative was to order the studies by the reported blood loss in the control group. However, this did not explain the heterogeneity between trials (Fig. 3).

Volume of hemorrhage in the control group, hemorrhage model, and risk of death in the control group were compared to identify which of these variables, relating to the severity of

Table 1 Included Trials

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Large animals (pigs and sheep)					
Alspaugh, 2000, ⁷ USA	Not specified	19 anesthetized swine. Swine of either gender averaging 50 kg in body weight, fasted overnight but allowed free access to water. Model of uncontrolled hemorrhage produced by inflicting splenic laceration and cryogenic brain injury.	Animals randomly assigned to two groups: 1. Prehospital resuscitation with Ringer's lactate. 2. Delayed resuscitation until after control of hemorrhage.	Death within 2 h	1. Prehospital, 3/9 2. Delayed resuscitation, 4/10
Bickell, 1992, ⁸ USA	Not specified	24 immature Yorkshire swine. After splenectomy, a stainless-steel wire was placed in the infrarenal aorta. The wire was pulled, producing a 5-mm aortotomy and spontaneous intraperitoneal hemorrhage.	Animals divided into three groups: 1. Untreated group. 2. Treated group: 4 mL/kg mixture of IV 7.5% NaCl and 6% Dextran 70 over 1 min. 3. Treated group: 80 mL/kg lactated Ringer's solution intravenously.	Death within 2 h	1. Untreated group, 0/8 2. 7.5% NaCl and 6% Dextran 70 group, 5/8 3. Lactated Ringer's, 8/8
Dronen, 1993, ¹⁴ USA	Not specified	17 3- to 4-mo-old Yorkshire swine. Immature swine were instrumented and subjected to severe blood loss (40–46 mL/kg); when the MAP was decreased to 30 mm Hg, a 4-mm tear was created in the infrarenal aorta, allowing free intraperitoneal bleeding.	Animals were randomized into two groups: 1. No resuscitation. 2. Resuscitation with normal saline infused at a rate of 6 mL/kg/min followed by shed blood at a rate of 2 mL/kg/min infused to maintain a MAP of 80 mm Hg.	Death within 1 h	1. No resuscitation, 7/8 2. Resuscitation, 7/9
Kowalenko, 1992, ²³ USA	Not specified	24 immature swine. Preresuscitative hemorrhage is accurately controlled from a femoral artery catheter. Once animal reaches a mean arterial pressure of 30 mm Hg, a 4-mm aortic tear is inflicted, allowing free intraperitoneal hemorrhage.	Animals were randomly divided into three groups: 1. No fluids. 2. Saline infusion at 6 mL/kg/min to reach MAP 40 mm Hg. 3. Saline infusion at 6 mL/kg/min to reach MAP 80 mm Hg.	Death within 1 h	1. No fluids, 7/8 2. MAP 40 mm Hg, 1/8 3. MAP 80 mm Hg, 5/8
Owens, 1995, ³⁵ USA	Not specified	20 immature Yorkshire swine. Swine were anesthetized, arterial and venous catheters were inserted. 25 mL/kg blood was withdrawn during a 30-min controlled hemorrhage, followed by a 20-min uncontrolled hemorrhage from a 5-mm aortotomy.	Animals were randomized into three groups: 1. No resuscitation. 2. Standard resuscitation: Lactated Ringer's infused to achieve and maintain 100% baseline cardiac index for 20 min. 3. Limited prehospital resuscitation: Lactated Ringer's infused to achieve and maintain 60% baseline cardiac index for 20 min. In the intraoperative phase, intraoperative resuscitation was continued for 120 min using lactated Ringer's to achieve 80% baseline CI.	Death within 2 h	1. No resuscitation, 1/6 2. Standard resuscitation, 1/6 3. Limited prehospital resuscitation, 0/8

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Riddez, 1998, ³⁸ Sweden	Not specified	32 swine. Inducing a 5-mm-long laceration in the infrarenal aorta induced uncontrolled hemorrhagic shock.	Animals were randomized into four groups: 1. No fluid resuscitation. 2. Ringer's solution in about the same amount as the expected blood loss per hour (1:1) over 2 h. 3. Ringer's solution in a ratio 2:1 of the expected blood loss over 2 h. 4. Ringer's solution in a ratio 3:1 of the expected blood loss over 2 h.	Death within 1 h	1. No fluid resuscitation, 4/8 2. Ringer's solution (1:1), 2/8 3. Ringer's solution (2:1), 2/8 4. Ringer's solution (3:1), 4/8
Sakles, 1997, ³⁹ USA	Not specified	16 adult sheep. Sheep were anesthetized and lacerating a branch of the pulmonary vein through an anterolateral thoracotomy induced uncontrolled hemorrhage.	Animals were randomly divided into two groups: 1. No resuscitation. 2. Immediate resuscitation: 30 mL/kg lactated Ringer's solution over a period of 10 min, repeated to achieve normotension.	Death within 2 h	1. No resuscitation, 1/8 2. Immediate resuscitation, 1/8
Silbergleit, 1996, ⁴¹ USA	Not specified	10 swine. Animals were anesthetized and uncontrolled continuous hemorrhage was induced via catheters in the femoral vessels.	Animals were randomly divided into two groups: 1. No fluid resuscitation. 2. Fluid resuscitation: 80 mL/kg lactated Ringer's during a resuscitation phase between 10 and 20 min postinjury.	Death within 1 h	1. No fluid resuscitation, 3/5 2. Fluid resuscitation, 4/5
Stern, 1993, ⁴⁶ USA	Alternation	27 immature Yorkshire swine. Near-fatal porcine aortic injury hemorrhage model. Preresuscitative hemorrhage is accurately controlled from a femoral artery catheter. Once animal reaches a predetermined physiologic endpoint, aortic tear is inflicted, allowing free intraperitoneal hemorrhage.	Animals were resuscitated with a saline infusion at 6 mL/kg/min as needed to maintain the desired endpoints: 1. MAP 40 mm Hg. 2. MAP 60 mm Hg. 3. MAP 80 mm Hg. After 30 min or a total saline resuscitation 90 mL/kg, the resuscitation fluid was changed to shed blood infused 2 mL/kg/min as needed to maintain the desired MAP.	Death within 1 h	1. MAP 40 mm Hg, 1/9 2. MAP 60 mm Hg, 2/9 3. MAP 80 mm Hg, 7/9
Stern, 1995, ⁴⁷ USA	Alternation	54 immature Yorkshire swine. Near-fatal porcine aortic injury hemorrhage model. Preresuscitative hemorrhage is accurately controlled from a femoral artery catheter. Once animal reaches a predetermined physiologic endpoint, aortic tear is inflicted, allowing free intraperitoneal hemorrhage.	Animals were divided into three groups: 1. MAP 40 mm Hg. 2. MAP 60 mm Hg. 3. MAP 80 mm Hg. Resuscitation fluid was either shed blood followed by normal saline or normal saline followed by shed blood.	Death within 1 h	1. MAP 40 mm Hg, 2/18 2. MAP 60 mm Hg, 3/18 3. MAP 80 mm Hg, 14/18
Stern, 2000, ⁴⁸ USA	Not specified	24 swine. Each swine underwent fluid percussion brain injury and uncontrolled hemorrhage to a mean arterial pressure of 30 mm Hg in the presence of a 4-mm aortic tear.	Animals were randomly assigned to one of three groups: 1. No resuscitation. 2. MAP 60 mm Hg. 3. MAP 80 mm Hg.	Death within 150 min	1. No resuscitation, 6/6 2. MAP 60 mm Hg, 1/9 3. MAP 80 mm Hg, 4/9

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Small animals (rats) Bilynskyj, 1992, ⁹ a,b,c,d,e, Brazil	Not specified	120 male Wistar rats. Uncontrolled hemorrhage was induced by 12% (e) or 50% tail resection (a,b,c,d).	Animals were divided into anesthetic groups (a+e, neurolidol/ketamine; b, pentobarbital; c, chloralose; d, urethane) and each of these were divided into two groups: 1. No fluid resuscitation. 2. Hypertonic saline (7.5%) 4 mL/kg IV. 15 min from start of bleeding.	Deaths within 4 h	a. No fluid, 4/10; fluid, 5/10 b. No fluid, 1/10; fluid, 0/10 c. No fluid, 0/10; fluid, 1/10 d. No fluid, 1/10; fluid, 0/10 e. No fluid, 2/10; fluid, 3/10
Burris, 1999, ¹⁰ USA	Not specified	61 male Sprague-Dawley rats. After instrumentation, the hemodynamically stable but lightly anesthetized rats were subjected to a vascular injury leading to uncontrolled hemorrhagic shock by piercing the infrarenal aorta with a 25-gauge needle, creating two standard sized holes, one on each side of the aorta.	Fluids infused at 2 mL/kg/min were turned off or on to maintain a mean arterial pressure of 40, 80, or 100 mm Hg in six groups: 1. No fluid. 2. Lactated Ringer's MAP 100 mm Hg. 3. Lactated Ringer's MAP 80 mm Hg. 4. Lactated Ringer's MAP 40 mm Hg. 5. Hypertonic NaCl/hetastarch (MAP 80) 6. Hypertonic NaCl/hetastarch (MAP 40)	Death within 2 h	1. No fluid, 9/11 2. Ringer's (MAP 100), 2/9 3. Ringer's (MAP 80), 0/11 4. Ringer's (MAP 40), 1/10 5. NaCl/hetastarch (MAP 80), 4/11 6. NaCl/hetastarch (MAP 40), 2/9
Capone, 1995a, ¹¹ USA	Randomized in blocks of four; allocation concealment not specified	40 male Sprague-Dawley rats. Uncontrolled hemorrhagic shock produced by a preliminary bleed (3 mL/100 g) followed by 75% tail amputation.	Experimental design consisted of three phases: Prehospital phase: from insult to hemostasis 60 minutes later. During this phase, uncontrolled hemorrhagic shock was produced so that different fluid resuscitation regimens could be tested. Hospital phase: started with hemostasis and continued for another 120 min, with unrestricted fluid resuscitation, including infusion of the donor blood to Hct 30% plus lactated Ringer's solution to achieve normotension. Observation phase extended from the end of the hospital phase to 3 days to evaluated outcome. 1. Untreated controls. 2. No prehospital fluid. 3. LR prehospital to maintain MAP at 40 mm Hg. 4. LR prehospital to maintain MAP at 80 mm Hg. Group 1 not resuscitated in hospital phase is excluded.	Death within 3 days	2. No fluid, 9/10 3. LR prehospital (MAP 40), 4/10 4. LR prehospital (MAP 80), 10/10

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Capone, 1995b, ¹² USA	Not specified	40 male Sprague-Dawley rats. Hemorrhage induced by an oblique sterile tail amputation of 75% its length, as measured from the tip.	Experimental design consisted of three phases: a "prehospital phase" (90 min of uncontrolled bleeding with or without lactated Ringer's solution) followed by a "hospital phase" (60 min including control of bleeding and fluid resuscitation including blood). 1. Untreated controls. 2. No prehospital fluid. 3. LR prehospital to maintain MAP at 40 mm Hg. 4. LR prehospital to maintain MAP at 80 mm Hg. Groups 2, 3, and 4 only were resuscitated in the hospital phase. Group 1 therefore not included.	Death within 3 days	2. No prehospital fluid, 1/10 3. LR prehospital (MAP 40), 0/10 4. LR prehospital (MAP 80), 3/10
Capone, 1995c, ¹¹ USA	Not specified	20 male Sprague-Dawley rats. Volume-controlled hemorrhage of 3 mL/100 g over 15 min followed by uncontrolled hemorrhage induced by an oblique sterile tail amputation of 75% its length, as measured from the tip.	Experimental design consisted of three phases: Prehospital phase: from insult to hemostasis 90 min later. During this phase, uncontrolled hemorrhagic shock was produced so that different fluid resuscitation regimens could be tested. Hospital phase: started with hemostasis and continued for another 60 min, with unrestricted fluid resuscitation, including infusion of the donor blood to Hct 30% plus lactated Ringer's solution to achieve normotension. Observation phase extended from the end of the hospital phase to 3 days to evaluated outcome. 1. No fluid resuscitation in either phase. 2. No resuscitation in prehospital phase, then in hospital phase all out fluid resuscitation as above.	Death within 3 days	1. No resuscitation, 10/10 2. Hospital resuscitation, 9/10
Craig, 1994, ¹³ USA	Not specified	45 male Sprague-Dawley rats. Rats were anesthetized and a femoral artery and vein were cannulated for blood pressure monitoring and fluid infusion. Through a midline abdominal incision, the distal ileocolic artery and vein were cut and allowed to bleed freely into the abdominal cavity. The abdomen was closed and the animals were randomized into five groups.	Animals randomly divided into five groups: 1. No resuscitation. 2. Small-volume lactated Ringer's solution. 3. Large-volume lactated Ringer's solution. 4. Small-volume hetastarch. 5. Large-volume hetastarch.	Death within 3 h	1. No resuscitation, 2/9 2. Small-volume lactated Ringer's solution, 3/9 3. Large-volume lactated Ringer's solution, 4/9 4. Small-volume hetastarch, 8/9 5. Large-volume hetastarch, 4/9

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Elgjo, 1996, ¹⁵ a,b, Norway	Not specified	39 male Wistar-Kyoto rats. Hypotension was induced by controlled withdrawal of blood until MAP reached 50 mm Hg. After 5 min, subjects were randomized into two groups for uncontrolled hemorrhage: (a) arterial hemorrhage—the abdominal aorta punctured with a 21-, 22-, or 23-gauge hypodermic needle; (b) venous hemorrhage—inferior caval vein puncture with an 18- or 19-gauge hypodermic needle.	Animals in each group were randomized into two groups: 1. No treatment. 2. Saline 8.0% 2 mL/kg IV at 0.4 mL/min.	Death within 4 h	a) Arterial hemorrhage 1. No treatment, 5/12 2. Saline 8.0%, 6/10 b) Venous hemorrhage 1. No treatment, 0/7 2. Saline 8.0%, 1/10
Feldman, 1997, ¹⁷ Israel	Not specified	56 male Sprague-Dawley rats. Halothane anesthesia and divided into groups with or without closed head trauma. Head trauma was delivered to the skull over the frontal portion of the left cerebral hemisphere by a weight drop device. The 30 rats that survived head injury were randomly divided into groups. Hemorrhagic hypotension was produced by 12% tail resection.	Randomly allocated to early or late fluid resuscitation with warmed lactated Ringer's solution. Volume infused was three times the total amount of blood lost. 1. Early resuscitation: IV fluids started 1.25 h after injury. 2. Delayed resuscitation: IV fluids started 2 h after injury.	Death within 2 h	No closed head injury 1. Early resuscitation, 0/10 2. Delayed resuscitation, 0/10 Closed head injury 1. Early resuscitation, 3/10 2. Delayed resuscitation, 3/10
Greene, 1998, ¹⁶ USA	Not specified	63 female Sprague-Dawley rats. The day before the study, the animals were cannulated through the jugular vein. Under light ether anesthesia the rats were restrained and hemorrhage was initiated by a 75% tail resection with a guillotine.	Rats were randomly divided into three groups: 1. Not resuscitated. 2. Isotonic saline 40 mL/kg. 3. Isotonic saline 80 mL/kg. Fluid resuscitation was initiated 15 min after tail resection and given over a period of 4 min.	Death within 6 h	1. Not resuscitated, 16/21 2. Isotonic saline 40 mL/kg, 15/21 3. Isotonic saline 80 mL/kg, 5/21
Gross, 1988, ¹⁸ Israel	Not specified	36 male Hebrew University rats. The rats were anesthetized and a midline laparotomy was performed. The ileocolic artery was identified and three of its major branches were incised longitudinally to induce uncontrolled intra-abdominal hemorrhage.	Animals were divided into three groups: 1. No resuscitation. 2. IV 0.9% saline 5 mL/kg at a rate of 0.4 mL/min. 3. IV 7.5% saline 5 mL/kg injected IV.	Death within 3 h	1. No resuscitation, 2/9 2. IV 0.9% saline, 3/9 3. IV 7.5% saline, 17/18
Gross, 1989, ¹⁹ Israel	Not specified	51 male Hebrew University rats. The rats were anesthetized and uncontrolled hemorrhagic shock was induced by sharp resection of 10% of the terminal portion of the tail.	Animals were divided into three groups: 1. No resuscitation. 2. After 5 min, IV infusion of 0.9% saline 5 mL/kg at a rate of 0.4 mL/min. 3. After 5 min, IV infusion of 7.5% saline 5 mL/kg injected intravenously.	Death within 3 h	1. No resuscitation, 3/15 2. IV 0.9% saline, 1/14 3. IV 7.5% saline, 16/22

