

Aspirin's Effectiveness Decreases during
Carotid Endarterectomy

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This thesis is based on my own independent work except
where otherwise acknowledged.

Sit down before fact as a little child, be prepared to give up every
preconceived notion... or you shall learn nothing.

Thomas H. Huxley
English biologist (1825 - 1895)

Abstract

Carotid Endarterectomy (CEA) is a well-established operation which reduces the risk of stroke in patients with atherosclerotic stenosis of the internal carotid artery. Paradoxically, the operation itself carries a risk of peri-operative stroke. Evidence suggests that patients with more reactive platelets may be at higher risk of post-operative thrombotic stroke.

A preceding study on platelet function during CEA showed a significant increase in aggregation in response to arachidonic acid (AA), (the substrate for the Cyclo-oxygenase (COX) pathway) by the end of the operation. To explore the hypothesis that the anti-platelet effect of aspirin is reduced during CEA, further aggregometry was performed at eight peri-operative time points. This showed that the anti-platelet effect of aspirin is significantly reduced within three minutes of the administration of intravenous unfractionated heparin (UFH) and persists into the post-operative period. Aggregation to AA (5mmol/L) increased from $3.94 \pm 2.20\%$ to $45.1 \pm 29.3\%$; $p < 0.0001$ following administration of UFH. This had never previously been described and contradicted all previous knowledge of aspirin's mechanism of action (irreversible acetylation of the Ser-529 residue of COX). As this finding was very unusual, a control group of patients undergoing peripheral artery angioplasty was also studied. Again, reduction in aspirin's anti-platelet effect was seen following administration of UFH. Aggregation increased from $5.6 \pm 4.5\%$ to $33.8 \pm 24.2\%$ ($p = 0.0001$).

A number of *in vitro*, *ex vivo* and ELISA studies were performed to determine the mechanism behind the changes in platelet aggregation. These showed that the changes could not be reproduced *in vitro*, were not due to re-activation or release of COX, or its isoform COX-2, but may be related to activation of the Lipoxygenase enzyme pathway.

Evidence suggests that Low Molecular Weight Heparin (LMWH) causes less platelet activation than UFH. The final part of this thesis therefore, was a pilot randomised trial comparing the effects of LMWH and UFH on the anti-platelet effect of aspirin. Although both groups showed an increased platelet aggregation in response to AA, there was a much less marked change in the platelets of patients who had received LMWH.

This reduction in aspirin's efficacy and resultant heightened platelet reactivity may be important, not just for patients undergoing CEA, but also may contribute to risk of thrombo-embolic complications in patients undergoing other vascular interventional procedures (surgery, angioplasty, stenting).

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Thank you to Stephen for everything else.

Abbreviations

5 AMP	5-prime Adenosine Monophosphate
AA	Arachidonic Acid
AAASPS	African American Antiplatelet Stroke Prevention Study
ACAS	Asymptomatic Carotid Atherosclerosis Study
ACEI	Angiotensin Converting Enzyme Inhibitor
ACST	Asymptomatic Carotid Surgery Trial
ADP	Adenosine Diphosphate
AHG	Anti-Haemophilic Globulin
AICLA	Accidents Ischémiques Cérébraux Liés à l'Atherosclerose
APTT	Activated Partial Thromboplastin Time
ARB	Angiotensin II Receptor Blockers
ARR	Absolute Risk Reduction
ATP	Adenosine Triphosphate
BMT	Best Medical Therapy
BMU	B-Mode Ultrasound
BP	Blood Pressure
CAD	Coronary Artery Disease
cAMP	cyclic Adenosine Monophosphate
CAPRIE	Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events
CATS	Canadian American Ticlopidine Study
CCA	Common Carotid Artery
CETC	Carotid Endarterectomy Trialists Collaboration
COL-ADP	Collagen-Adenosine Diphosphate
COL-EPI	Collagen-Epinephrine
COX	Cyclo-Oxygenase
CT	Closure Time
CWD	Continuous Wave Doppler
DIC	Disseminated Intravascular Coagulation
ECA	External Carotid Artery
ECST	European Carotid Surgery Trial
EEG	Electroencephalogram
EGF	Epidermal Growth Factor

ELISA	Enzyme-Linked Immuno-Sorbent Assay
ESPS	European Stroke Prevention Study
FSF	Fibrin Stabilising Factor
GA	General Anaesthetic
GALA	General Anaesthetic versus Local Anaesthetic for CEA trial
GP	Glyco-protein
HBA1c	Glycosolated Haemoglobin
HDL-C	High Density Lipo-protein C
HETE	Hydroxyeicosatetraenoic acid
HMWK	High Molecular Weight Kallikrein
HPETE	Hydroperoxyeicosatetraenoic acid
ICA	Internal Carotid Artery
IQR	Inter-quartile Range
LA	Local Anaesthetic
LDL-C	LowDensity Lipo-protein C
MATCH	Management of AtheroThrombosis with Clopidogrel in High-risk patients with recent transient ischaemic attack or ischaemic stroke
MCA	Middle Cerebral Artery
MHz	MegaHertz
MI	Myocardial Infarction
MONICA	Multinational MONItoring of trends and determinants in Cardiovascular disease
MR	Modified Release
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NCEP III	National Cholesterol Education Programme
NHS	National Health Service
NICE	National Institute for Clinical Evidence
NNT	Number Needed to Treat
NSF	National Service Framework
PDGF	Platelet-Derived Growth Factor
PF4	Platelet Factor 4
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2
PGI2	Prostaglandin I2
PK	Prekallikrein

PRP	Platelet Rich Plasma
PTFE	Polytetrafluoroethylene
RPM	Revolutions Per Minute
RRR	Relative Risk Reduction
SLE	Systemic Lupus Erythematosus
SPCA	Serum Prothrombin Conversion Accelerator
TASS	Ticlopidine Aspirin Stroke Study
TCD	Transcranial Doppler
TFPI	Tissue Factor Plasma Inhibitor
TIA	Transient Ischaemic Attack
TPA	Tissue Plasminogen Activator
TRAP	Thrombin Receptor Activating Peptide
TxA2	Thromboxane A2
TxB2	Thromboxane B2
VEGF	Vascular Endothelial Growth Factor
vWF	vonWillebrand Factor
WHO	World Health Organisation

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Appendix 1 Ethical Committee Approval, Patient Information and Consent Forms

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Abstracts and Publications Arising from this Work

Publications

Platelet inhibition by aspirin is reversed in patients during carotid surgery: a form of transient aspirin resistance?

DA Payne, CI Jones, SE Webster, PD Hayes, AR Naylor, AH Goodall
Thrombosis and Haemostasis, 2004. Jul;92(1):89-96

“Aspirin’s anti-platelet effect is irreversible”: time to re-think a ‘beautiful hypothesis’?

SE Webster, PD Hayes, DA Payne, AH Goodall, AR Naylor
BJS May 2004, Vol 91, Suppl 1. Page 33

The Anti-Platelet effect of Aspirin is Significantly Reduced following Administration of Heparin during Carotid Endarterectomy

SE Webster, DA Payne, CI Jones, PD Hayes, PRF Bell, AH Goodall, AR Naylor
Journal of Vascular Surgery, 2004 Sep;40(3):463-8

Presentations

Randomised Control Trial of Dextran versus Heparinised-Saline as an irrigant during Carotid Endarterectomy

SE Webster, DA Payne, PD Hayes, AR Naylor
Midlands Vascular Society, Birmingham UK, March 2003

Administration of Unfractionated Heparin during Carotid Endarterectomy can overcome the anti-platelet effect of Aspirin

SE Webster, DA Payne, PD Hayes, PRF Bell, AR Naylor
American Association of Vascular Surgeons, Chicago, Illinois, June 2003

Administration of Unfractionated Heparin during Carotid Endarterectomy can overcome the anti-platelet effect of Aspirin (POSTER)

SE Webster, DA Payne, PD Hayes, PRF Bell, AR Naylor
International Society of Thrombosis and Haemostasis, Birmingham, UK July 2003

Administration of Unfractionated Heparin during Carotid Endarterectomy can overcome the anti-platelet effect of Aspirin

SE Webster, DA Payne, PD Hayes, PRF Bell, AR Naylor
Annual Meeting of the Stroke Association, ICC Birmingham, September 2003

The transient aspirin resistance seen following administration of heparin during carotid endarterectomy is not due to induction of COX-2 (POSTER)

SE Webster, DA Payne, PD Hayes, V Liapis, AH Goodall, AR Naylor
Annual Meeting of the Royal College of Surgeons of Edinburgh, November 2003

Aspirin's anti-platelet effect is irreversible: Time to re-think a beautiful hypothesis?
SE Webster, DA Payne, PD Hayes, AH Goodall, AR Naylor
Association of Surgeons of Great Britain and Ireland, Annual Meeting, Harrogate, UK
April 2004

Metabolism of Arachidonic Acid Through 12-LOX in Platelets may account for Transient
Aspirin Resistance Linked to Treatment with Heparin
SE Webster, CI Jones, PD Hayes, AR Naylor, Goodall AH
ISTH XXth Meeting, Sydney, Australia (Poster), August 2004

Aspirin's Anti-platelet Effect is Reversible – The Effect of Unfractionated Heparin versus
Low Molecular Weight Heparin
SE Webster, DA Payne, PD Hayes, AH Goodall, AR Naylor
The Stroke Association 9th Scientific Conference, Churchill College, Cambridge, UK,
September 2004

Aspirin's anti-platelet effect is *not* irreversible – *the effect of Unfractionated Heparin on
the aspirinated platelet*
Sally Ward-Booth, David Payne, Paul Hayes, Chris Jones, Alison Goodall, Ross Naylor
Invited presentation to the Annual meeting of the Aspirin Foundation, Royal College of
Physicians, London, UK, April 2005

Prizes

Prize for Best Poster

The transient aspirin resistance seen following administration of heparin during carotid
endarterectomy is not due to induction of COX-2 (POSTER)
SE Webster, DA Payne, PD Hayes, V Liapis, AH Goodall, AR Naylor
Annual Meeting of the Royal College of Surgeons of Edinburgh, November 2003

Moynihan Prize Session

Aspirin's anti-platelet effect is irreversible: Time to re-think a beautiful hypothesis?
SE Webster, DA Payne, PD Hayes, AH Goodall, AR Naylor
Association of Surgeons of Great Britain and Ireland, Annual Meeting, Harrogate, UK,
April 2004

Chapter 1 Introduction

1.1 Stroke and Transient Ischaemic Attack

1.1.1 Definition

A stroke is a syndrome defined as the “rapidly developing clinical signs of focal (or global) disturbance lasting 24 hours or longer, or leading to death with no apparent cause other than that of vascular origin” (World Health Organisation (WHO) MONICA project principal investigators, 1988). A stroke is the clinical manifestation of a wide range of pathologies of vascular origin, that is, the cerebral damage is caused by either ischaemia or haemorrhage. The symptoms and signs are variable in extent and severity, depending on the degree of vascular interruption. If the symptoms last less than twenty four hours the clinical event is defined as a Transient Ischaemic Attack (TIA). A TIA is a significant event, warning of an underlying pathology that increases the risk of stroke.

1.1.2 Epidemiology

Stroke is the one of the most common causes of death in the developed world (WHO Report 2004) and is the main cause of chronic disability. It causes approximately 10/100000 deaths per year in people aged over 40 years, and 1000/100000 in those aged over 75 years. (Office of National Statistics (ONS) Mortality statistics: Cause 1998). In the United Kingdom each year, around 20,000 TIAs will occur, and 110,000 people will suffer a first stroke. 30% of people suffering a stroke will die within three months of the event, and 35% are left with significant levels of disability (National Service Framework (NSF) guidelines). It is estimated that there are 300,000 people with moderate to severe disability in England and as many as 900,000 with any sort of disability. (Report from National Audit Office, Department of Health, Reducing Brain Damage: Faster Access to better stroke care, 2005/6). Worldwide, there are over 9 million survivors of stroke. (Sarti C et al, 2000).

These statistics lead to a vast cost to society, with stroke patients requiring acute medical care, rehabilitation and long-term care with resultant loss of earnings and workforce. It is estimated that the annual cost to the NHS is over £2.8 billion, compared with £1.9 billion for Coronary Heart Disease (Report from National

Audit Office, Department of Health, Reducing Brain Damage: Faster Access to better stroke care, 2005/6).

1.1.3 Aetiology

The clinical syndrome of stroke may be caused by different underlying pathologies (shown in table 1.1.1). Cerebral ischaemia accounts for approximately 85% (Bamford J et al, 1990), with the most common cause of ischaemic stroke being thrombo-embolism from atheromatous disease of the internal carotid artery - one of the main vessels supplying the brain (Sacco RL et al, 1989). Complications of internal carotid artery (ICA) atherosclerotic disease, such as plaque rupture, occlusion or platelet emboli cause as many as 70% of all strokes. The work in this thesis is concerned with stroke caused by atherosclerosis of the ICA and the following sections will be thus directed.

1.1.4 Clinical Classification of Stroke

The severity of symptoms of the event depends on the size of the embolus or haemorrhage and the area of the brain affected. The outcomes of this disease therefore range from transient to permanent, sub-clinical to fatal. A number of clinical pictures have been defined (table 1.1.2).

1.1.5 Risk Factors

As there is no way of predicting which patients will suffer a severe event, and there being no curative treatment for a completed stroke, much of the management of the disease is directed towards stroke prevention. Recognition of risk factors for stroke is needed for the identification of patients at high risk and those who would benefit from lifestyle modifications or drug therapies to reduce their risk. In 2006, an article by Goldstein et al reviewed the evidence for risk factors, both modifiable and non-modifiable, and summarises the current recommendations for treatment. An overview is given below, with tables reproduced from the article. Table 1.1.3 outlines the recommendations for Treatable Vascular Risk Factors, and table 1.1.4 the recommendations for modifiable behavioural risk factors.