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Gross, 1990 ²⁰ Israel	Not specified	94 male Hebrew University rats. The rats were anesthetized and a midline laparotomy was performed. The ileocolic artery was identified and three of its major branches were incised longitudinally to induce uncontrolled intra-abdominal hemorrhage. The rats were divided into two groups according to whether or not the abdomen was closed after induction of hemorrhage.	Rats in each of the two groups were divided into six groups: 1. Untreated. 2. 5 mL/kg 7.5% NaCl after 5 min. 3. 5 mL/kg 7.5% NaCl after 15 min. 4. 15 mL/kg 7.5% NaCl after 30 min. 5. 5 mL/kg 7.5% NaCl after 60 min. 6. 5 mL/kg 7.5% NaCl after 120 min.	Death within 2 h	Abdomen closed 1. Untreated, 2/5 2. 5 mL/kg 7.5% NaCl after 5 min, 12/14 3. 5 mL/kg 7.5% NaCl after 15 min, 7/8 4. 15 mL/kg 7.5% NaCl after 30 min, 5/9 5. 5 mL/kg 7.5% NaCl after 60 min, 5/9 6. 5 mL/kg 7.5% NaCl after 120 min, 7/9 Abdomen open 1. Untreated, 0/5 2. 5 mL/kg 7.5% NaCl after 5 min, 9/9 3. 5 mL/kg 7.5% NaCl after 15 min, 1/6 4. 15 mL/kg 7.5% NaCl after 30 min, 1/6 5. 5 mL/kg 7.5% NaCl after 60 min, 2/8 6. 5 mL/kg 7.5% NaCl after 120 min, 1/6
Haizlip, 1999, ²¹ USA	Not specified	30 male rats. Uncontrolled intraperitoneal hemorrhage was induced by division of the distal branches of the ileocolic artery and vein.	Animals were randomized into three groups: 1. No fluid. 2. 0.5 mL LR every 5 min if MAP < 80 mm Hg. 3. 2.0 mL LR every 5 min if MAP < 80 mm Hg.	Death within 3 h	1. No fluid, 1/10 2. 0.5 mL LR, 4/10 3. 2.0 mL LR, 0/10
Kim, 1997, ²² USA	Not specified	45 rats (5 excluded due to catheter insertion problems) After an initial volume-controlled hemorrhage (3 mL/100 g), uncontrolled hemorrhagic shock was induced by 75% tail resection.	Animals were randomized into four groups: Normothermia (37.5°C) 1. No fluid resuscitation. 2. Lactated Ringer's to maintain MAP 40 mm Hg. Hypothermia (30°C) 1. No fluid resuscitation. 2. Lactated Ringer's to maintain MAP 40 mm Hg.	Death within 72 h	Normothermia (37.5°C) 1. No resuscitation, 10/10 2. MAP 40 mm Hg, 9/10 Hypothermia (30°C) 1. No resuscitation, 7/10 2. MAP 40 mm Hg, 3/10
Krausz, 1991, ²⁴ a,b Israel	Not specified	37 male Hebrew University rats. Either (a) 8% or (b) 50% tail resection induced uncontrolled hemorrhagic shock.	Animals were randomly divided into four groups. (a) 8% tail resection. 1. No resuscitation. 2. Saline 7.5% 5 mL/kg. (b) 50% tail resection. 1. No resuscitation. 2. Saline 7.5% 5 mL/kg.	Death within 4 h	(a) 8% tail resection 1. No resuscitation, 0/8 2. Saline 7.5% 5 mL/kg, 0/8 (b) 50% tail resection 1. No resuscitation, 0/9 2. Saline 7.5% 5 mL/kg, 7/12

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Krausz, 1992a, ²⁵ Israel	Not specified	33 male Hebrew University rats. Uncontrolled hemorrhagic shock was induced by resection of 12% of the terminal portion of the tail.	Animals randomly divided into four groups: 1. Untreated 2. 41.5 mL/kg 0.9% NaCl after 15 min. 3. 5 mL/kg 7.5% NaCl after 15 min. 4. 41.5 mL/kg 0.9% NaCl and 5 mL/kg 7.5% NaCl after 15 min	Death within 4 h	1. Untreated, 2/9 2. 41.5 mL/kg 0.9% NaCl after 15 min, 0/8 3. 5 mL/kg 7.5% NaCl after 15 min, 5/7 4. 41.5 mL/kg 0.9% NaCl and 5 mL/kg 7.5% NaCl after 15 min, 1/9
Krausz, 1992b, ²⁶ Israel	Not specified	37 male Hebrew University rats. Uncontrolled hemorrhagic shock was induced by 10% tail resection.	Animals were randomly divided into five groups giving 5 mL/kg 7.5% NaCl after: 1. Untreated. 2. Saline after 5 min. 3. Saline after 15 min. 4. Saline after 30 min. 5. Saline after 60 min. 6. Saline after 120 min.	Death within 4 h	1. Untreated, 2/15 2. Saline after 5 min, 12/15 3. Saline after 15 min, 8/15 4. Saline after 30 min, 0/10 5. Saline after 60 min, 0/10 6. Saline after 120 min, 1/10
Krausz, 1992c, ²⁷ Israel	Not specified	25 male Hebrew University rats. Uncontrolled hemorrhagic shock was induced by 12% tail resection.	Animals were randomly divided into three groups: 1. Untreated. 2. 41.5 mL/kg 0.9% NaCl. 3. 5.0 mL/kg 7.5% NaCl.	Death within 4 h	1. Untreated, 4/13 2. 0.9% NaCl, 2/6 3. 7.5% NaCl, 6/6
Krausz, 1994, ²⁸ Israel	Not specified	16 male Hebrew University rats. Uncontrolled hemorrhagic shock was induced by transcutaneous tear of two branches of the ileocolic artery.	Animals randomly divided into two groups: 1. Untreated. 2. Saline (7.5%) 5 mL/kg after 10 min.	Death within 4 h	1. Untreated, 3/8 2. Saline 7.5%, 6/8
Krausz, 1995, ²⁹ Israel	Not specified	32 male Hebrew University rats. Uncontrolled hemorrhagic shock was induced by transcutaneous tear of two branches of the ileocolic artery.	Animals randomly divided into four groups: 1. Untreated. 2. 5 mL/kg 7.5% saline after 5 min. 3. 5 mL/kg 9.2% sodium acetate after 5 min. 4. 41.5 mL/kg 0.9% saline infused in 15 min.	Death within 4 h	1. Untreated, 2/8 2. 5 mL/kg 7.5% saline after 5 min, 6/8 3. 5 mL/kg, 9.2% sodium acetate after 5 min, 4/8 4. 41.5 mL/kg 0.9% saline infused in 15 min, 2/8
Krausz, 2000, ³⁰ Israel	Not specified	50 male Sprague-Dawley rats. Uncontrolled hemorrhagic shock was induced by moderate splenic injury.	Animals randomly divided into five groups: 1. Untreated. 2. 41.5 mL/kg lactated Ringer's after 10 min. 3. 5 mL/kg 7.5% NaCl after 10 min. 4. 7.5 mL/kg hydroxyethyl starch 6% after 10 min. 5. 15 mL/kg hydroxyethyl starch 6% within 10 min.	Death within 4 h	Only cumulative death data reported
Leppaniemi, 1996a, ³¹ USA	Not specified	40 male Sprague-Dawley rats. Uncontrolled hemorrhagic shock was induced by a 25-gauge needle puncture to the infrarenal aorta.	Animals were randomly divided into three groups: 1. No resuscitation. 2. Lactated Ringer's 60 mL/kg at 1.5 mL/min. 3. 7.5% saline 5 mL/kg at 1.5 mL/min.	Death within 6 h	1. No resuscitation, 6/8 2. Lactated Ringer's 60 mL/kg at 1.5 mL/min, 2/8 3. 7.5% saline 5 mL/kg at 1.5 mL/min, 6/8

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Leppaniemi, 1996b, ³² USA	Not specified	40 male Sprague-Dawley rats. Uncontrolled hemorrhagic shock was induced in anesthetized animals by a 25-gauge needle puncture to the infrarenal aorta.	Animals were randomly divided into six groups: 1. No resuscitation. 2. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 2.5 min. 3. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 5 min. 4. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 10 min. 5. Lactated Ringer's 60 mL/kg at 3.0 mL/min and given at 5 min. 6. Lactated Ringer's 60 mL/kg at 3.0 mL/min and given at 10 min.	Death within 3 h	1. No resuscitation, 7/9 2. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 2.5 min, 2/6 3. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 5 min, 1/6 4. Lactated Ringer's 60 mL/kg at 1.5 mL/min and given at 10 min, 1/6 5. Lactated Ringer's 60 mL/kg at 3.0 mL/min and given at 5 min, 5/6 6. Lactated Ringer's 60 mL/kg at 3.0 mL/min and given at 10 min, 0/6
Marshall, 1997, ³³ USA	Not specified	32 male Sprague-Dawley rats. Uncontrolled hemorrhagic shock was induced by preliminary bleed of 3 mL/100 g followed by 75% tail amputation. Experiment comprised three phases: (1) prehospital phase with uncontrolled bleeding and resuscitation to either 40 or 80 mm Hg; (2) hospital phase with control of bleeding and resuscitation to a mean arterial pressure of more than 80 mm Hg; (3) a 3-day observation phase.	Animals were randomly divided into four groups: 1. MAP 40 mm Hg with lactated Ringer's. 2. MAP 40 mm Hg with lactated Ringer's and blood. 3. MAP 80 mm Hg with lactated Ringer's. 4. MAP 80 mm Hg with lactated Ringer's and blood.	Death within 3 days	1. MAP 40 mm (LR), 2/8 2. MAP 40 mm Hg (LR + B), 1/8 3. MAP 80 mm Hg (LR), 8/8 4. MAP 80 mm Hg (LR + B), 0/8
Matsuoka, 1996, ³⁴ USA	Not specified	120 adult male Sprague-Dawley rats. Creating a standardized liver injury at the beginning of the shock period induced uncontrolled hemorrhage. A midline celiotomy was performed and about 65% of the median and left lateral lobes of the liver were removed.	Animals were randomized into four groups: 1. No resuscitation. 2. Lactated Ringer's solution 4 mL/kg. 3. Lactated Ringer's solution 24 mL/kg. 4. 7.5% saline 4 mL/kg.	Death within 4 h	1. No resuscitation, 15/30 2. Lactated Ringer's solution 4 mL/kg, 14/30 3. Lactated Ringer's solution 24 mL/kg, 12/30 4. 7.5% saline 4 mL/kg, 3/30
Rabinovici, 1989, ³⁶ Israel	Not specified	50 male Hebrew University rats. Rats were anesthetized and uncontrolled hemorrhage was induced by sharp resection of 10% of the terminal portion of the tail.	Animals were randomly divided into five groups: 1. No treatment. 2. After 5 min, 5 mL/kg 0.9% saline at 0.4 mL/min. 3. After 5 min, 5 mL/kg 6% Dextran 70 at 0.4 mL/min. 4. After 5 min, 5 mL/kg 7.5% saline was infused. 5. After 5 min, 5 mL/kg 7.5% saline in 6% Dextran 70 was infused.	Death within 4 h	1. No treatment, 1/10 2. After 5 min, 5 mL/kg 0.9% saline at 0.4 mL/min, 2/10 3. After 5 min, 5 mL/kg 6% Dextran 70 at 0.4 mL/min, 5/10 4. After 5 min, 5 mL/kg 7.5% saline was infused, 6/10 5. After 5 min, 5 mL/kg 7.5% saline in 6% Dextran 70 was infused, 6/10

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Rabinovici, 1991, ³⁷ USA	Not specified	21 male Sprague-Dawley rats. Rats were anesthetized and uncontrolled hemorrhage was induced by sharp resection of 15% of the terminal portion of the tail.	Animals were randomly divided into three groups: 1. No treatment. 2. Saline 7.5% 5 mL/kg 15 min after tail resection. 3. Saline 7.5% 41.5 mL/kg 15 min after tail resection.	Time to death data only hours	Death data not reported.
Selby, 1996, ⁴⁰ USA	Not specified	24 Sprague-Dawley rats. Uncontrolled hemorrhage was induced by sharp 75% tail resection.	Animals were randomly divided into four groups: 1. No resuscitation. 2. Isotonic saline 40 mL/kg in 4 min. 3. Isotonic saline 80 mL/kg in 4 min. 4. Isotonic saline 80 mL/kg in 1 min.	Death within 3 h	Death data not reported.
Sindlinger, 1993, ⁴² USA	Not specified	45 female Sprague-Dawley rats. Animals were anesthetized with pentobarbital and uncontrolled hemorrhage was induced by 75% tail resection with a guillotine.	Animals were randomly divided into three groups: 1. No resuscitation. 2. Saline 0.9% 40 mL/kg over a 4-min interval 15 min after hemorrhage. 3. Saline 0.9% 80 mL/kg over a 4-min interval 15 min after hemorrhage.	Death within 6 h	1. No resuscitation, 11/15 2. Saline 40 mL/kg, 6/15 3. Saline 80 mL/kg, 8/15
Solomonov, 2000, ⁴³ Israel	Not specified	45 adult Sprague-Dawley rats. Creating a standardized massive splenic injury induced uncontrolled hemorrhagic shock by two transverse incisions in the rat's spleen.	Animals were randomly divided into three groups: 1. No resuscitation. 2. Treated after 15 min with 41.5 mL/kg 0.9% saline infused within 10 min. 3. Treated after 15 min with 5 mL/kg 7.5% saline infused within 10 min.	Death within 1 h	1. No resuscitation, 2/15 2. Saline 41.5 mL/kg, 5/15 3. Saline 5 mL/kg, 9/15
Soucy, 1995, ⁴⁴ a,b,c USA	Not specified	135 female Sprague-Dawley rats. Uncontrolled hemorrhage was induced by 75% tail resection.	Animals were divided into three groups: (a) no anesthesia, (b) pentobarbital anesthesia, and (c) droperidol and ketamine anesthesia. Each of the groups was then randomly divided into three subgroups: 1. No resuscitation. 2. Isotonic saline 40 mL/kg. 3. Isotonic saline 80 mL/kg. Each given 15 min after initiation of hemorrhage.	Death within 3 h	(a) No anesthesia 1. No resuscitation, 14/15 2. Saline 40 mL/kg, 11/15 3. Saline 80 mL/kg, 5/15 (b) Pentobarbital anesthesia 1. No resuscitation, 11/15 2. Saline 40 mL/kg, 6/15 3. Saline 80 mL/kg, 8/15 (c) Droperidol and ketamine 1. No resuscitation, 15/15 2. Saline 40 mL/kg, 10/15 3. Saline 80 mL/kg, 5/15
Soucy, 1999, ⁴⁵ USA	Not specified	43 female Sprague-Dawley rats. Uncontrolled hemorrhage was induced by 75% tail resection.	Animals were randomized into five groups: 1. No resuscitation. 2. Moderate volume (80 mL/kg), slow infusion (0.82 mL/min). 3. Moderate volume (80 mL/kg), fast infusion (4.4 mL/min). 4. High volume (283 mL/kg), slow infusion (0.82 mL/min). 5. High volume (283 mL/kg), fast infusion (4.4 mL/min).	Death within 3 h	1. No resuscitation, 10/12 2. Moderate/slow, 4/6 3. Moderate/fast, 4/10 4. High/slow, 7/8 5. High/fast, 5/7

Table 1 Included Trials (Continued)

Study ID	Allocation Method	Animal Model	Intervention	Outcomes	Mortality Results
Talmor, 1999, ⁴⁹ USA	Not specified	207 male Sprague-Dawley rats. Rats were divided into groups with or without closed head injury. Uncontrolled hemorrhagic shock was induced 25% tail resection.	Animals were randomly assigned to head injury or no head injury groups. These groups were then subdivided into fluid or no fluid or resuscitation blood pressure targets.	Death within 24 h	Closed head injury 1. No resuscitation, 3/11 2. Saline 3 mL/mL blood lost, 2/10 No head injury 1. No resuscitation, 0.8 2. Saline 3 mL/mL blood lost, 1/9 Closed head injury 1. Target MAP 70 mm Hg, 10/18 2. Target MAP 80 mm Hg, 26/34 3. Target MAP 90 mm Hg, 10/10 No head injury 1. Target MAP 70 mm Hg, 1/9 2. Target MAP 80 mm Hg, 6/14 3. Target MAP 90 mm Hg, 10/10

MAP, mean arterial pressure; IV, intravenous; CI, cardiac index; Hct, hematocrit; LR, lactated Ringer's.

injury, best explained the observed heterogeneity. The hemorrhage model produced the greatest reduction in the observed heterogeneity (between-study variance, τ^2) from 0.295 to 0.081. This 73% reduction is compared with 27% for volume of hemorrhage in the control group and 56% for risk of death in the control group.

Using hemorrhage model as the best indicator of severity of injury, animal species, volume of fluid administered in the intervention group, type of fluid administered, and follow-up time were considered for the meta-regression model. At each step, the variable that explained the most residual variance was incorporated into the model until the between-study variance was 0. Follow-up time and volume of fluid infused explained the remaining heterogeneity.

The crude stratum-specific pooled risk ratios changed a little when adjusted for the species used, the type of fluid infused, and the time at which the outcome was assessed (Table 2). The adjusted pooled risk ratio of death in the fluid-resuscitated group versus the no-fluid group, for those animals with an injury to the aorta was 0.48 (95% confidence interval [CI], 0.33–0.71). The corresponding figures for less severe injuries (<50% tail resection, and other vascular injuries) were 1.86 (95% CI, 1.13–3.07) and 1.70 (95% CI, 1.01–2.85).

Normotensive against Hypotensive Resuscitation

There were nine trials comparing normotensive versus hypotensive resuscitation. The pooled risk ratio for hypotensive compared with normotensive resuscitation was 0.37 (95% CI, 0.27–0.52) (Fig. 4). There was no evidence of heterogeneity ($p = 0.38$; χ^2 , 8.58; df , 8).

DISCUSSION

The effect of fluid resuscitation on the risk of death in animal models of uncontrolled hemorrhage appears to be related to the severity of hemorrhage. When hemorrhage is severe (injury to the aorta or >50% of the rat tail removed), fluid resuscitation reduces the risk of death; but when hemorrhage is less severe (injury to vessels other than the aorta or <50% of the rat tail removed), the risk of death is increased. This suggests that the risks and benefits of fluid resuscitation are finely balanced. For those animals with less severe injury, the potential risks of fluid resuscitation—increasing hydrostatic pressure and impaired clotting leading to increased blood loss and diluting the oxygen-carrying capacity of the blood—appears to outweigh the benefits of improved tissue perfusion and reduced tissue ischemia. The opposite seems to hold for the severest injuries. The effect of hypotensive resuscitation was to reduce the risk of death in all the trials. This suggests that using a lower than normal blood pressure as a guide to fluid resuscitation consistently reduces the risk of death regardless of the severity of injury. This could be by ensuring that the benefits of fluid resuscitation remain the same and reducing the potential risks.

Review: Animal (for haemorrhage model analysis only)
Comparison: 03 Heterogeneity
Outcome: 01 Mortality

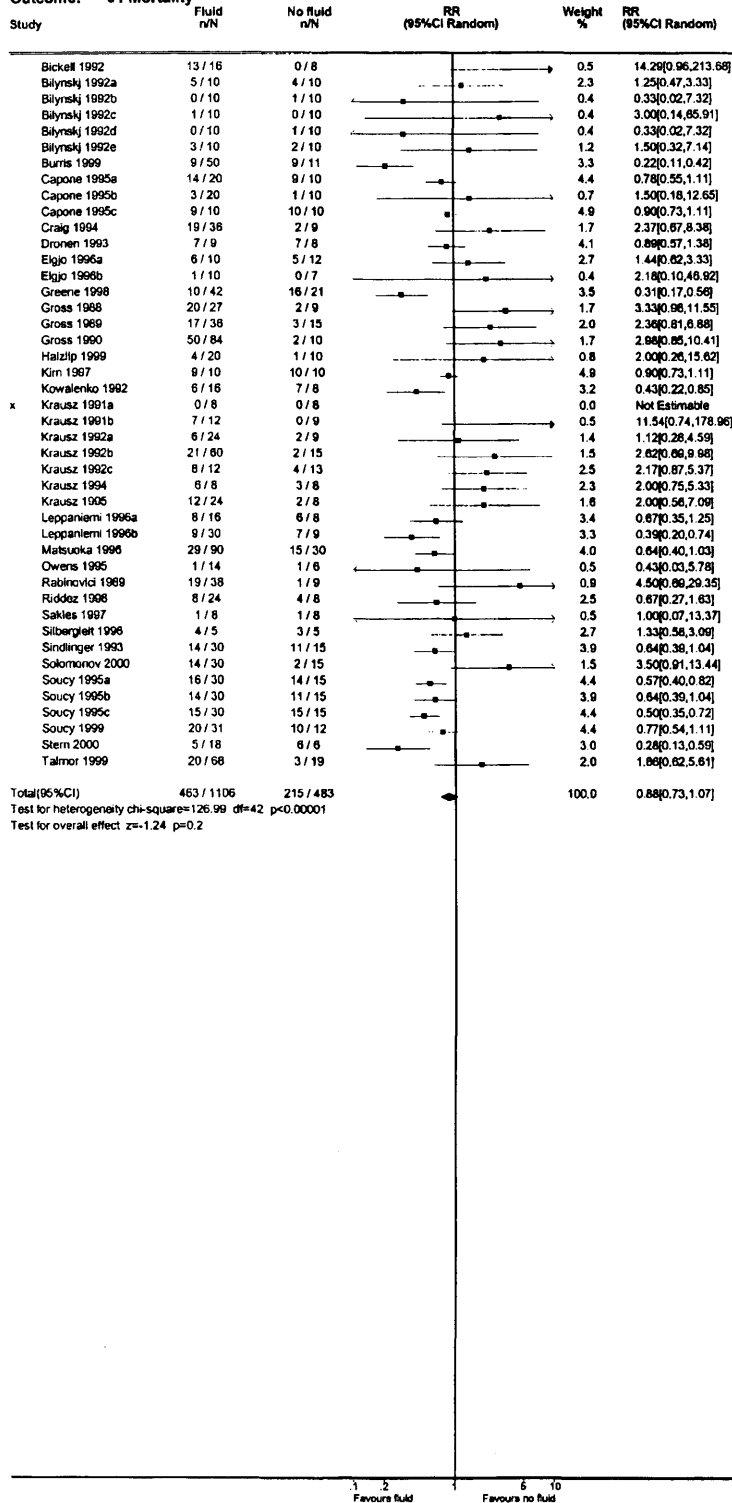


Fig. 1. Risk ratios for the included studies.

Review: Animal (for haemorrhage model analysis only)
Comparison: 01 Results by model type
Outcome: 01 Death

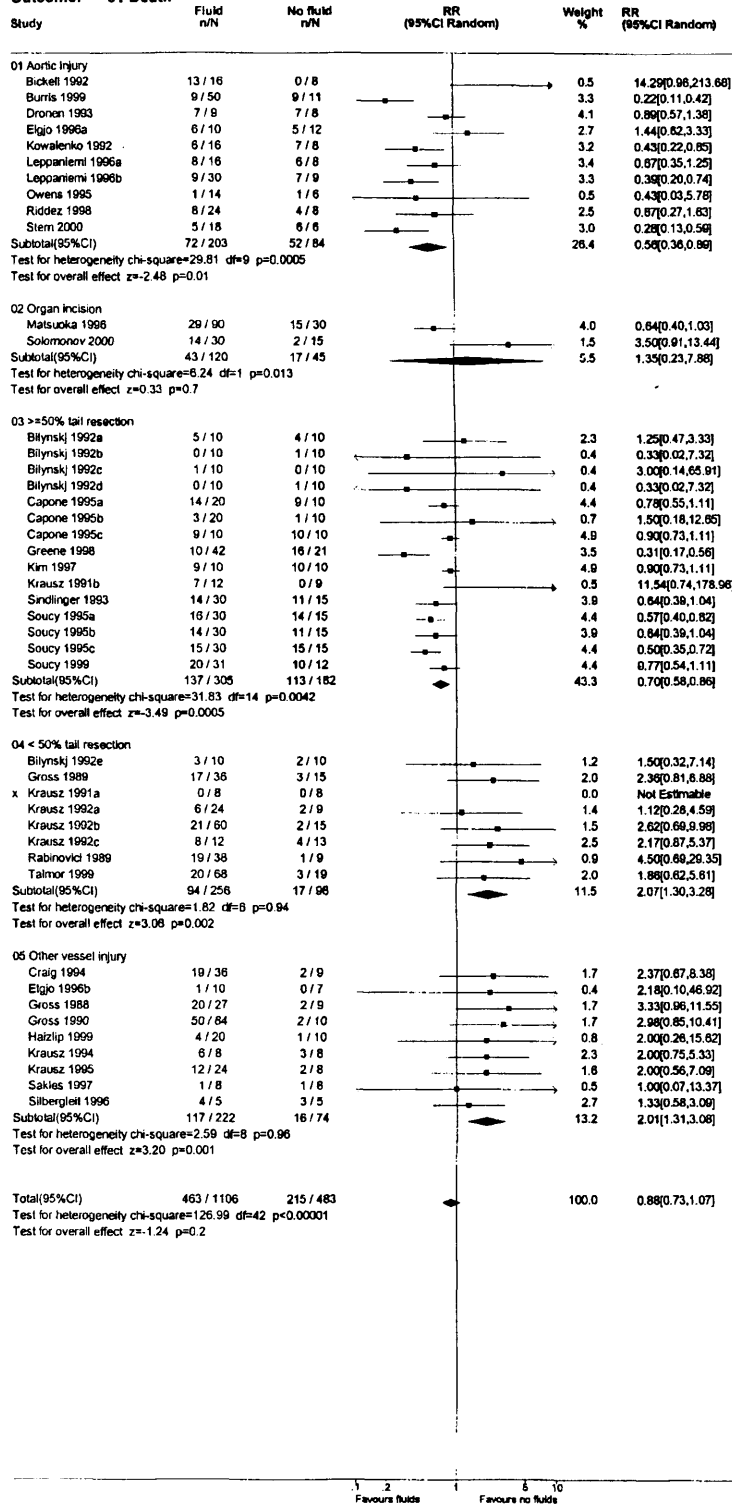


Fig. 2. Risk ratios for the included studies stratified by hemorrhage model.

Review: Animal (for haemorrhage model analysis only)
 Comparison: 08 Ordered by blood loss in the control group
 Outcome: 01 Mortality

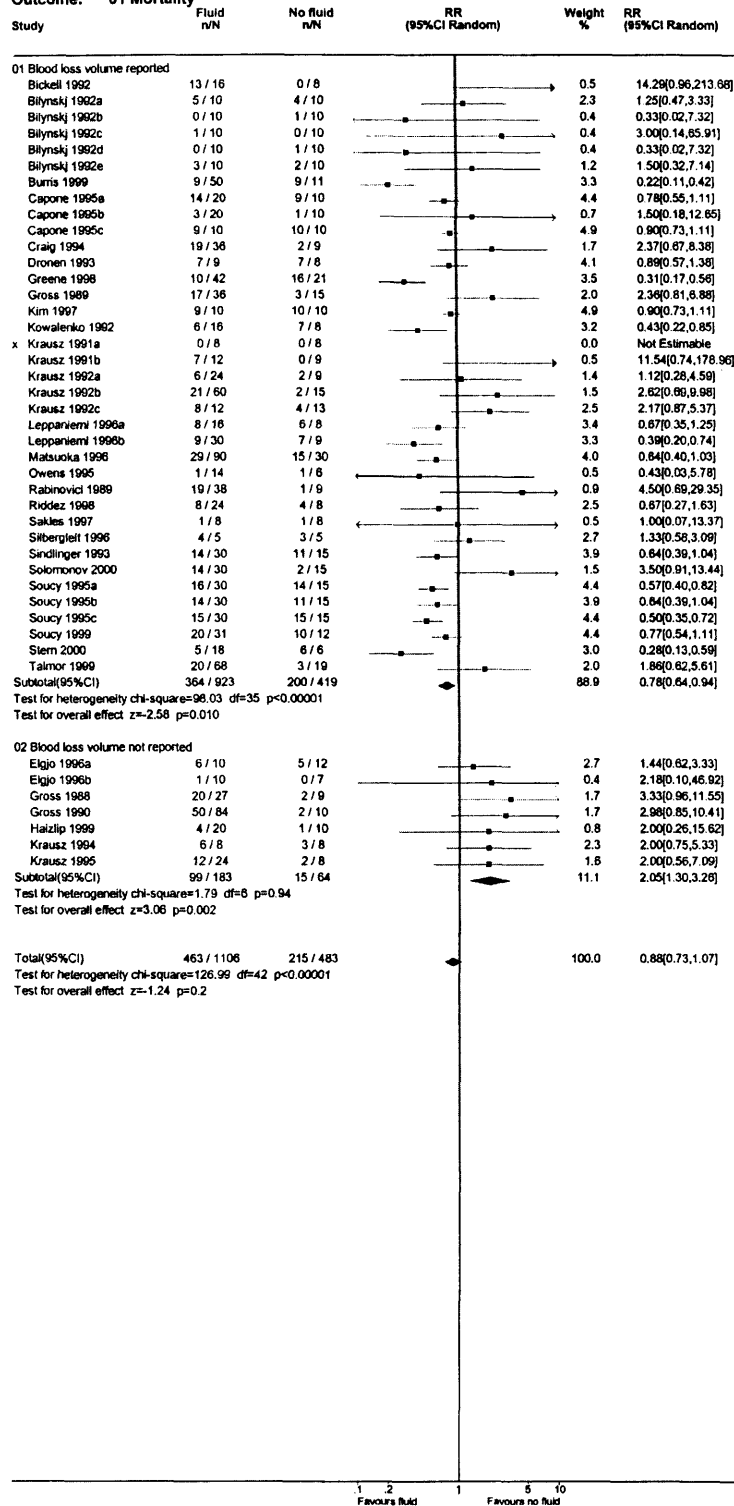


Fig. 3. Risk ratios for the included studies ordered by volume of blood loss reported in the control group.