Table 1.1.1 Aetiological Classification of Stroke

Type	Cause	Example
Ischaemic	Cardio-embolic	Atrial Fibrillation
		Mural thrombus
		Paradoxical embolus via patent foramen ovale
		Infective Endocarditis
	Athero-thromboembolic	Carotid Artery Atherosclerosis / Dissection
		Vertebral Artery Atherosclerosis / Dissection
		Cerebral Artery Occlusion
	Small Vessel Disease	Hypertensive Vasculopathy
		Diabetic Vasculopathy
		Vasculitis
	Haematological	Polycythaemia / Leukaemia / Paraproteinaemia / Sickle cell Disease / Disseminated Intravascular Coagulation
	Others	Venous Thrombosis / Trauma / Moya-Moya Syndrome / Inflammatory Vascular Disease (Giant Cell Arteritis, Takayasu's Disease, Systemic Lupus Erythematosis)
Haemorrhagic	Sub-arachnoid haemorrhage	Arterio-venous malformation
		Aneurysm
	Parenchymal	Hypertensive
		Amyloid Angiopathy

Table 1.1.2 Clinical Syndromes resulting from Stroke

Type	Definition
Total Anterior Circulation Stroke	All of: contralateral hemiplegia / hemiparesis contralateral hemisensory loss disturbance of higher cerebral function
Partial Anterior Circulation Stroke	Any of: motor or sensory deficit and hemi-anopia motor or sensory deficit and higher cortical dysfunction higher cortical dysfunction with or without hemi-anopia pure motor deficit e.g. monoparesis
Lacunar Stroke (Occlusion of a single deep perforating artery)	Maximum deficit from a single vessel event with no associated visual field defect, no cortical dysfunction and no brainstem disturbance
Posterior	Any of: ipsilateral cranial nerve palsy with contralateral long tract signs bilateral motor or sensory deficit disorder of conjugate eye movements cerebellar dysfunction isolated hemi-anopia cortical blindness

Non-Modifiable

Age

Age is the most powerful, if non-modifiable risk factor for infarction, intra-cerebral haemorrhage and sub-arachnoid haemorrhage (Bamford et al 1990). Most strokes occur in the over 75 years age group.

Sex

Stroke is more prevalent in males than females, (Brown et al 1996), except at the extremes of age – 35 to 44 years and those over 85 years when females have a slightly higher rate (Sacco et al 1998).

Race

Blacks, Hispanic Americans, Chinese and Japanese races have all been identified as having higher stroke incidence rates. (Goldstein et al, 2001).

Family History

A family history of stroke increases the risk of stroke and is likely to be related to a complex interaction of genetic and environmental factors (Goldstein et al, 2001).

Modifiable

Hypertension

Evidence from studies looking at both diastolic and systolic blood pressure measurements (Framingham studies – Kannel et al 1981, Wolf et al 1985) has shown that hypertension is a significant risk factor for stroke. MacMahon et al (1990) suggested that stroke risk doubles with each 7.5mm mercury rise in diastolic pressure and Shimizu et al (1984) observed that in addition to the actual blood pressure, the increase in blood pressure with time is also a risk factor. The mechanism by which hypertension causes an increased risk of stroke may be due to the increased severity of atheroma and/or the presence of micro-vascular disease in the small penetrating arteries within the brain. (Reed et al 1988, Hower et al 1991).

Smoking

The smoking of tobacco increases the risk of stroke; this increase occurring in a dose-dependant fashion. (Shinton & Beevers 1989)

Cardiac Disease

The presence of cardiac disease indicates a higher risk of stroke; there is a five-fold increase in the incidence of stroke in patients with atrial fibrillation, (Framingham Study) and a higher incidence is also seen in patients with coronary artery disease and cardiac failure (Kannel et al 1983).

TIA's

A TIA is, by definition, a stroke lasting less than 24 hours, and is often a warning sign of a future event – such as a further TIA or stroke. This risk is particularly high in the first three months following a TIA with Coull et al (2004) describing the risk at one week as 8.0%, 11.5% by one month and 17.3% by 3 months. The risk of stroke in the first five years following a TIA is 24-29%. (Heyman et al, 1984; Dennis et al, 1990; Whisnant et al, 2002)

Diabetes Mellitus

Patients with diabetes are at double the risk of stroke. (Kannel and McGee 1979).

Lipids

Although most of the increased incidence seen in patients with high lipids is due to the association with other patho-physiological conditions, there is probably an independent increased risk also (Qizilbash 1992). More recently, the importance of “statins”, the group of HMA-co-reductase drugs, used initially for their lipid-lowering properties, but now recognised as plaque-stabilising drugs (Vaughn CJ 2003) has been shown. It is now recommended that all patients at risk of occlusive vascular disease should be considered for statin therapy. (NSF guidelines, CHD chapter 2).

Others

Other factors, such as obesity, physical inactivity, poor diet, alcohol, hyperhomocysteinaemia, drug abuse, use of the Oral Contraceptive Pill or HRT and factors increasing blood viscosity are more loosely associated with an increased risk of stroke. (Larry et al, 2001)

1.1.6 Stroke Prevention

As well as modifying the risk factors outlined in 1.1.5, the two mainstays of preventing stroke (in those caused by atherosclerosis of the internal carotid artery) are anti-thrombotic drugs and surgery. The following sections will describe haemostasis, thrombosis, anti-thrombotic therapies and carotid endarterectomy.

Table 1.1.3 Recommendations for reducing the risk of further stroke and other vascular events in patients with previous stroke or TIA, by treating modifiable vascular risk factors

(See Table 1.1.5 for description of class and level of evidence)

Risk Factor	Recommendation	Class / Level of Evidence
Hypertension	Anti-hypertensives are recommended in persons who have had an ischaemic stroke and are beyond the hyperacute period	Class I Level A
	Because this benefit extends to persons with or without hypertension, anti-hypertensives should be considered for all ischaemic stroke and TIA patients	Class IIa Level B
	Absolute target BP level and reduction are uncertain and should be individualised, but benefit has been associated with an average reduction of $\approx 10/5$ mm Hg and normal BP levels have been defined as $<120/80$	Class IIa Level B
	Several lifestyle modifications have been associated with BP reductions and should be used with anti-hypertensive therapy	Class IIb Level C
	Optimal drug regimen remains uncertain; however, available data support the use of diuretics and combination of diuretics and an ACEI. Choice of specific drugs and targets should be individualized on the basis of reviewed data	Class I Level A

Diabetes	More rigorous control of blood pressure and lipids should be considered in diabetics	Class IIA Level B
	Although all major anti-hypertensives are suitable for the control of BP, most patients will require more than one agent. ACEIs and ARBs are more effective in reducing the progression of renal disease and are first-choice for diabetics.	Class I Level A
	Glucose control is recommended to near-normoglycaemic levels among diabetics with ischaemic stroke or TIA.	Class I Level A
	The goal for HbA1c should be $\leq 7\%$	Class IIA Level B
Cholesterol	Patients with elevated cholesterol, co-morbid CAD, or evidence of an atherosclerotic origin should be managed according to NCEP III guidelines, which include lifestyle and dietary modification and medication recommendations.	Class I Level A
	Statin agents are recommended and the goal for cholesterol lowering is an LDL-C of $<100\text{mg/dL}$ and LDL-C $< 70\text{mg/dL}$ for very high risk persons with multiple risk factors	Class I Level A
	Patients with ischaemic stroke or TIA presumed to be due to an atherosclerotic origin but with no pre-existing indications for statins are reasonable to consider for treatment with a statin	Class IIA Level B
	Ischaemic stroke or TIA patients with low HDL-C may be considered for treatment with niacin or gemfibrozil	Class IIB Level B

Table 1.1.4 Recommendations for Modifiable Behavioural Risk Factors

Risk Factor	Recommendation	Class/Level of Evidence
Smoking	All ischaemic stroke or TIA patients who have smoked in the past year should be strongly encouraged not to smoke	Class I Level C
	Avoid environmental smoke	Class IIa Level C
	Counselling, nicotine replacement products	Class IIa Level B
Alcohol	Patients with prior ischaemic stroke or TIA who are heavy drinkers should eliminate / reduce their intake	Class I Level A
	Light to moderate levels of alcohol may be considered	Class IIb Level C
Obesity	Weight reduction may be considered for all overweight ischaemic stroke or TIA patients aiming for BMI of 18.5 to 24.9 Kg/m ² and a waist circumference of less than 35 inches in women and less than 40 inches in men	Class IIb Level C
Physical Activity	At least 30 minutes of moderate-intensity physical exercise most days may be considered to reduce risk factors and co-morbid conditions that increase the likelihood of recurrence of stroke	Class IIb Level C

Table 1.1.5 Definitions for Classes and Levels of Evidence used in AHA Recommendations

Class I	Conditions for which there is evidence for and/or general agreement that the procedure or treatment is useful or effective	
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/ efficacy of a procedure or treatment	
	Class IIa	Weight of evidence is in favour of the procedure
	Class IIb	Usefulness/efficacy is less well established by evidence or opinion
Class III	Conditions for which there is evidence and/or general agreement that the procedure or treatment is not useful/effective and in some cases may be harmful	
Level of evidence A	Data derived from multiple randomized trial or non-randomised studies	
Level of evidence B	Data derived from a single randomised trial or nonrandomised studies	
Level of evidence C	Expert opinion or case studies	

1.2 Platelets, Haemostasis and Thrombosis

1.2.1 Platelets

Platelets were first identified by Donné in 1842 but although many scientists documented thrombi and considered the role of platelets (Wharton-Jones et al *from Platelets in thrombotic and non-thrombotic disorders*), further understanding of their structure, function and relationship to pathological processes did not occur until the mid-twentieth century. In 1948, Duguid suggested that mural thrombi consisting of platelets and fibrin became incorporated into the vessel wall and were involved in the pathogenesis of atherosclerotic lesions. Fisher in 1959 described platelet-rich emboli passing through retinal vessels during episodes of transient monocular blindness. Through research over the last few decades, there is now detailed information about the structure and function of platelets, and their pathogenic contribution to atherosclerotic processes.

Platelets are small anucleate cells formed in the bone marrow from megakaryocytes. They usually circulate singly and until activated are non-adhesive. The normal platelet count is 150 to 400×10^9 , with each platelet having a lifespan of around ten days before being removed from the circulation by the spleen.

In the inactivated state, the platelet has a disc-shape. It has been described as having three regions – the *peripheral zone*, consisting of external and internal membrane systems that make up the exposed surface of the platelet and walls of the complex open canicular systems, the *sol-gel zone* or matrix of the platelet cytoplasm, which provides structural support and a contractile system and the *organelle zone*, consisting of granules, electron-dense bodies, peroxisomes, lysosomes, glycosomes and mitochondria.

The secretion granules are of vital importance in promoting platelet aggregation, and are of three main types – α -granules, dense granules and lysosomes. α -Granules initially arise in the trans-golgi network and contain a variety of components involved in haemostasis and wound healing (fibrinogen, vWF, thrombospondin, fibronectin, β -thromboglobulin, platelet factor 4, VEGF, PDGF, EGF), adhesive molecules (including fibrinogen, fibronectin, thrombospondin), growth factors (β -transforming growth factor, platelet-derived growth factor), coagulation factors (V, VII), and other proteins (platelet factor 4, platelet basic protein, β -thromboglobulin,

connective tissue activating protein III, neutrophil activating peptide 2). Dense granules contain ATP, ADP, calcium, pyrophosphate and serotonin. Lysosomes are scarcer and contain acid hydrolases, such as acid phosphatase, β -galactosidase and β -glucuronidase.

Following activation, platelets undergo a shape change, becoming more spherical with pseudopodia to facilitate aggregation.

Platelet prostanoid receptors

Prostanoids (thromboxane and prostaglandins) are oxygenated metabolites of the polyunsaturated, essential fatty acid, arachidonic acid (AA). Prostaglandin G/H synthase (also known as cyclo-oxygenase / COX) catalyses the rate limiting step in the synthesis of prostanoids. The major arachidonic acid metabolite in platelets is thromboxane A₂, a potent platelet activator, which is also produced in response to activation by thrombin and ADP.

The availability of AA is the rate-limiting step in platelets. AA is released from membrane phospholipids in activated platelets mainly by phospholipase A₂. It is then converted into Prostaglandin H₂ (by the action of cyclo-oxygenase 1 or 2), then to Thromboxane A₂ by thromboxane synthase. TxA₂ is biochemically unstable and is rapidly transformed non-enzymatically to its hydrolysis product thromboxane B₂. (Patrignani and Sciulli, *Platelets in thrombotic and non-thrombotic disorders*, 2002).

Lipoxygenase, another enzyme contained in the platelet uses AA as a substrate. The end products of this enzyme pathway are 12- and 15- HETE (Hydroxyeicosatetraenoic acid), via 12-HPETE (12-hydroperoxy-eicosatetraenoic acid) or 15-HPETE (15-hydroperoxyeicosatetraenoic acid). Although the functions of these molecules are poorly understood, HPETE has been reported to convert the response of sub-threshold concentrations of AA to full aggregation at physiological concentrations (Calzada et al, 1997, 2001), mediated through potentiation of the metabolism of AA to TxA₂. HETE has also been shown to increase the adhesivity of the platelet (Buchanan et al, 1986).

1.2.2 Haemostasis and Thrombosis

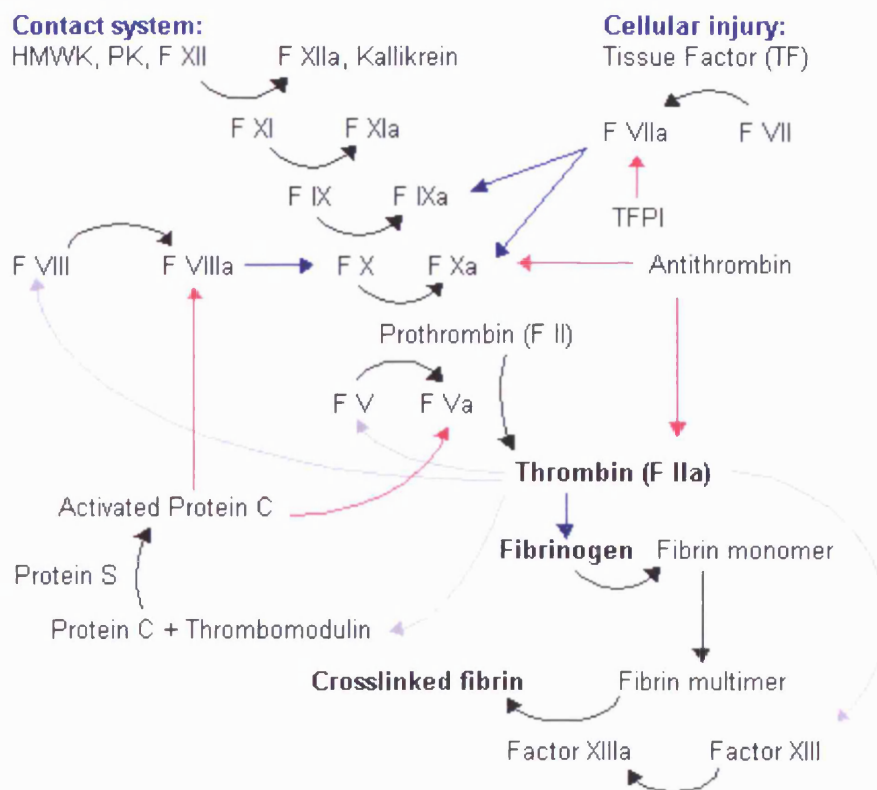
Haemostasis is a complex process involving platelet activation and aggregation and interactions between many clotting factors. Whilst vital for life, these processes also play a significant part in the formation and complications of atherosclerotic plaques. At sites of vascular injury or atherosclerotic plaque rupture, there is exposure of the sub-endothelial matrix. This allows vWF in the sub-endothelium to interact with GPIb-IX-V receptors on the platelet surface, tethering platelets to the vascular matrix of collagen and glycosaminoglycans. Thrombin receptors on the platelet surface are activated inducing release of intra-cellular calcium and activating protein kinase C.