Table 2 Crude and Adjusted Pooled Risk Ratios of the Risk of Death in Those Resuscitated with Fluid versus Those with No Fluid, Stratified by Hemorrhage Model

Hemorrhage Model	Crude RR (95% CI)	p Value	Adjusted RR (95% CI) ^a	p Value
Aortic injury	0.56 (0.36–0.89)	0.01	0.48 (0.33–0.71)	<0.001
Organ incision	1.35 (0.23–7.88)	0.7	0.76 (0.49–1.18)	0.229
≥50% tail resection	0.70 (0.58–0.86)	0.0005	0.69 (0.38–1.25)	0.221
<50% tail resection	2.07 (1.30–3.28)	0.002	1.86 (1.13–3.07)	0.015
Other vessel injury	2.01 (1.31–3.08)	0.001	1.70 (1.01–2.85)	0.046

^a Adjusted for resuscitation fluid used, time at which mortality was assessed, and the animal used.

Limitations of the Systematic Review

The most fundamental limitation of this study is that the included trials involve animal models, and so the way they relate to real human injuries is unclear. Although the species used did not explain much of the heterogeneity between trials and the pooled risk ratios were adjusted for this, these results cannot necessarily be extrapolated to humans. These animal models do not reflect the complexity of multiple injuries or comorbidities and would not represent the quality of health care a human would experience in hemorrhagic shock. Equally, a wide variety of anesthetics were used, with only two articles directly comparing the effects of anesthetics on small numbers of animals.^{9,45} This was not a prespecified source of heterogeneity and so interpretation is difficult. Different anesthetics have different profiles of cardiovascular and respiratory depression. Depressing these reflexes is likely to result in worse shock for any given fluid infused, compared with a conscious animal or human. It is also likely to result in the need for more fluid to achieve a given fluid target. However, stratification by anesthetic suggested that it was not a major explanatory factor.

Because the outcomes were assessed within the first 3 days, care is required before extrapolating these results to long-term mortality. In humans, it is known that deaths from trauma occur over several weeks, with those dying after a couple of days dying of organ failure and sepsis.⁴ A hypotensive resuscitation strategy could increase these deaths further. However, with improved critical care unit management of organ failure and sepsis, this might be less of a risk in humans. Where possible, data were extracted to be analyzed on an intention-to-treat basis. However, we cannot be certain how individual authors dealt with the issue of animals dying before fluid therapy was begun.

Results from the exploration of heterogeneity should always be treated skeptically. They are equivalent to subgroup analysis within a trial. The overinterpretation of such results needs to be carefully avoided. The results can, at most, be considered as interesting hypotheses for further trials rather than results in themselves.

We used a traditional meta-analysis for the presentation of the subgroup analyses in which individual studies have standard errors and these (together with the between-study variance for Fig. 2) are used to determine the study-specific weights. To deal with zeros, 0.5 is added where necessary.

The study by Bickell et al. in Figure 1 demonstrates the weakness of this approach. The results are 13 of 16 versus 0 of 8, giving a risk ratio of 14.29 (95% CI, 0.96–213.68). The point estimate and upper confidence limit should be infinity, and this highly significant result should not have a confidence interval that encompasses 1. Therefore, the reported risk ratios and confidence intervals for the individual trials should be interpreted with caution where no deaths occurred in either the control or the intervention arm. However, this concern does not carry over to the pooled effect estimates.

Comparison with Human Systematic Reviews

To assess the applicability of an animal systematic review to humans, a comparison with the human literature is useful. A systematic review of randomized, controlled trials investigating the timing or volume of fluid resuscitation in humans provided no evidence to support early or larger volume fluid administration.⁵ The advantage of this systematic review is that it reflects the available evidence in humans. The disadvantage is that it consisted of only five trials, two of which only reported coagulation data. Systematic reviews of other strategies to normalize the blood pressure in bleeding trauma patients all suggest that they could be harmful.⁵² These results provide some reassurance that this animal systematic review may be applicable to humans.

Implications of the Results

This systematic review demonstrates that the effect of fluid resuscitation on the risk of death in animal trials depends on the severity of hemorrhage. If this is applicable to humans, results from fluid versus no-fluid resuscitation trials may be highly dependent on the exact nature of the injuries in the recruited participants.

A large number of people could potentially be saved if hypotensive resuscitation proved to be of benefit in humans. If it led to just a 5% absolute risk reduction (the animal studies demonstrated a 41% absolute risk reduction) from 20% to 15% and only 50% of the 3 million people dying each year from hemorrhagic shock could be resuscitated to a normotensive target, this would mean an extra 375,000 people per year could survive.

It seems a reasonable hypothesis that as the trials of hypotensive versus normotensive resuscitation gave consistent results, the same might occur with human trials as well.

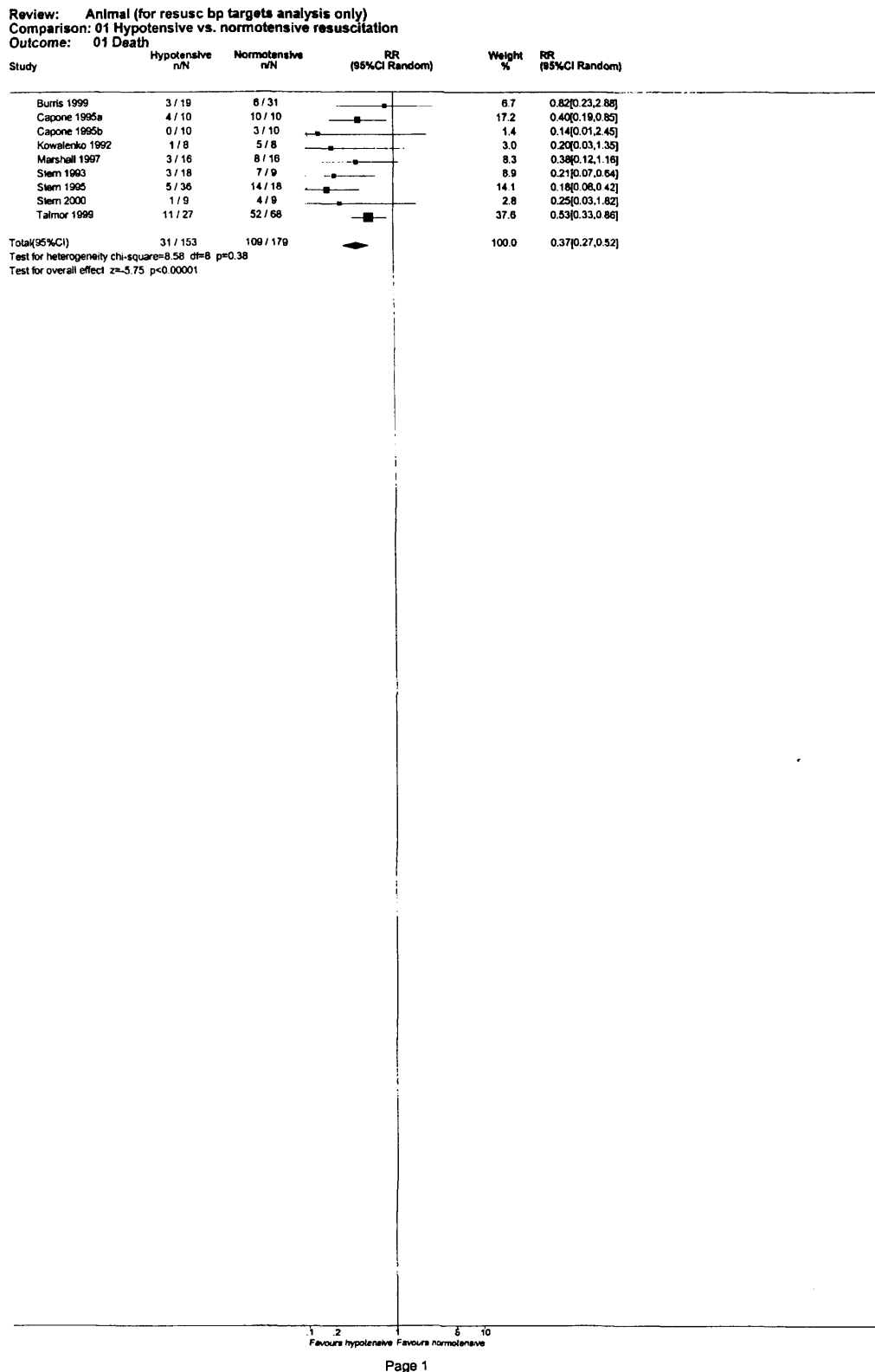


Fig. 4. Risk ratios for the studies comparing hypotensive versus normotensive resuscitation.

The human systematic reviews suggesting potential harm of blood pressure normalization strategies add to the need for trials in this area. Discovering acceptable blood pressure targets in patients of different ages with different comorbidities that lead to long-term mortality and morbidity reduction could be useful.

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06 Efficacy of substitution therapy with PPSB concentrate in intensive care patients

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Introduction: Substitution of clotting factors is one of the most costly therapies in ICU treatment. However, efficacy of such treatment in clinical practice is poorly investigated. In a multi-centre trial we evaluated if substitution of PPSB-concentrate is able to raise clotting parameters.

Methods: In a multi-centre observational trial in 27 Austrian Hospitals and following ethics committee approval 280 patients (median age 58 [6–93]) were included in the study. During the observation period all patients requiring substitution with PPSB concentrate were included. No trigger-levels and no prescribed dosage was defined but patients received a PPSB-concentrate containing the vitamine K dependent factors II, VII, IX, X and the vitamin-dependent inhibitors C and S (Prothromplex® Total S-TIM 4, Baxter) as

regarded clinically necessary by the physician concerned. Change in coagulation parameters, dosage applied, and adverse side-effects were registered. Statistical analysis based on the intention-to-treat principle while using the last value technique.

Results: Median dose applied was 21.4 IU/kg and lead to a median rise in Quick test from 38 to 62%. No side effects were reported. The increase was clinically sufficient in all cases and no further substitution was required.

Conclusion: For the first time in a representative number of patients we showed that the substitution of 1 IU/kg bodyweight PPSB concentrate raised Quick test by about 1%. Use of PPSB concentrate is save and no side effects, especially thrombosis were reported.

P107 Assessment of rVlla as a universal haemostatic agent in a model of haemodilution

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Haemostatic failure, secondary to large volume fluid replacement, is a major component to the mortality and morbidity associated with blunt trauma. Progressive bleeding in multiply injured patients is due to both dilution effects and specific inhibitory effects on platelet function of the colloids used. Recombinant factor Vlla (rVlla) is seen increasingly as a possible universal haemostatic agent that could act to reverse or prevent haemostatic failure associated with dilution and the direct effects of the colloids within the 'Golden' hour of haemorrhagic shock.

We have conducted a pilot preclinical study to evaluate the potential role of rVlla as a universal haemostatic agent in a model of large volume fluid replacement using thrombelastography (TEG). TEG is a method of global haemostasis assessment, providing information on the rate of clot formation, clot strength and durability.

Whole blood samples from normal donors were tested undiluted (100%) or diluted (50% and 80%) with standard colloid replacement solutions (Haemacel, Albumin, Gelofusine, Hydroxyethyl starch) and N/Saline. Global haemostasis was assessed in the TEG, $\pm 90 \mu\text{g/kg}$ rVlla added. In undiluted blood (100%) there were no statistically significant changes in any TEG parameter when rVlla was added. At dilutions of $> 50\%$ addition of rVlla significantly improved the kinetics of clot formation and rate of platelet reactivity, $P < 0.05$, although time to the start of coagulation and final clot strength were not significantly different. The beneficial effects of addition of rVlla did not differ between different fluid replacement solutions.

Addition of rVlla therefore appears to improve markers of global haemostasis in this model of large volume fluid replacement. Further work is required to assess its potential value as a universal haemostatic agent in the setting of blunt trauma and large volume fluid replacement.

P108 Measurement of serum transferrin receptor (sTfR) in critically ill patients

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Introduction: Measurement of sTfR is an important new hematological parameter. Laboratory studies have indicated that sTfR values are elevated in anemias associated with enhanced erythropoiesis and tissue iron deficiency only. The aim of this study was to demonstrate whether iron deficiency, expressed through elevations of sTfR, constitutes an important etiologic component of ICU patients' anemia.

Methods: Twenty-seven patients were studied (10 male, 17 female), mean age 65.4 ± 4.0 years and mean APACHE II score of 16.5 ± 1.0 . Five patients had sepsis syndrome, 12 severe sepsis, 8 had septic shock and 2 patients suffered from multiple organ failure. Patients who presented with a bleeding episode were excluded from the study. We measured sTfR, hemoglobin, hematocrit, serum iron concentration and ferritin and calculated APACHE

II and sepsis score on day 1, 4 and 8 of ICU stay. For the measurement of sTfR, monoclonal antibodies were used.

Results: All critically ill patients had sTfR values at the lower level of the normal range ($0.93 \pm 0.5 \text{ mg/l}$, with normal values ranging between 0.94 and 1.28 mg/l). Though all sTfR values were found in the normal range, variations of transferrin receptors were correlated to sepsis score, hemoglobin and hematocrit on corresponding days. Statistical analysis revealed no significant difference.

Conclusion: Investigation of anemia in critically ill patients includes bone marrow examination for iron status determination. This invasive procedure can now be substituted by transferrin receptors' measurement, since ferritin levels are not reliable in septic patients, as it is the case with all acute phase proteins.

Methods: The anti-Xa kinetic (0, 1, 3, 6, 12 hours) following 40 mg of enoxaparin SC was investigated in 16 ICU patients (group 1; age 61.1 ± 16 years; m/f 7/9, APACHE II 20.9 ± 7 , mechanical ventilation $n=15$, vasopressors $n=13$) and 13 non critically ill patients on the general ward (group 2; age 61.7 ± 9 years, m/f 7/6) requiring prophylactic anticoagulation. Patients with impaired renal function or requiring hemofiltration and those requiring therapeutic anticoagulation were not eligible.

Results: Mean anti-Xa levels were consistently lower in group 1 vs group 2 on ANOVA ($P=0.001$ between groups and over time) as

was the $AUC_{0-12 \text{ hours}}$ (2.6 ± 1 vs 4.2 ± 1.7 U/ml*h, group 1 vs 2, $P=0.008$). BMI (25.7 ± 5 vs 24 ± 6 kg/m²) and creatinine clearance (67.5 ± 31 vs 67.7 ± 27 mg/dl) were comparable in both groups ($P=ns$). The peak anti-Xa level 3 hours after administration was negatively correlated to the BMI ($r=-0.41$, $P<0.03$) and the norepinephrine dose ($r=-0.36$, $P=0.056$).