Platelet adhesion and activation of the GP IIb-IIIa receptor follow, with a rise in the intra-cytoplasmic concentration of calcium ions leading to platelet shape change from disc to sphere, followed by centralisation of the platelet granules and release of their contents (ADP, β -thromboglobulin, PF4). AA is freed from membrane phospholipids and is converted via PGH₂ to Thromboxane A₂ by the enzymes COX-1 and thromboxane synthase respectively. (Patrignani and Sciulli, *Platelets in thrombotic and non-thrombotic disorders*, 2002).

Platelet to platelet interaction is facilitated by shape change and mediated by the membrane protein GPIIb-IIIa complex. Activation of this receptor is the final common pathway in platelet aggregation; when activated by an inside-out signalling mechanism, GPIIb-IIIa can bind soluble fibrinogen. Once bound, the fibrinogen forms a bridge to another receptor on an adjacent platelet. Feedback amplification loops lead to platelet recruitment with the release of platelet agonists such as ADP and serotonin from α -granules.

The coagulation cascade is summarised in figure 1.2.1. Table 1.2.1 shows the factors involved in coagulation.

Figure 1.2.1 Coagulation Cascade



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The coagulation cascade, where HMWK = High molecular weight kininogen, PK = Prekallikrein, TFPI = Tissue factor pathway inhibitor. Black arrow represents conversion/activation of factor. Red arrows represent action of inhibitors. Blue arrows represent reactions catalyzed by activated factor. Grey arrows represent various functions of thrombin.

Table 1.2.1 Factors involved in coagulation

Factor	Other Names
Pre-Kallikrein	Fletcher factor
HMWK	Contact activation factor / Fitzgerald / Flaageac Wilheims
I	Fibrinogen
II	Prothrombin
III	Tissue Factor
IV	Calcium
V	Pro-accelerin, labile factor, accelerator globulin
VI (Va)	Accelerin
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA), co-thromboplastin
VIII	Anti-haemophiliac Factor A, Antihaemophilic globulin (AHG)
IX	Christmas factor
X	Stuart-Prower Factor
XI	Plasma thromboplastin antecedent
XII	Hageman Factor
XIII	Protransglutaminase, fibrin stabilising factor (FSF), fibrinoligase

1.3 Anti-thrombotic therapy for non-cardioembolic stroke or TIA

1.3.1 Overview

There are a multitude of drugs that can affect platelet function (see table 1.3.1). They work in a number of different ways – they may raise cyclic nucleotides, interfere with arachidonic acid metabolism, or affect activation or adhesion receptors. A few of the most relevant and clinically used will be described in more detail, together with the evidence of their relative efficacy.

Table 1.3.1 Drugs that may affect platelet function (*Cazenave and Gachet, 1997*)

Type	Mechanism of action	Examples
Drugs interfering with arachidonic acid metabolism	Cyclo-oxygenase (COX-1) inhibition	Aspirin, sulphinpyrazone, indomethacin, ibuprofen
	Phospholipase A2 inhibition	Mepacrine, hydrocortisone, methylprednisolone
	Thromboxane synthase inhibition	Dazoxiben, ozagrel
	Thromboxane synthase inhibition and TXA2 receptor antagonists	Ridogrel, nidogrel, isbogrel
Drugs interfering with activation receptors	Platelet ADP receptor inhibition	Thienopyridines (Ticlopidine, clopidogrel)
	Thrombin receptor inhibition	TRAP
	Alpha2-adrenergic receptor inhibition	Dihydroergocryptine, yohimbine
	5-HT2 receptor inhibition	Ketanserine, sarpogrelate
	PAF receptor inhibition	Glinkolides
Drugs interfering with adhesion receptors	GPIIb/IIIa antagonists	Abciximab, peptidomimetics (tirofiban), disintegrins (trigramin)
	GPIb-V-IX antagonists	Recombinant vWF
Drugs raising cyclic nucleotides	Adenylyl cyclase activation	PGE1 (alprostadi), PGI2 (epoprostenol), prostaglandin analogues (iloprost, beraprost)
	Cyclic nucleotide phosphodiesterase inhibition	Caffeine, theophylline, dipyridamole etc
	Guanylyl cyclase activation	Nitrous oxide, nitrate derivatives (isosorbide dinitrate)

1.3.2 Aspirin

For many years Aspirin has been used for its therapeutic properties, initially being developed for its anti-pyretic and analgesic properties (Jack 1997). John Vane and Bengt Samuelson's description of the biochemistry and pharmacology of arachidonic acid metabolism in the early 1970s and Majerus' subsequent work to describe the molecular mechanism of action of action led to aspirin being re-discovered as an anti-platelet drug.

Aspirin irreversibly binds to and inactivates cyclo-oxygenase 1 (COX-1), by the acetylation of the serine residue Ser529 (Patrono, 1994). This prevents access of the substrate (AA) to the catalytic site of the enzyme. Since platelets do not carry genetic material they are incapable of synthesizing new enzymes, so the aspirin acts on each platelet for its lifetime. It is probable that aspirin also inhibits the COX-1 in megakaryocytes (Demers et al, 1980), so, given a daily platelet turnover of 10%, a once daily dosage is effective at greatly reducing thromboxane-A₂; therefore platelet aggregation and potential thrombotic complications of atherosclerosis.

Aspirin Resistance

The concept of "aspirin resistance" has provoked recent study and debate. The term can be used to describe a number of different phenomena – the ability of aspirin to 1) protect individuals from thrombotic complications, 2) to cause prolongation of bleeding time, 3) to inhibit platelet aggregation *ex vivo* or 4) to inhibit platelet thromboxane production (Weber 2002), and as yet there is no widely accepted definition. This lack of definition leads to difficulty in "diagnosing" aspirin resistance; indeed, aspirin remains the first line anti-platelet therapy with no accepted test to determine its efficacy in any individual patient. Weber went on to propose a typological classification based on laboratory tests of platelet function in patients and normal subjects. By assessing both platelet aggregation in response to collagen and platelet thromboxane production, he defined three types of resistance – Type 1 (pharmacokinetic) – those subjects in which oral aspirin did not suppress aggregation or thromboxane production, but in whom addition of *in vitro* aspirin caused suppression of both, Type 2 (pharmacodynamic) - where no response was seen neither with oral dosage nor *in vitro* aspirin and Type 3 (pseudo-resistance) where samples showed a decreased thromboxane production in the presence of

normal aggregation. These definitions have yet to be accepted into clinical use and have not been substantiated in other studies.

There is some observational evidence that patients with evidence of aspirin resistance on laboratory tests of platelet function have poorer clinical outcomes. Grottemeyer in 1993 reported a series of 180 stroke patients on aspirin. Measurements of platelet reactivity were made 12 hours following aspirin and patients were followed up over a 24-month period, with major endpoints being stroke, myocardial infarction or vascular death. Major endpoints were seen in only 4% of the aspirin responders, but in 40% ($p < 0.0001$) of those with “aspirin resistance”. Another study reported in 2003 by Gum followed 326 patients with stable cardiovascular disease on aspirin. At enrolment, platelet aggregometry was performed, with aspirin resistance being defined as aggregation to $10\mu\text{M}$ ADP $\geq 70\%$ and aggregation to 0.5mg/ml AA of $\geq 20\%$. Of those studied, 5.2% were aspirin resistant, and this group showed an increased risk of death, MI or CVA compared with those that were aspirin sensitive (24% versus 10%). It would seem that laboratory tests suggesting a lower anti-platelet effect of aspirin have some clinical relevance, but the cause is still unknown. It has been postulated that aspirin resistance may be due to lack of compliance, too low a dose, poor intestinal absorption or metabolism, other drug interactions, other sources of thromboxane production or platelet activation, altered thromboxane metabolism, increased platelet turnover or genetic polymorphisms (Hankey, 2006). However, it is likely that aspirin resistance is related to a number of different causes and is not an easily definable “resistant or sensitive” problem; rather a gradation of degree of platelet responsiveness within the complex multifactorial setting of cardiovascular disease. As there is no agreed management (suggested methods include increased dose of aspirin, increase dosing frequency, change to alternative anti-platelet therapy) for patients found to be aspirin resistant in laboratory tests, the role of screening has not yet been defined.

1.3.3 Dipyridamole

Dipyridamole is a pyrimidopyrimidine derivative with vasodilator and anti-platelet properties. It is thought to work by three different mechanisms; firstly, by inhibition of cyclic nucleotide phosphodiesterase, the enzyme that degrades cAMP to 5-AMP. This therefore leads to an increased level of intra-platelet cAMP which works as a

platelet inhibitor. Secondly, dipyridamole can block the uptake of adenosine, which usually acts at A₂ receptors to stimulate platelet adenylyl cyclase and thus increases cAMP. Thirdly, the drug can directly stimulate PGI₂ synthesis and protect against its degradation.

1.3.4 Thienopyridines (Ticlopidine and clopidogrel)

The Thienopyridines Ticlopidine and Clopidogrel are drugs with a similar chemical structure and range of pharmacological activity. (Defreyn et al 1989, Quinn and Fitzgerald 1999, Savi and Herbert 2000). By means of an irreversible covalent modification of the platelet receptor P2Y₁₂, they selectively inhibit ADP-induced platelet aggregation and ADP-mediated amplification, with no direct effects on arachidonic acid metabolism. The two drugs are very similar, with Clopidogrel having a safer side effect profile. Ticlopidine has been known to cause hypercholesterolemia, thrombocytopenia, neutropenia and aplastic anaemia. (Muller et al 2001).

1.3.5 GPIIb/IIIa Antagonists

GPIIb/IIIa is the most abundant protein on the platelet surface and is also present on α -granules. The receptor is a member of the integrin group of adhesion receptors, consisting of two subunits, both with large extra-cellular domains, short cytoplasmic domains and single trans-membrane domains linking the two. The GPIIb/IIIa receptor is seen as the most important of platelet receptors to mediate aggregation, as it may be stimulated by many agonists (e.g. collagen, vWF, fibrinogen, thrombin, ADP, epinephrine, serotonin, TxA₂) and by shear stress forces in narrowed vessels. Via “inside-out” signalling, there is an up-regulation of the receptor’s affinity for soluble forms of adhesive proteins such as fibronectin, vWF, vitronectin and fibrinogen. This phase is followed by an “outside-in” signalling pathway, which leads to consolidation and stabilisation of the platelet aggregate.

The recognition of the importance of this receptor, and its’ role in thrombosis formation has inspired the development of a family of GPIIb/IIIa receptor antagonist drugs. These include ABCIXIMAB (Reopro TM), which is a monoclonal antibody, TIROFIBAN (Aggrastat TM), a synthetic molecule and EPTIFIBATIDE

(Integrilin TM), a synthetic molecule based on the structure of the venom of a Pygmy rattle snake.

These drugs are powerful anti-platelet drugs and are currently reserved for in-hospital use, especially following invasive therapies, such as coronary artery stenting or angioplasty.

1.3.6 Heparin

J. Mclean, a medical student, discovered heparin in 1916. His journal entry documenting the discovery read:-

“After more tests and then preparation of other batches of heparophosphatide, I went one morning to the door of Dr Howell’s office, and standing there (he was seated at his desk), I said “Dr Howell, I have discovered anti-thrombin”. He was most sceptical, so I had the deinner, John Schweinhant, bleed a cat. Into a small beaker full of its blood, I stirred all of a protein batch of heparophosphatides, and I placed this on Dr Howell’s laboratory table and asked him to call me when it clotted. It never did clot. [It was heparin].”

Mc Lean J 1959 Circulation XIX:75

Heparin continues to make the news, and almost one hundred years on, its interactions with platelets are still not fully understood.

The heparins are a heterogeneous group of sulphated mucopolysaccharides, obtained from porcine intestinal mucosa or bovine lung tissue. They work by binding to the epithelial cell surface membrane and potentiating the action of anti-thrombin III. Anti-thrombin III is a plasma protein inhibitor, which inhibits clotting factor proteins (forming 1:1 stable complexes). These complex-forming reactions are usually slow, but are accelerated 1000 times by heparin due to a conformational change in the anti-thrombin III. Heparin also inhibits factor Xa. The two main forms of heparin – UFH (unfractionated, high molecular weight) and low molecular weight (LMWH) have different properties; UFH has a higher affinity for anti-thrombin III, whereas LMWH inhibits Xa. Short term side effects of heparin include bleeding, transient thrombocytopenia and Heparin-induced thrombocytopenia (HIT) – the latter being caused by heparin-platelet interactions,

either non-immune or immune-mediated. In the long-term, heparin can cause osteoporosis.

1.3.7 Dextran

The mechanism of action of Dextran has, until recently, been unclear. It was first used in the 1950's as a plasma-volume expander, but was soon noted to confer beneficial anti-thrombotic properties. (Bergqvist, 1982). Subsequent studies have shown that Dextran reduces platelet adhesiveness, and increases lysability of *ex vivo*-formed thrombi. (Noorman et al, 1997). These effects were thought to be due to the effect of Dextran on Factor VIII as Dextran infusions decrease factor VIII related antigen and ristocetin co-factor activity.

It has also been suggested that the effects of Dextran are due to impairment of the factor VIII bound to platelet surface, preventing it's usual action of supporting platelet adhesion to the exposed sub-endothelium and initiating and stabilising ristocetin-induced platelet aggregates (Magnus et al, 1979). There is good evidence from recent work that Dextran is also an antagonist for the mannose receptor in the liver, thereby inhibiting tissue-type Plasminogen activator (tPA) binding and clearance. This leads to higher levels of free, active tPA in the circulation (Noorman et al, 1997). tPA is a potent fibrinolytic agent working by converting Plasminogen into plasmin, which then cleaves fibrin into soluble degradation products.

1.3.8 Warfarin

Warfarin is a derivative of coumarin, a chemical found naturally in many plants e.g. woodruff (Rubiaceae). It was originally developed commercially as a rat poison. It inhibits the enzyme epoxide reductase (which is responsible for the reduction of Vitamin K, necessary for the synthesis of coagulation factors II, VII, IX, X, Protein C and Protein S) in the liver.