Conclusion: It is cautiously concluded that the SC administration of established doses of prophylactic enoxaparin might not be appropriate in the critically ill patient requiring vasopressor support and mechanical ventilation.

P127 Follow up study in the assessment of rVIIa as a universal haemostatic agent in a model of haemodilution

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Introduction: Large volume fluid replacement to treat haemorrhagic shock can result in haemostatic failure due both to a dilution effect [1] and an intrinsic effect [2,3]. Recombinant factor VIIa (rVIIa) is seen increasingly as a possible universal haemostatic agent that could act to reverse or even prevent the dilution and intrinsic effects of fluids used within the 'golden' hour of haemorrhagic shock. Our preliminary findings demonstrated that rVIIa appeared to improve markers of global haemostasis in a model of large volume fluid replacement. This follow up study assessed what effects rVIIa had on global haemostasis and the electron microscopic appearances of clot formation when haemaccel or sodium chloride were used as diluents to create a model of large volume fluid replacement.

Methods: One hundred whole blood samples from normal donors were tested undiluted, or diluted to 50% and 80% using haemaccel or sodium chloride (NaCl). Each sample was tested with and without addition of 90 µg/kg of rVIIa. Global haemostasis was assessed using thrombelastography (TEG). Parameters measured were: Time to initial fibrin formation (R), Time to 20 mm clot amplitude (K), Rapidity of fibrin build up and cross-linking (α), Maximum clot amplitude (MA), Time to MA (TMA), and Clot firmness (G). Haemostatic testing was terminated when maximum clot amplitude was reached and samples were then examined by electron microscopy.

Results: One hundred samples analysed. Twenty samples for each of five fluid groups. Each sample tested \pm rVIIa. Without addition of

rVIIa, an intrinsic effect with worsening of TEG parameters was noted with increasing dilution in the fluid groups. Addition of rVIIa produced significant improvement in TEG parameters: $P<0.05$ R, K, α , TMA for all fluid groups; $P<0.05$ MA and G for 80% Haem, 50% and 80% NaCl. Moreover with increasing dilution there was a greater relative improvement in TEG parameters in rVIIa added groups. When fluid groups were compared to each other to see if the beneficial effects of rVIIa produced a difference, no significant difference was found between groups except for 50% haemaccel compared with 50% NaCl where K, MA and G were significantly better ($P<0.05$) in the haemaccel group. Electron microscopy demonstrated a reversal of both the dilution and intrinsic inhibitory effects, with increased fibrin deposition and meshwork with greater cross-linking following addition of rVIIa.

Conclusion: In this *in vitro* model of large volume fluid replacement with associated haemodilution, the addition of rVIIa appeared to improve markers of global haemostasis and caused increased fibrin deposition with a tighter resultant meshwork on electron microscopy. Further work is required to assess the potential value of rVIIa as a universal haemostatic agent in trauma settings involving large volume fluid resuscitation.

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P128 Recombinant activated factor VII (rFVIIa-NovoSeven®-NovoNordisk) treatment of bleeding complications in intensive care unit

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Recombinant activated factor VII (rFVIIa-NovoSeven®-NovoNordisk) was first developed to treat severe bleeding episodes occurring in patients with haemophilia A or B with inhibitor. Complexed with tissue factor, it activates the factor X and allows transformation of prothrombin into thrombin, independently of factors VIII and IX. The success of rFVIIa in controlling haemophilic bleedings has led to use it, punctually for other serious bleedings in liver transplantation, cardiac surgery or in cirrhotic patients.

We report five cases of patients hospitalised in intensive care unit, with haemorrhagic shock or severe haemostasis disorder. Despite of massive transfusions and usual resuscitation therapeutics, their clinical course was pejorative. Then, they were given a 90 µg/kg rFVIIa intravenous dose. See Table overleaf.

A dramatic improvement occurred in a few hours. The majority of haemorrhages stopped after one or two rFVIIa doses administration. The immediate correction of haemostasis disorders allowed a reduction in blood transfusions amount and a decreasing of amine support requiring. One patient presented a thrombosis of the portal venous system quickly corrected, but no other adverse event could be attributed to rFVIIa.

rFVIIa-NovoSeven® appears to be of great interest in the treatment of uncontrolled haemorrhage in intensive care units. It can be considered as an efficient and safe therapy. Nevertheless, because of its high price and of the very preliminary nature of the data we report, further investigations are necessary before using this product as a routine treatment.

Assessment of rVIIa as a universal haemostatic agent in an *in vitro* model of Haemodilution

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Introduction

Haemostatic failure, secondary to large volume fluid replacement in trauma, is a major contributor to the morbidity and mortality associated with trauma. Volume replacement in the shocked patient, especially with colloids, has been suggested to increase mortality.^{1,2,3} Previous studies have suggested that in combination both the volumetric and intrinsic qualities of these colloids may lead to ongoing haemorrhage by their effects upon the haemostatic process.^{4,5,6,7,8,9,10}

In the setting of major trauma. Advanced Trauma Life Support (ATLS) guidelines on hypovolaemic shock require restoration of the circulating volume to ensure adequate tissue perfusion. Several authors have emphasised that the intrinsic properties of these colloids directly affect haemostasis by affecting clot quality when measured by the thrombolystogram^{4,5,6,8,9}

Recently, new therapeutic options have become available which may give the clinician the opportunity to improve clot quality early on and thereby reduce the risk of further haemorrhage. Such options could have the potential to overcome both the volumetric and intrinsic effect of these early resuscitative fluids, prior to definite treatment. Amongst these is recombinant Vila (NovoSeven). Recombinant Vila is a clotting factor that complexes with tissue factor (TF) and initiates coagulation with the production of thrombin¹¹ which ultimately leads to fibrin formation and a stable blood clot. Although rVIIa is licensed for use in patients with inhibitors to clotting factors VIII and IX¹², it is increasingly seen as a possible universal haemostatic agent. Anecdotal reports in the literature have shown infusion of rVIIa to be effective at arresting severe haemorrhage in the context of a major trauma,^{13,14,15,16} unremitting gastrointestinal haemorrhage^{17,18} and post surgical bleeding.^{19,20,21} Mechanisms of its haemostatic action in such situations is not entirely clear but may be due to a combination of inhibition of TFPI pathways and enhancement of thrombin generation on platelet surfaces.^{22,23,24}

The majority of the experience to date is case report based²⁵ and there is limited experimental data that shows that recombinant Vila has a role in preventing or treating the multifactoral haemostatic failure that occurs secondary to large volume fluid replacement in the setting of major trauma or major blood loss.

Our study aimed to create a "worst case" scenario to determine whether rVIIa is able to improve both the volumetric and intrinsic effects of two common, resuscitative fluids. An *in vitro* model was constructed using the thrombolystogram to assess global haemostasis. Our model uses crystalloid and colloid (gelatins), which have previously been reported as affecting clot quality,^{26,27} dilutions much larger than previously used before, no TF and the currently licensed dose of rVIIa. Improvement in clot quality was also assessed using scanning electron microscopy to determine the effect of rVIIa upon clot quality and structure.

Materials and Methods

Sample collection

Ethical approval was granted from the Local Research Ethics Committee. Venous blood was collected from 20 normal donors who did not have any pre existing conditions and who were currently not on any medication that was likely to affect coagulation e.g. aspirin, NSAIDs. Blood was drawn into 3.8% trisodium citrated at a 1:10 ratio.

Dilutional model

Dilutions for the model were ascertained by reviewing the typical total volumes of fluids that patients received following major trauma within the preop and operative phase within our institution. A review of the trauma database showed that people with an ISS (Injury Severity Score) >16 received a fluid load that equated to approximately 80% haemodilution. Patients with ISS <16 received around a 50% haemodilution. Haemaccel (3.5% w/v polygeline) and Sodium Chloride (0.9% w/v) were chosen for the model as typical of the type of fluids that trauma victims would receive within a typical Accident and Emergency Department when the ATLS guidelines are followed.²⁸

rVIIa (NovoNordisk, Pease Pottage, UK) was reconstituted as per manufacturers instructions and then further diluted to a give final concentration of 90ug/kg in 6 ul in the test system.

Blood samples were divided into five categories for testing; undiluted blood, blood diluted with Haemaccel (Beacon Ltd) to 50% and 80% dilutions, and blood diluted with 0.9% NaCl (Baxter Healthcare UK) to 50% and 80%. Each sample was freshly prepared at the time of testing.

Thrombelastography.

Global haemostasis was assessed using the Haemoscope TEG analyser 5000 series (Medicell, London). For testing, plastic cups (total volume 360ul) were placed in cup wells and 334ul of sample were added to the cups. Coagulation was initiated by the addition of 20ul 0.2M calcium chloride +/- 6ul rVIIa

Cups were then oscillated in the instrument through an angle $4^{\circ} 45'$, each rotation lasting 10 seconds. As the clot formed the developing fibrin platelet bonds linked the torque of the rotating cup to a pin suspended in the sample figure 1. The strength of these fibrin platelet bonds affected the magnitude of the pin motion, such that strong clots moved the pin directly in phase with the cup motion. As the clot lysed, the bonds were broken and the transfer of cup motion was diminished. Rotational movement of the pin was monitored and interpreted by a computer, to produce a haemostatic profile both graphically see figure 2 and numerically. The derived values provide information on the rate of clot formation, clot strength and durability. Parameters measured were TMA, G, K and α angle (see figure 2).

At the point when maximum clot amplitude was reached, testing was terminated, the cups were removed from the TEG analyser and blood samples tipped into 4% glutaraldehyde solution for fixation prior to examination by scanning electron microscope.

Figure 1. Diagram illustrating the mechanics of TEG operation.²⁹

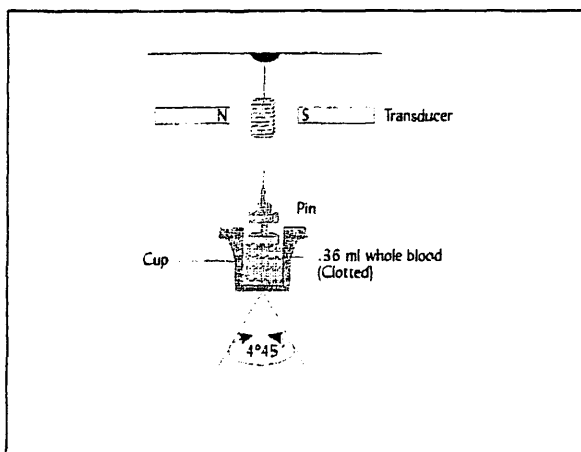
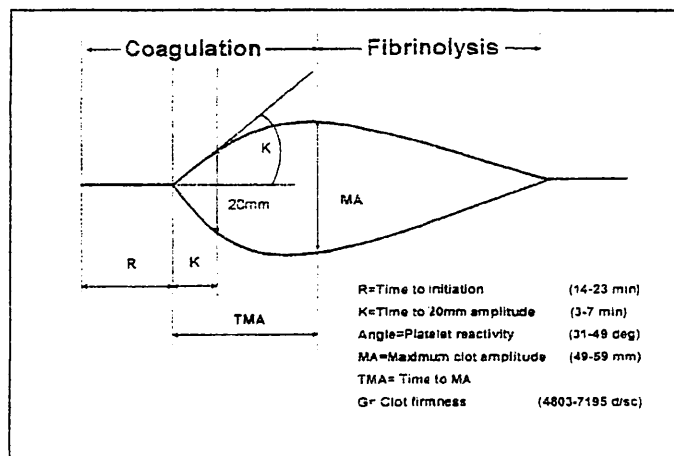


Figure 2. A normal TEG tracing. Phases of coagulation are outlined and derivation of parameters. Normal ranges are given in parenthesis.²⁹



Scanning Electron Microscopy

Jeol 100 CX electron microscope was utilised to allow examination of the clot ultrastructure. Samples were initially fixed and dehydrated using Hexamethyldisilazane chemical dehydration process. Dehydrated samples were mounted onto a stub, which enabled the sample to be held tightly in the rod to facilitate introduction to the electron beam. Stubs, with attached samples, were sputter coated with a fine layer of a gold/palladium mix. Coating with a metal layer is necessary to allow the secondary electrons to be "bounced" back off the surface of a sample and be then collected by the electron microscope detector to produce the scanning electron microscopic image. All images were taken at the same resolution; photography using Ilford FP4 roll film was used to keep a record of the images.

All SEM images obtained in this study and included in the summary showed that there was a direct correlation with the corresponding results obtained on the thrombolystogram. Both the intrinsic and dilutionary effects of the recussitation fluids were clearly seen and matched in both the TEG and the SEM. Furthermore the clear therapeutic effect of the FVIIa of overcoming the individual effects of these fluids was clearly seen in both the TEG and the SEM. The images included in the summary were those which best illustrated these effects

Statistical Analysis

Data analysis was conducted using SPSS v11.0 except for confidence intervals that used Altmans' computer package.³⁰ Data was mixed between normal and non-normal, skewed distribution. Wilcoxon Signed Rank Test was used as the data was classed as being paired and nonparametric. P values were calculated and were deemed to be statistically significant with values of $p < 0.05$. Group differences were calculated and then compared to whole blood as percentage change to allow a clinically relevant comparison. All values were rounded up to one decimal place and are medians.

Results

Effect of diluent

All parameters were effected by dilution; the greater the dilution, the greater the negative effect upon coagulation (clot formation and clot quality). Parameters affected the most were G (table & graph d) and K (Table & graph a); at 80% NaCl dilution G was reduced by 74% and K was reduced by 123%.

Type of Diluent

Diluents had differing effects upon coagulation; NaCl had a greater negative effect upon blood than Haemaccel. G (table & graph d) was reduced by saline by 46% compared to 8% in the Haemaccel group (50% dilution group).

Effect of rVIIa

In all cases, at all dilutions, rVIIa improved the derived parameters although the improvement did not return the diluted parameters back to those of whole blood. In undiluted whole blood, addition of rVIIa increased the coagulability of the blood, the largest percentage changes occurred in the clot kinetic parameters, a (table & graph b) and K (table & graph a), 20% ($p < 0.008$) and 22% ($p < 0.001$) respectively. Overall measurements of clot formation, G (table & graph d) and TMA (table & graph c) were also improved but not

to the extent of the clot lysis.

At the varying dilutions it was the clot kinetic measurements that showed the greatest improvement upon the addition of rVIIa. K (table & graph a) showed the greatest improvement with addition of rVIIa and also showed the greatest proportional improvement with increasing dilution. At 50% dilution the improvement was 29% ($p<0.003$) for Haemaccel and 16% ($p<0.001$) for NaCl, with an increase to 57% ($p<0.001$) and 67% ($p<0.05$) improvement at 80% dilution respectively. Proportional improvements were similar at fixed dilutions irrespective of the diluent. Haemaccel, whilst benefiting from rVIIa, did so to a lesser extent in comparison with saline, albeit the differences were small. K (table & graph a) always improved to a greater degree than a (table & graph b) especially at the higher concentrations, which saw improvements ranging from 57% ($p<0.001$) to 67% ($p<0.005$) dependant upon the diluent.