A summary of anti-coagulant drugs is shown in Table 1.3.2

Table 1.3.2 Anti-coagulant Drugs

Classification		Specific agent	Mechanism of action
Thrombin Inhibitors	Indirect	Heparin	Binds to Anti-thrombin-III, inhibits thrombin and Factor Xa
		LMWH	Preferentially inhibits Factor Xa
	Direct	Hirudin	Binds at two specific sites on thrombin, inhibits both clot-bound and circulating thrombin
Oral anti-coagulant		Warfarin	Prevents modification of vitamin K dependant coagulation factors (II, VII, IX, X)

1.4 Evidence for the use of anti-platelet agents for stroke prevention

1.4.1 Overview

There have been many studies comparing different anti-platelet agents with both placebo groups and each other. In a meta-analysis of twenty-one randomised control trials involving over 18000 patients anti-platelet drugs gave a relative odds reduction of 28% (nonfatal) or 16% (fatal) when compared with placebo (Antithrombotic Trialists's Collaboration, 2002). Recent National Institute for Clinical Excellence (NICE) guidelines recommend the combination of modified release (MR) Dipyridamole and Aspirin in people who have had an ischaemic stroke or a transient ischaemic attack for a period of 2 years from the most recent event as part of the prevention of occlusive vascular events. After this period of time, or if MR Dipyridamole causes adverse effects, they recommend long-term treatment with low-dose aspirin. Clopidogrel alone should be used as part of prevention of occlusive vascular events in people who are intolerant of low-dose aspirin and either have experienced an occlusive vascular event or have symptomatic peripheral arterial disease (NICE guidelines May 2005).

Four anti-platelet agents have been shown to decrease the risk of further ischaemic stroke in patients who have suffered TIA or stroke. The evidence for each will be discussed.

1.4.2 Aspirin

Two randomised control trials comparing different doses of aspirin for preventing further stroke after a patient has suffered a completed stroke or TIA have shown that all doses are effective at reducing events, but higher doses increase the risk of gastro-intestinal bleeding.

Meta-analyses of more than fifty secondary prevention trials have shown aspirin to prevent death from cardiovascular disease by approximately 15% and non-fatal vascular events by about 30% (Anti-platelet trialists' Collaboration 1994) and that anti-platelet (Aspirin) therapy compared with placebo reduces the risk of stroke by 23% in patients with a history of TIA or stroke. Aspirin remains the standard first-line treatment for the prevention of vascular events, though the dose is still a question of much discussion, with advocates for doses ranging from 37.5mg to 1300mg. (Usually 75 to 150mg is recommended).

1.4.3 Ticlopidine

There have been three randomised control trials involving Ticlopidine. The Canadian American Ticlopidine Study (CATS) looked at 1053 patients with previous ischaemic stroke and compared Ticlopidine 250mg twice daily with placebo. There was a relative reduction in risk of 23% with Ticlopidine.

The Ticlopidine Aspirin Stroke Study (TASS) compared Ticlopidine 250mg twice daily with aspirin 650mg twice daily in 3069 patients with recent minor stroke or TIA. The study showed a 21% relative risk reduction in stroke over 3 years. In a study of 1800 black patients with recent stroke– the African American aspirin Stroke Prevention Study (AAASPS), Ticlopidine 250mg twice daily was no better than Aspirin 650mg once daily in preventing stroke, MI or vascular death over a 2 year period.

In all of these studies, however, important side effects of Ticlopidine were noted. These included diarrhoea, rash, bleeding or neutropenia (2%). Severe neutropenia affected less than 1% of patients and was usually reversible.

1.4.4 Clopidogrel

The Clopidogrel versus Aspirin in Patients at risk of ischaemic events (CAPRIE) randomised 19000 patients with stroke, MI or peripheral vascular disease to aspirin 325mg or clopidogrel 75mg once daily. Overall there was a relative risk reduction of 8.7% for stroke, MI or vascular death with Clopidogrel rather than Aspirin. However, on subgroup analysis for stroke patients the benefit became non-significant. The authors concluded that there was perhaps more relative benefit with clopidogrel in those patients with pre-existing stroke or MI. Clopidogrel has fewer side effects than Ticlopidine, and neutropenia was not reported.

1.4.5 Combination Dipyridamole and Aspirin

Several smaller studies have compared Aspirin with Dipyridamole and evaluated them as dual therapy. In the French Toulouse Study, 440 patients with previous TIA were randomised to aspirin 900mg once daily, aspirin with dihydroergotamine, aspirin with dipyridamole or dihydroergotamine alone. No significant difference in outcome was observed between the groups.

The Accidents Ischemiques cerebraux liés à l'athérosclérose (AICLA) trial randomised 604 patients with previous TIA or Stroke to placebo, aspirin 1g once daily, or aspirin 1g once daily with dipyridamole 225mg once daily. Aspirin or aspirin with dipyridamole reduced the risk of further stroke with no apparent additional benefit for the added dipyridamole.

The first European Stroke Prevention Study (ESPS-1) compared placebo with a once daily dose of 975mg of aspirin plus once daily dipyridamole 225mg in 2500 patients. A reduced risk of stroke of 38% and reduced risk of death 33% were seen in the treatment group.

The second study - ESPS-2 took 6602 patients with prior stroke or TIA, and randomised them into four groups to compare Aspirin 50mg plus Dipyridamole 400mg once daily, Aspirin alone, Dipyridamole alone and placebo. The overall stroke risk was reduced by 37% for combination treatment, 18% Aspirin alone and 16% Dipyridamole alone.

1.4.6 Combination Clopidogrel and Aspirin

7599 patients with previous stroke or TIA were given either Clopidogrel 75mg once daily or Clopidogrel 75mg with aspirin 75mg once daily in the MATCH trial (Management of AtheroThrombosis with Clopidogrel in High-risk patients with TIA or Stroke). The composite primary endpoint was ischaemic stroke, MI, vascular death or re-hospitalisation secondary to ischaemic events. No significant benefit of combination therapy over clopidogrel alone was seen, but this group did show an increased risk of major haemorrhage, with 1.3% increase in life-threatening bleeding.

A summary of the recommendations for anti-platelet therapy is shown in table 1.4.1

Table 1.4.1 Summary of Recommendations for Anti-platelet therapy in patients at risk of non-cardiogenic thrombo-embolic stroke