Electron Microscope Results

Improvement in thrombus formation following addition of rVIIa is also shown qualitatively in scanning electron microscopic examination of samples. All of these samples were taken at the same resolution and the trends seen in these pictures were seen throughout the series. Representative images are shown in figures 1a to 5b. For undiluted blood, control without addition of rVIIa (figure 1a), the fibrin network appeared looser and diminished compared with the rVIIa added sample (figure 1b), where the fibrin meshwork was more abundant with greater cross linking. Ultra structural appearance of the 50% (figure 2a) and 80% (figure 4a) Haemaccel samples also demonstrated a looser fibrin meshwork, which was reversed with the addition of rVIIa producing a tighter, denser meshwork (figure 2b, 4b). Similar appearances were seen for 50% (figure 3a-b) and 80% NaCl (figure 5a-b).

Parameter K

<u>Fluid Type</u>	<u>Actual Value</u>	<u>Treatment Effect</u>	<u>P value</u>	<u>95% Confidence Intervals</u>	<u>Diff to WB (Actual Value)</u>	<u>% Diff to WB</u>	<u>% improvement by adding rFVIIa to the diluent</u>
WB	6.5						
WB with rVIIa	5.1	1.4*	<0.001	0.6 to 1.6	1.4*	21.7*	21.7*
50% Haemacell	5.8				0.7*	4.8*	
with rVIIa	4.3	1.5*	<0.003	0.6 to 2.1	2.2*	33.3*	28.5*
50% NaCl	6.1				0.4*	5.4*	
with rVIIa	5.1	1*	<0.001	0.8 to 2.1	1.4*	20.9*	15.5*
80% Haemacell	12.1				-5.6**	-86.8**	
with rVIIa	8.4	3.7*	<0.001	1.9 to 5.1	-1.9**	-30.2**	56.6*
80% NaCl	14.4 n=11				-7.9**	-122.5**	
with rVIIa	10.1 n=13	4.3*	<0.05	-0.1 to 7.2	-3.6**	-55.8**	66.7*

Results Table A

*Improved

**Worse

<u>Parameter Angle</u>							
<u>Fluid Type</u>	<u>Actual Value</u>	<u>Treatment Effect</u>	<u>P value</u>	<u>95% Confidence Intervals</u>	<u>Diff to WB (Actual Value)</u>	<u>% Diff to WB</u>	<u>% improvement by adding rFVIIa to the diluent</u>
WB	31						
WB with rVIIa	37.3	-6.3*	<0.008	-7.6 to -1.6	-6.3*	-20.2*	20.2*
50% Haemacell	34.5						
with rVIIa	41.3	-6.8*	<0.006	-8.9 to -2.2	-3.5*	-11.3*	21.8*
50% NaCl	31.8						
with rVIIa	37	-5.3*	<0.001	-8.7 to -4.2	-0.8*	-2.4*	17*
80% Haemacell	20						
with rVIIa	27.5	-7.5*	<0.001	-8.4 to -3.6	11**	35.9**	24.6*
80% NaCl	17.3						
with rVIIa	23	-5.8*	<0.004	-10.4 to -2.1	13.7**	44.4**	18.6*

Results Table B.

*Improved

**Worse

Parameter TMA

<u>Fluid Type</u>	<u>Actual Value</u>	<u>Treatment Effect</u>	<u>P value</u>	<u>95% Confidence Intervals (based on median values)</u>	<u>Diff to WB (Actual Value)</u>	<u>% Diff to WB</u>	<u>% improvement by adding rFVIIa to the diluent</u>
WB	30.3						
WB with rVIIa	29.6	0.7	<0.04	-0.8 to 7.6	0.7*	2.3*	2.3*
50% Haemacell with rVIIa	26 23.2	2.8	<0.02	0.5 to 3.6	4.3* 7.1*	14.4* 23.6*	9.2*
50% NaCl with rVIIa	27.1 26	1.2	<0.001	0.7 to 2.3	3.2* 4.3*	10.7* 14.5*	3.8*
80% Haemacell with rVIIa	26.8 25	1.8	<0.001	0.1 to 2.2	3.5* 5.3*	11.7* 17.7*	6*
80% NaCl with rVIIa	25.8 23.9	1.9	<0.05	0.3 to 4.2	4.5* 6.4*	14.9* 21.1*	6.2*

Results Table C

*Improved

**Worse

Parameter G

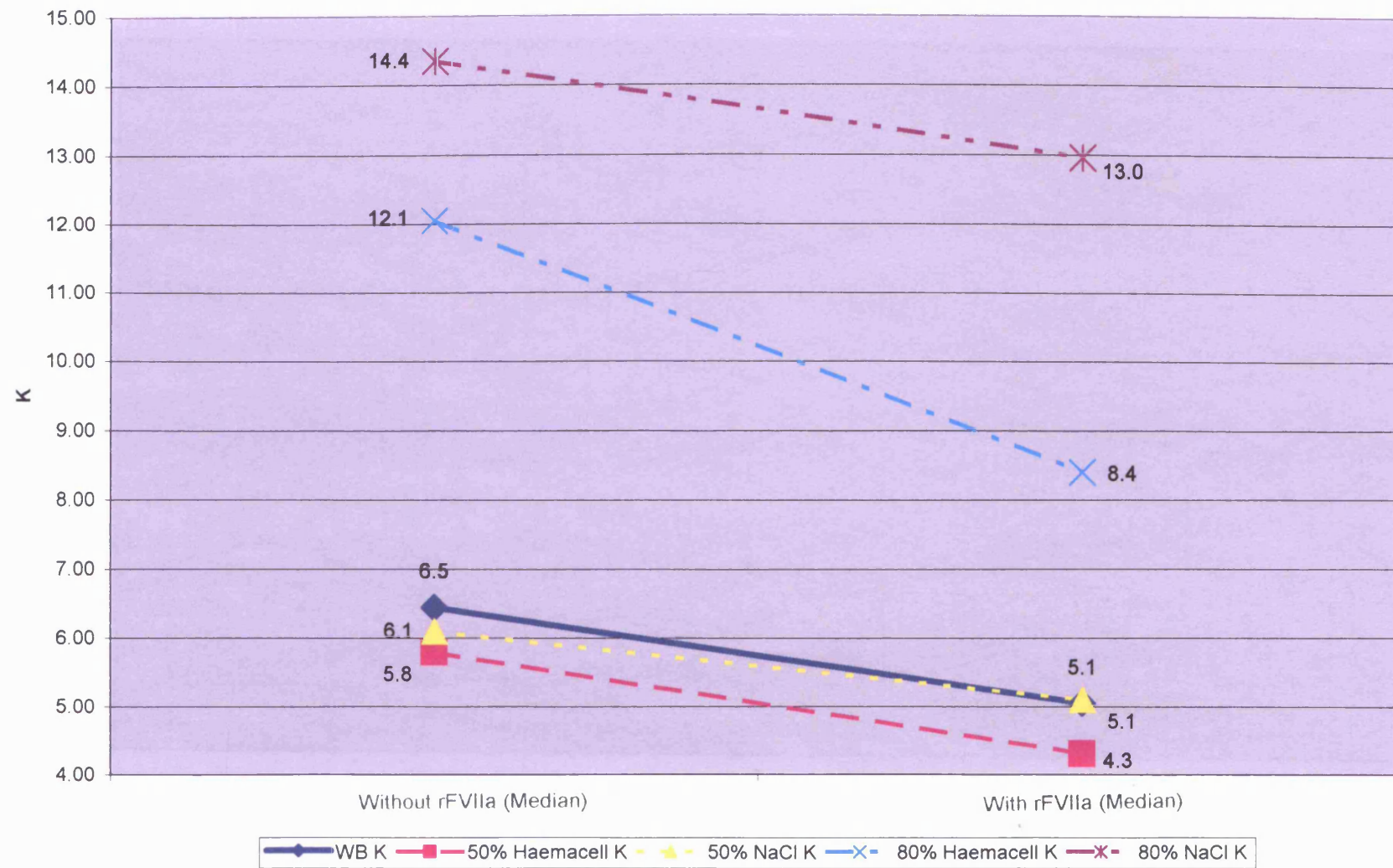
Fluid Type	Actual Value	Treatment Effect	P value	95% Confidence Intervals (based on median values)	Diff to WB (Actual Value)	% Diff to WB	% improvement by adding rFVIIa to the diluent
WB with rVIIa	5313.7	-312.7*	<0.3	-162.3 to 483.3	-312.7*	6.3*	6.3*
50% Haemacell	4615.4				386**	7.7**	
with rVIIa	4569.6	45.8**	<0.6	-296.7 to 227.5	431.4**	8.6**	-0.9**
50% NaCl	2692.8				2308.3**	46.2**	
with rVIIa	2874	-181.2*	<0.001	162.9 to 352.9	2127**	42.5**	3.7*
80% Haemacell	1666.95				4147.6**	66.7**	
with rVIIa	1920.5	-253.6*	<0.001	104.4 to 301	3080.5**	61.6**	5.1*
80% NaCl	1289.6				3711.4**	74.2**	
with rVIIa	1349.3	-59.7*	<0.008	49.7 to 217.2	3651.8**	73**	1.2*

Results Table D

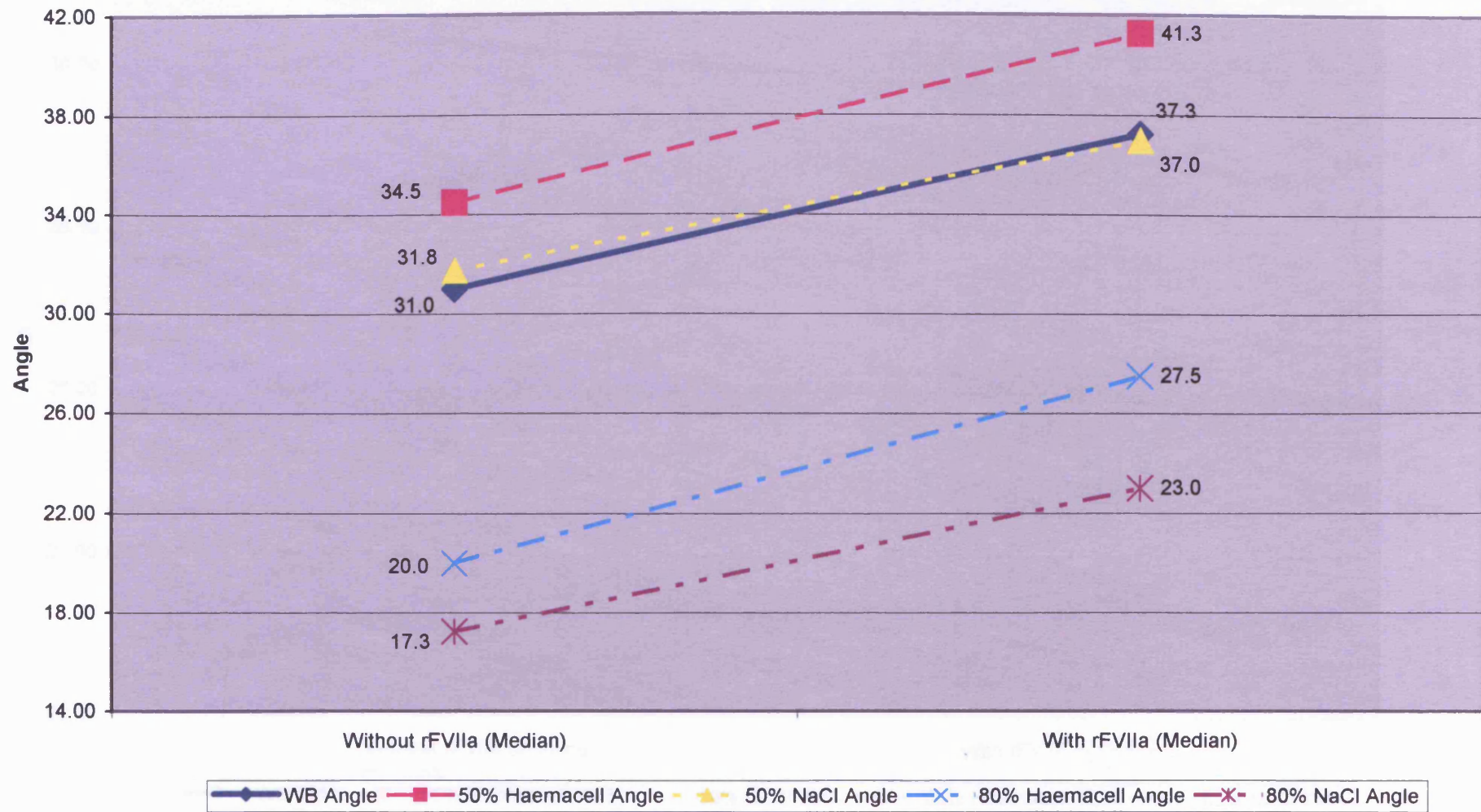
*Improved

**Worse

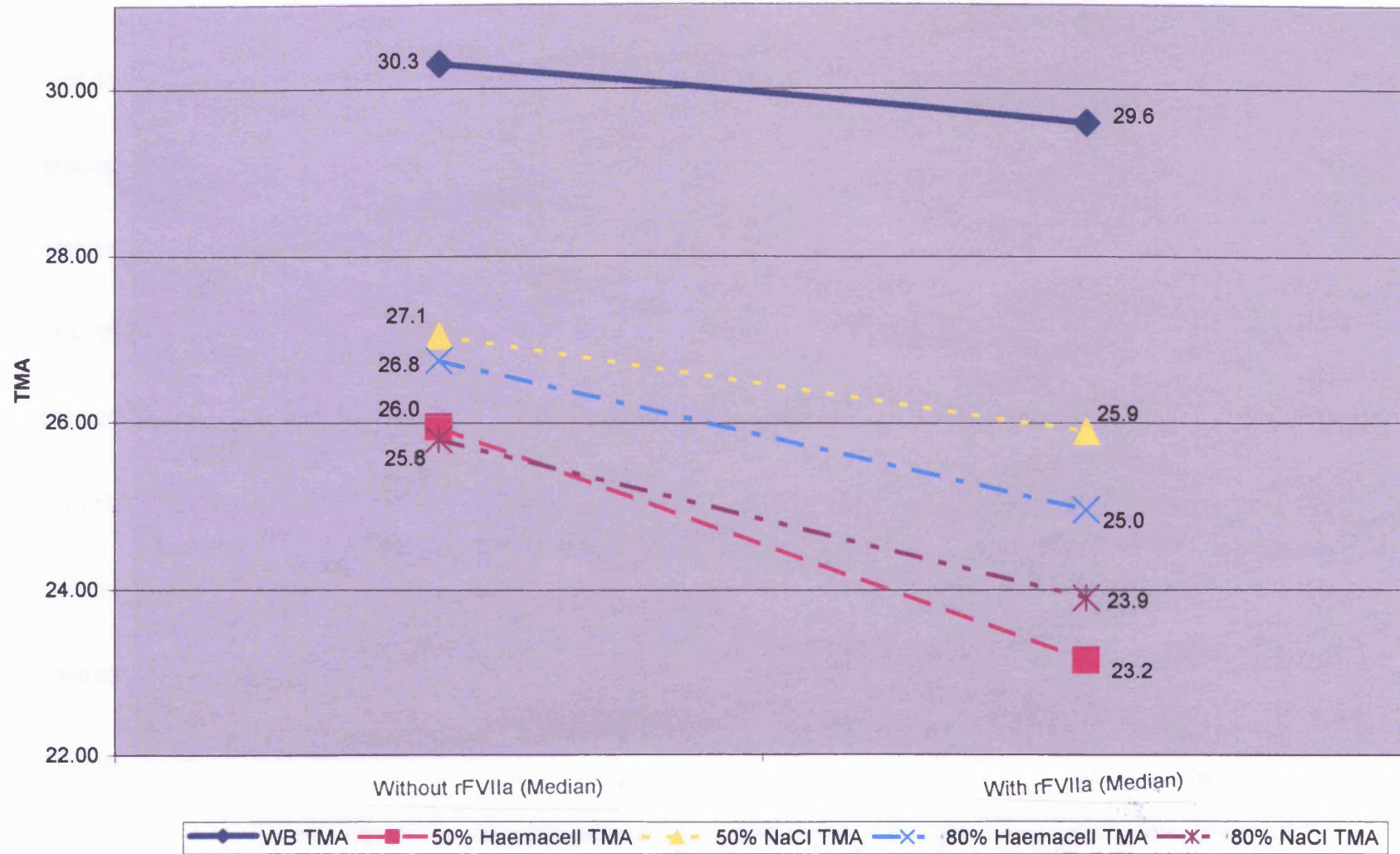
Graph A. Effect of NovoSeven Upon TEG K Value.



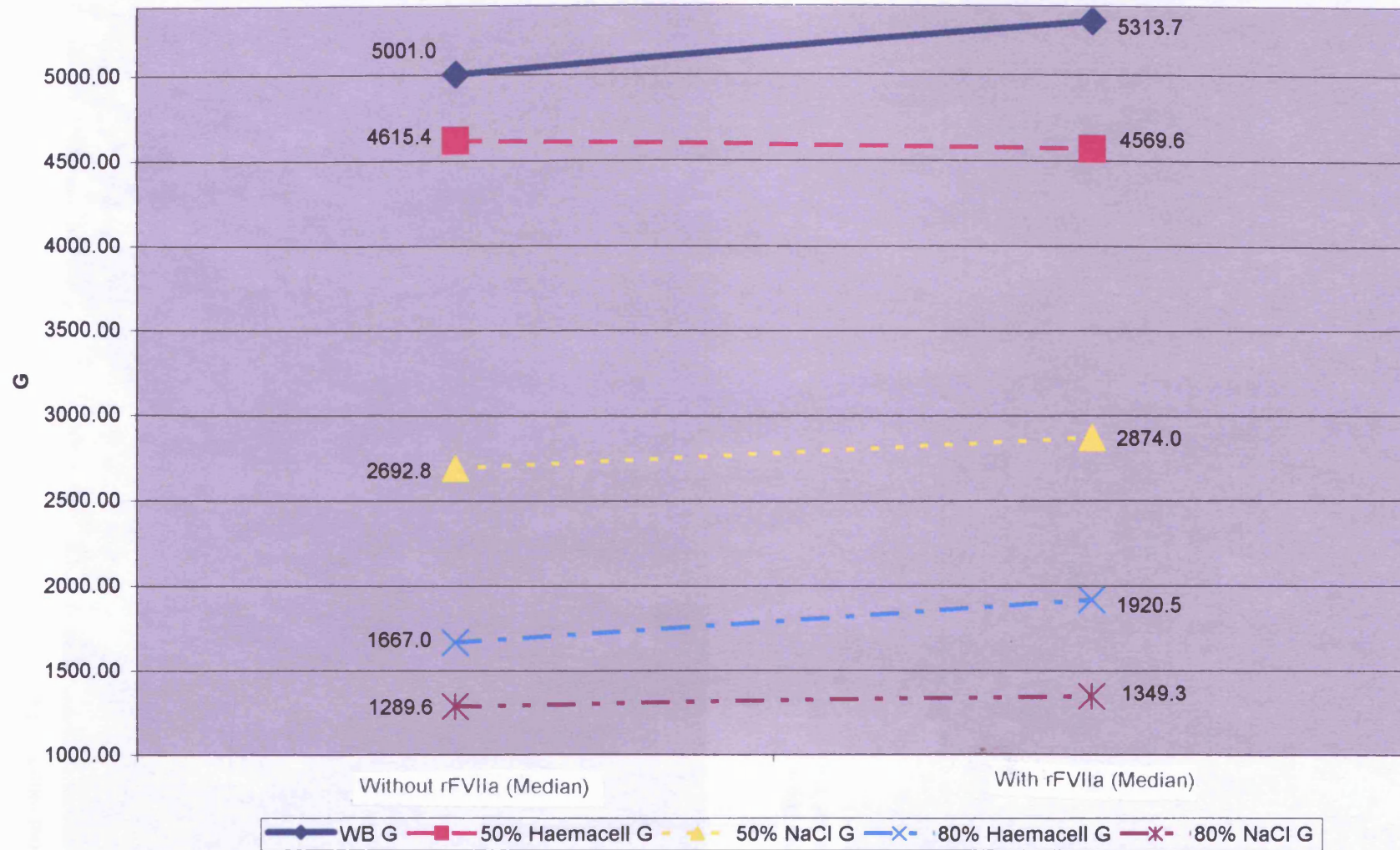
Graph B. Effect of NovoSeven Upon TEG Angle.



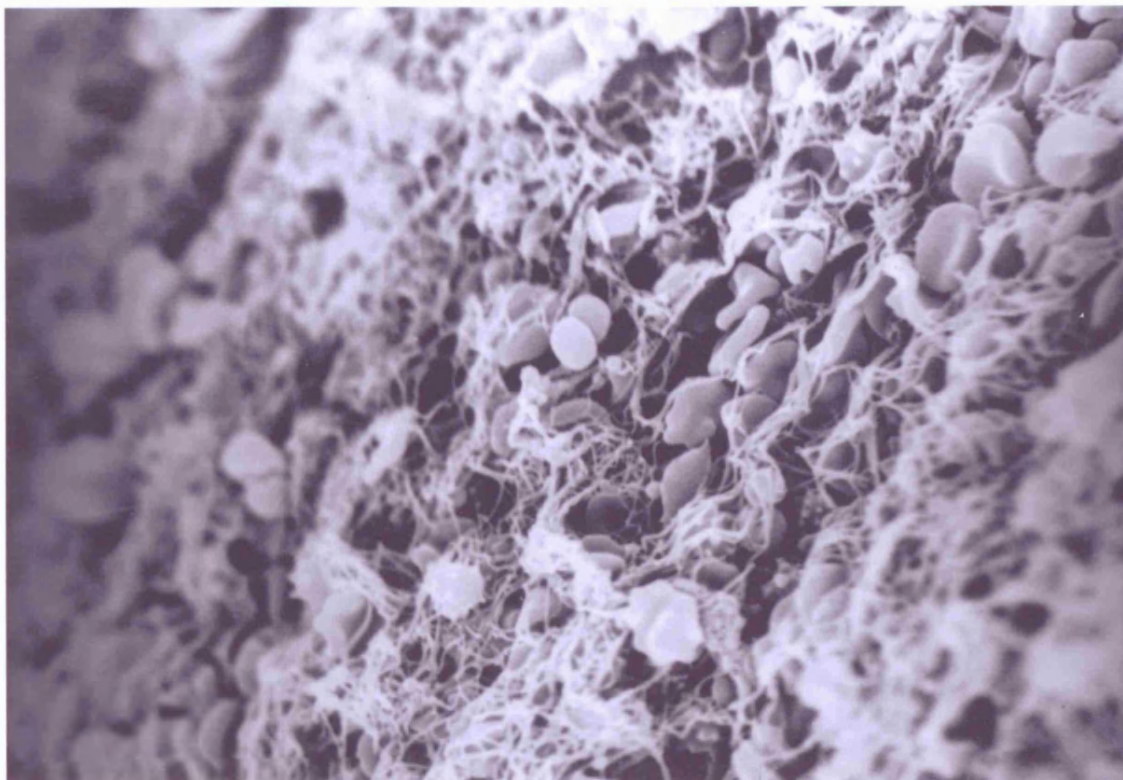
Graph C. Effect of NovoSeven Upon TEG TMA.



Graph D. Effect of NovoSeven Upon TEG G.



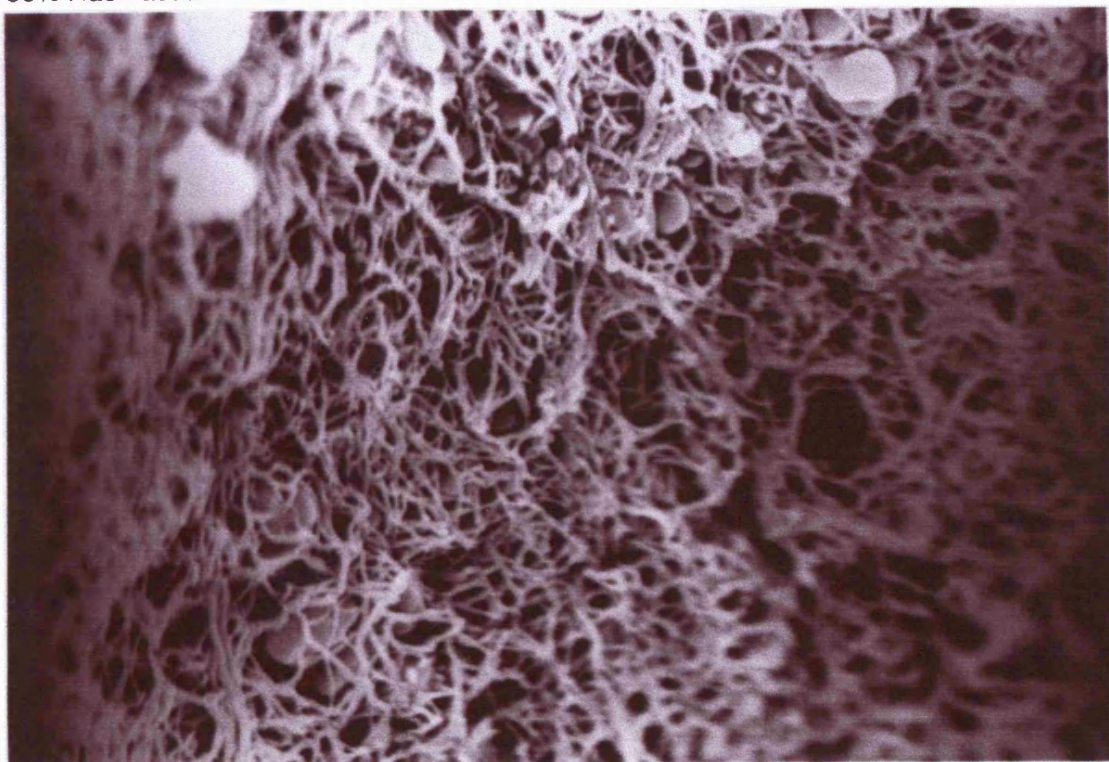
Whole blood 1a



Whole blood
+ Factor VIIa 1b



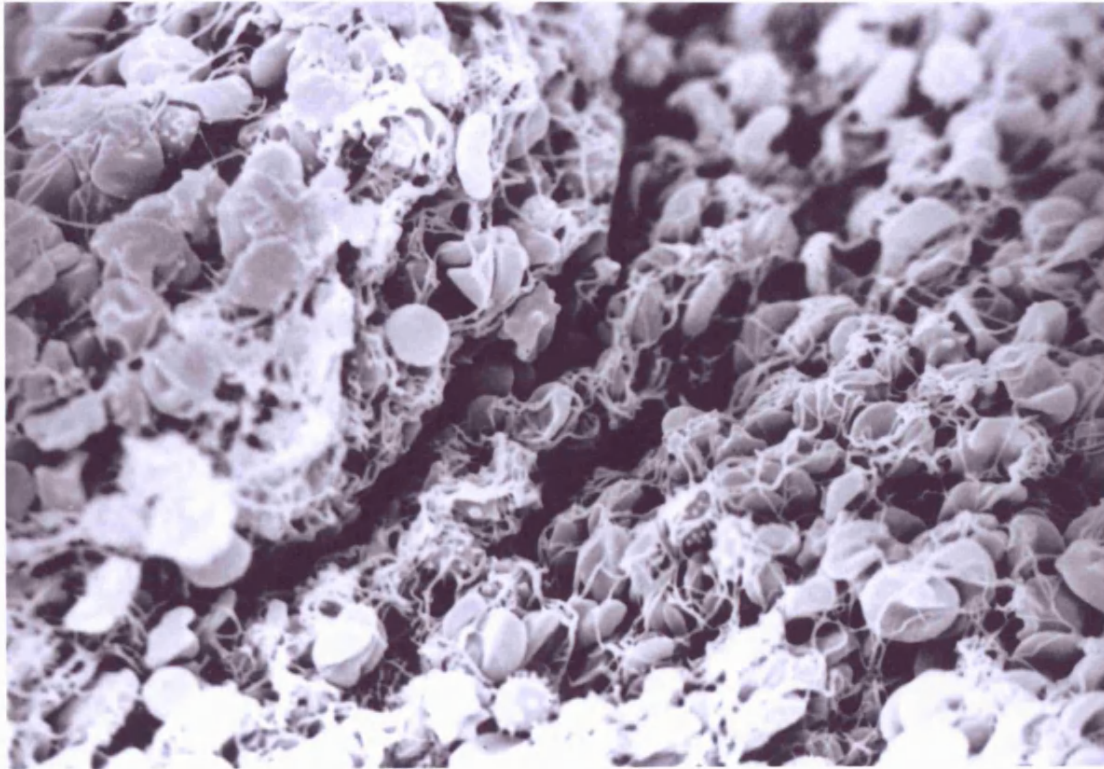
50% Blood
50% Haemaccel 2a



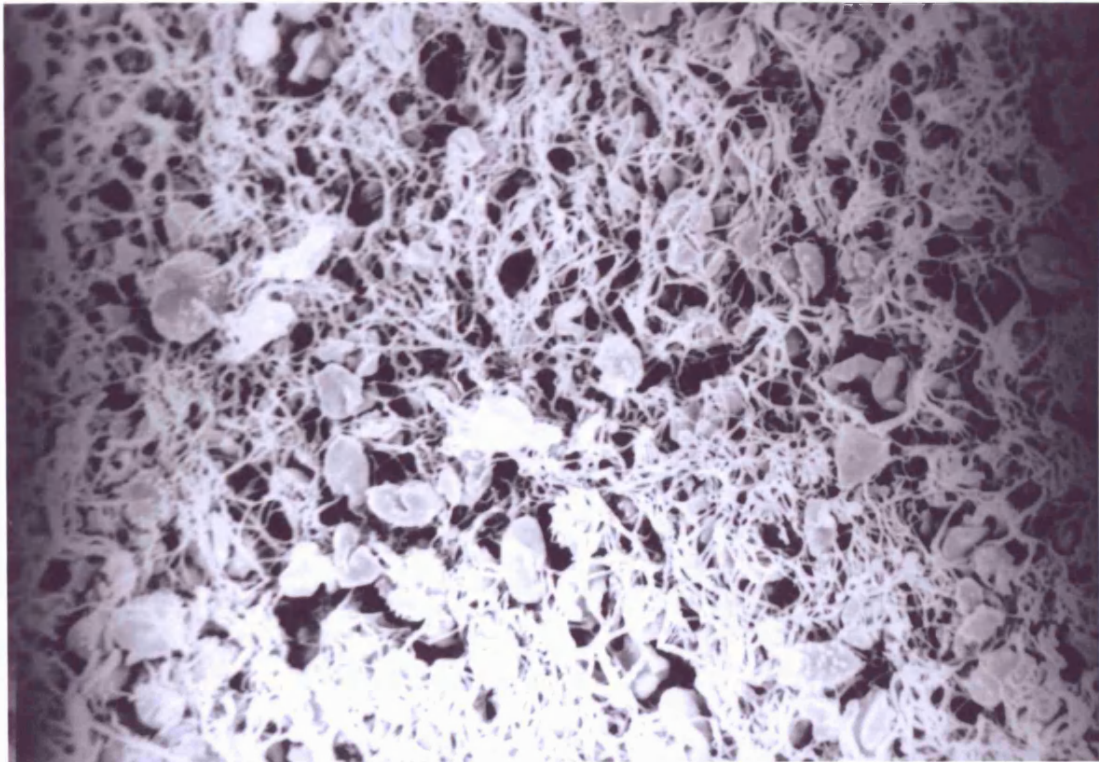
50% Blood
50% Haemaccel
+ Factor VIIa 2b



50% Sodium Chloride
50% Blood 3a



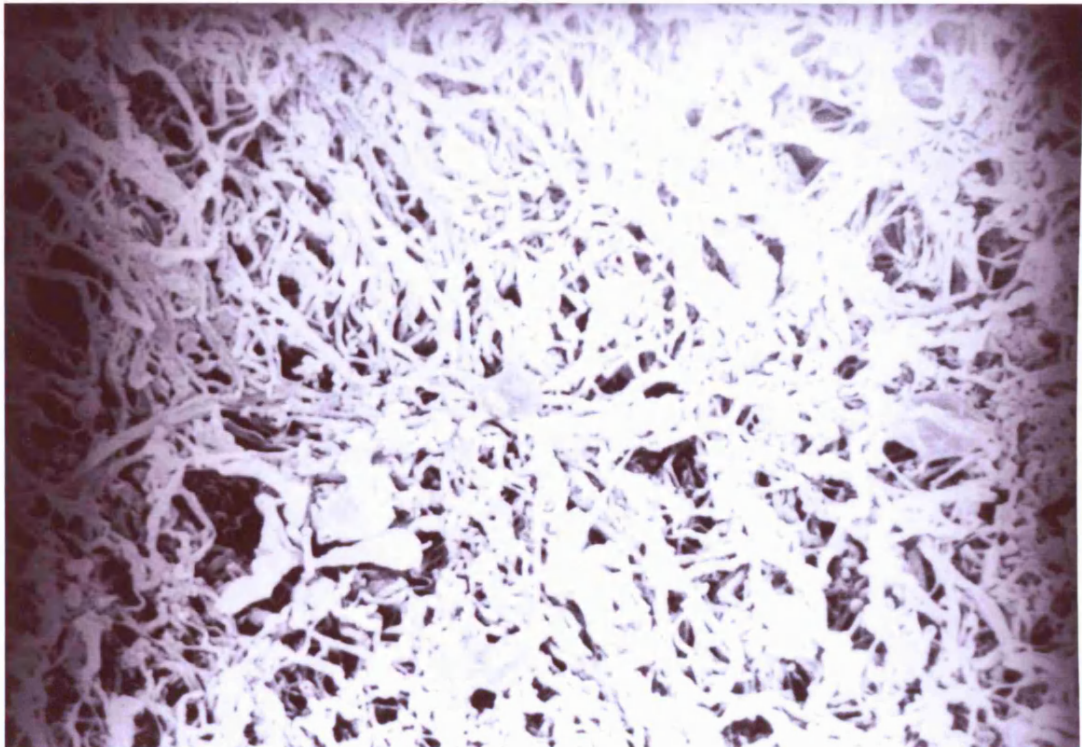
50% Sodium Chloride
50% Blood
+ Factor VIIa 3b



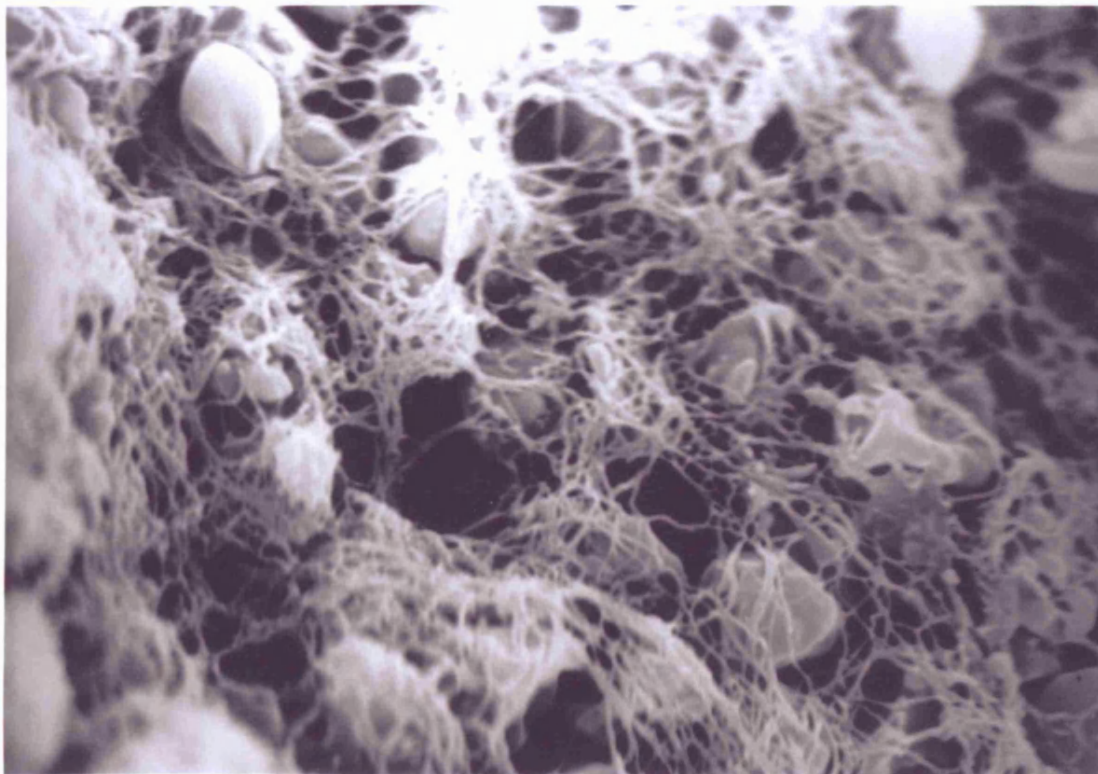
20% Blood
80% Haemaccel 4a



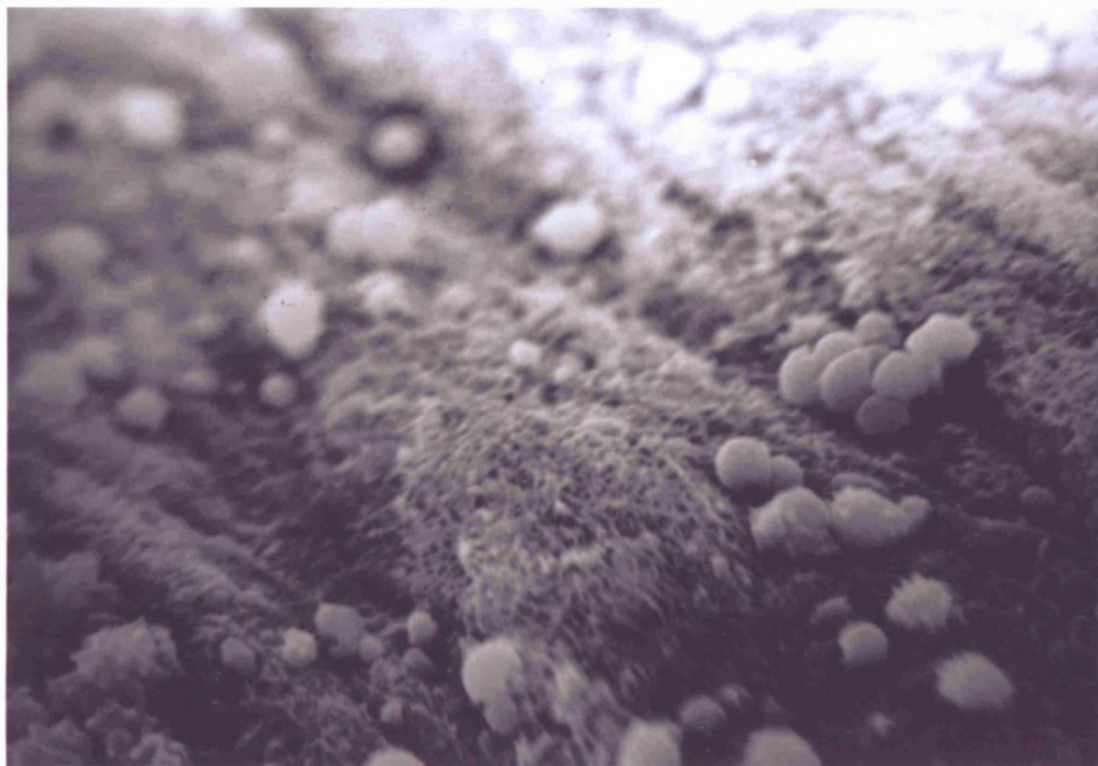
20% Blood
80% Haemaccel
+ Factor VIIa 4b



80% Sodium Chloride
20% Blood 5a



80% Sodium Chloride
20% Blood
+ Factor VIIa 5b



Discussion

Aims of this study were to examine if rVIIa could reduce, if not reverse, the volumetric and intrinsic effects of these two IV fluids that are used in resuscitation of the hypovolaemic patient. Our model demonstrates that the ability to form a good quality clot is impaired with dilution; the greater the dilution the more pronounced the effect. Haemacell and saline had differing effects upon the TEG parameters and ultimately clot quality. rVIIa is, to some degree, able to reverse the effects of dilution although the diluted blood is never able to regain the native profile of whole blood.

TEG provides a global view of haemostasis and reflects the interaction of all components of the haemostatic process and has been used both clinically and experimentally including, surgery^{31,32,33,34,35,36,37,38,39} and trauma.⁴⁰ TEG analysis was chosen as the actions of both fluids and rVIIa are known to effect both the plasma and cellular components of coagulation.^{41,42,43,44}

In this study rVIIa influences all parameters measured but the most significant changes are seen in α and K, which represent kinetics of clot formation. Such results are to be expected as addition of rVIIa would lead to a thrombin burst and thus a subsequent increase in both the speed and rapidity of fibrin deposition. K is a measure of the speed of clot formation to reach a certain arbitrary level of clot strength, depending more on fibrinogen level than platelet function.²⁹ α is related to K as they are both a function of clot polymerisation and is more comprehensive than K in measuring the rapidity of fibrin build-up and cross linking.²⁹ rVIIa leads to greater thrombin generation and thus a steeper angle. Interestingly, the relative magnitude of the improvement, following addition of rVIIa, was similar when comparing the same degree of dilution irrespective of the diluent.

TMA and G reflect "the end product" and hence clot quality; improvements were seen but not to the same extent as a and K. G depends upon the clot kinetics but is a mathematical calculation based upon MA (maximum amplitude). MA is a direct function of the maximum dynamic properties of fibrin and platelet bonding via the GPIIb/IIIa and represents the strength of the fibrin clot.²⁹ In dilutions as profound as our study, the relative concentrations of essential substrates will be small in comparison to whole blood; there will be only a finite degree of improvement that will be possible. rVIIa appears to aid haemostasis creating a clot more quickly, but the final 'quality' will ultimately depend upon the absolute concentration of other key components such as fibrinogen and platelets.

Our study differs from previous data that has demonstrated reduced clot quality with gelatin-based plasma substitutes.⁴ Our results are different to these studies for several reasons. Dilutional models previously used do not include dilutions of up to 80%; dilutions up to 40% promote haemostasis, above 40% there is a rapid reduction in the ability to create good quality clot;^{5,6} some researchers feel this may be due inhibition of the fibrinolytic pathway.^{5,6} Many of the studies used starches and gelatins, some of which are related to Haemaccel but very few actually used Haemacell which may be important as Haemaccel has high concentrations of calcium⁴¹ As yet there is little evidence to offer an explanation as to why our results may show Haemaccel to be a superior resuscitative fluid with regards to clot quality.⁴² A few studies have seen similar haemostatic changes such as ours.⁴³ Evans et al⁴¹ illustrated that Haemaccel was a potent platelet inhibitor but again the dilutions were only equivalent to 40%; it may be that at greater dilutions a biphasic effect occurs; the once inhibitory effect of calcium may actually promote coagulation.^{44,45} Haemaccel contains 6.25mmols of ionised calcium/l, previous in vitro work at 40% dilution with this gelatin fluid showed an increase ionised calcium level of 2.8mmol/l.⁴⁶ As we have used much greater dilution volumes, our levels of ionized calcium achieved will lead to much higher concentration; this in turn could account for the differing intrinsic effects that our study has seen. Indeed previous studies have shown that calcium has differing biphasic effects upon platelet aggregation and coagulation at differing plasma concentrations.^{44,45}

Recombinant Vila, at a final concentration 90ug/kg, was able to improve the haemostatic parameters but was not able to fully reverse the dilutional coagulopathy. Our study has several limitations; clearly it is a static, artificial system and does not take into account the physiological resolution of the dilutional state. Although the results are statistically significant they are also conservative in their estimation as to the potential positive effect of rVIIa. rVIIa needs to be complexed with tissue factor (TF) in order for it to work with maximum efficiency^{22,23,24} and this model was developed from normal healthy individuals. Our in vitro model used a system with little or no TF and thus the effects seen are due to thrombin generation on the platelets by the rVIIa alone; this reaction requires high doses of Vila and is a very inefficient way of generating thrombin;^{22,23,24} in trauma there will be a widespread liberation of TF from damaged cells allowing massive thrombin generation by exogenous rVIIa.

Concerns have been expressed about potential uses of rVIIa in respect to widespread thrombus formation when rVIIa is administered in bleeding. However to date there is no data to suggest there is clear evidence of a systemic activation of the haemostatic mechanism.^{1b,17>1s} Indeed a recent randomized study showed that there were no increases in thromboembolic events.²¹ Case reports, whose patients have had extensive blood loss following trauma^{13,16,25} and animal models studies^{14,17,18,47} have received rVIIa with subsequent reductions in clinical blood loss and coagulation parameter improvement.

The arrest of haemorrhage following trauma is a fine balance between thrombogenesis and fibrinolysis to enable the haemostatic mechanisms to achieve good clot quality. All processes of this haemostatic mechanism can be altered by the volume and intrinsic effects of intravenous resuscitative fluids. rVIIa is an exciting therapeutic option by which the clinician may be able to control haemorrhage by the improvement of

clot quality and reduce the volume of fluid replacement. rVIIa could provide the clinician with another tool to deal with the management of the shocked, dilutional coagulopathic patient, increasing "The Golden Hour" and helping to treat this common cause of mortality and morbidity in patients.

Our studies confirm that rVIIa, in an in vitro model of large fluid dilution can overcome and reverse the dilutional and intrinsic effects of these fluids improving global parameters, clot quality and structure. However what occurs in vitro may not occur in vivo and care must be taken in extrapolating in vitro data to the clinical scenario.

Declarations

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