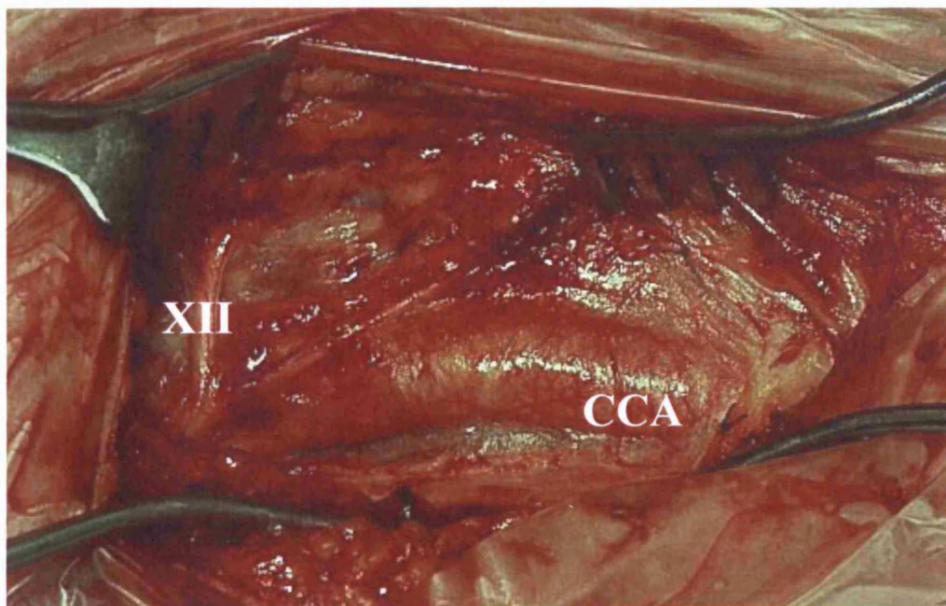
Recommendations	Class/ Level of Evidence
For patients with non-cardio embolic ischaemic stroke or TIA, anti-platelet agents rather than oral anti-coagulation are recommended to reduce the risk of recurrent stroke and other cardiovascular events	Class I Level A
Aspirin 50 to 325 mg daily, the combination of Aspirin and extended-release Dipyridamole, and Clopidogrel are all acceptable options for initial therapy	Class IIa Level A
Compared with Aspirin alone, both the combination of Aspirin and extended-release Dipyridamole, and Clopidogrel are safe. The combination of Aspirin and extended-release Dipyridamole is suggested over Aspirin alone	Class IIa Level A
Clopidogrel may be considered over Aspirin alone on the basis of direct-comparison trials. Selection of an anti-platelet agent should be individualised based on patient risk factor profiles and tolerance	Class IIb Level B
Addition of Aspirin to Clopidogrel increases the risk of haemorrhage and is not routinely recommended for ischaemic stroke or TIA patients	Class III Level A
For patients allergic to Aspirin, Clopidogrel is reasonable	Class III Level A
For patients who have an ischaemic event while taking Aspirin, there is no evidence that increasing the dose of Aspirin provides additional benefit. Although alternative anti-platelet agents are often considered for non-cardio embolic patients, no single agent or combination has been well studied in patients who have had an event while receiving Aspirin	Class IIa Level B

1.5 Carotid Endarterectomy

1.5.1 Introduction

As described previously, 80% of all strokes are caused by ischaemia, with up to 50% of these being due to thrombo-embolic occlusion of the internal carotid or middle cerebral arteries (Dennis et al, 1990). Atherosclerotic plaques are typically found at areas of turbulent flow, i.e. arterial bifurcations. Carotid disease is usually seen at the origin of the internal carotid artery, where the common carotid artery divides into its internal and external branches (figures 1.5.1). Atherosclerotic stenosis of the carotid artery can cause a stroke despite best medical therapy, particularly when the atherosclerotic plaque ruptures or ulcerates exposing the highly thrombogenic core. This can precipitate thrombosis with occlusion or embolisation of platelet thrombi, with the resultant disruption to cerebral perfusion.

Figure 1.5.1 Photograph of carotid artery anatomy



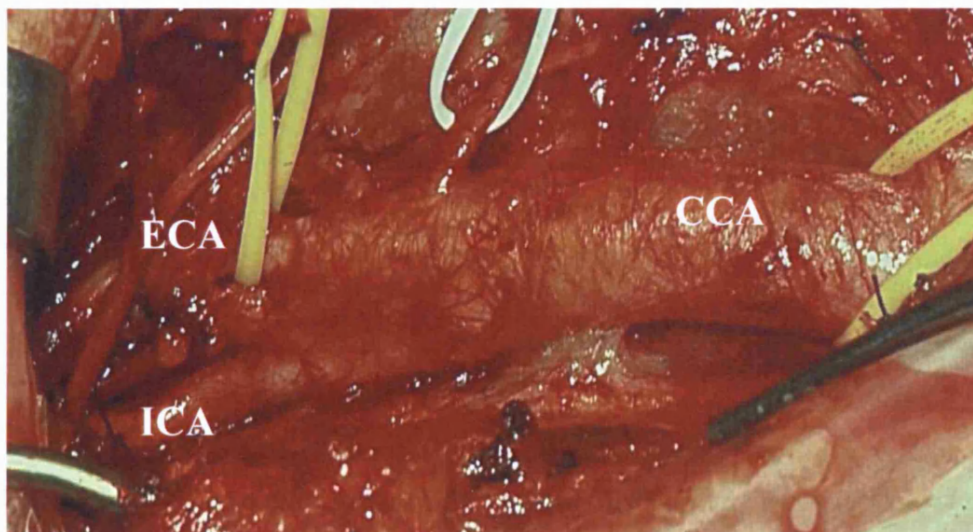
The figure shows the Common Carotid Artery (CCA) and Hypoglossal or Twelfth Cranial Nerve (XII) during carotid endarterectomy. Photograph courtesy of Professor AR Naylor.

1.5.2 Description of Carotid Endarterectomy

The role of surgery to remove atheromatous plaques from the internal carotid artery and so prevent stroke, has evolved over the last fifty years, following the first surgical reconstruction of the carotid artery, reported in 1954 by Eastcott et al in 1954.

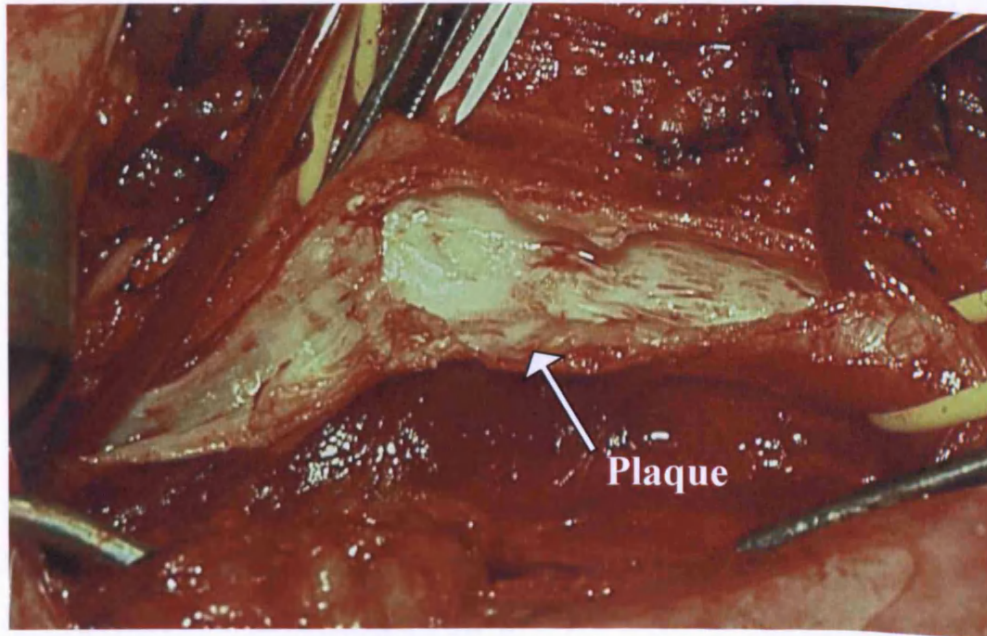
To simplify, carotid endarterectomy involves an incision along the anterior border of the sterno-cleidomastoid muscle, dissection of deeper structures of the neck to expose the vessels with subsequent arteriotomy and removal of the atherosclerotic plaque. The endarterectomy zone is then washed; the arteriotomy closed and flow to the brain restored (figures 1.5.1-5). There are many controversies relating to the peri-operative management of CEA patients, and to the intra-operative techniques, such as general anaesthetic versus local anaesthetic, routine or selective shunting, and methods of arteriotomy closure and monitoring. These debates and relevant evidence will be discussed later in this chapter. The Leicester practice for CEA and peri-operative patient management will be outlined in more detail at the end of the section on monitoring and quality control, after the studies leading up to our current practice have been described.

Figure 1.5.2 Photograph of Common Carotid Artery and its branches



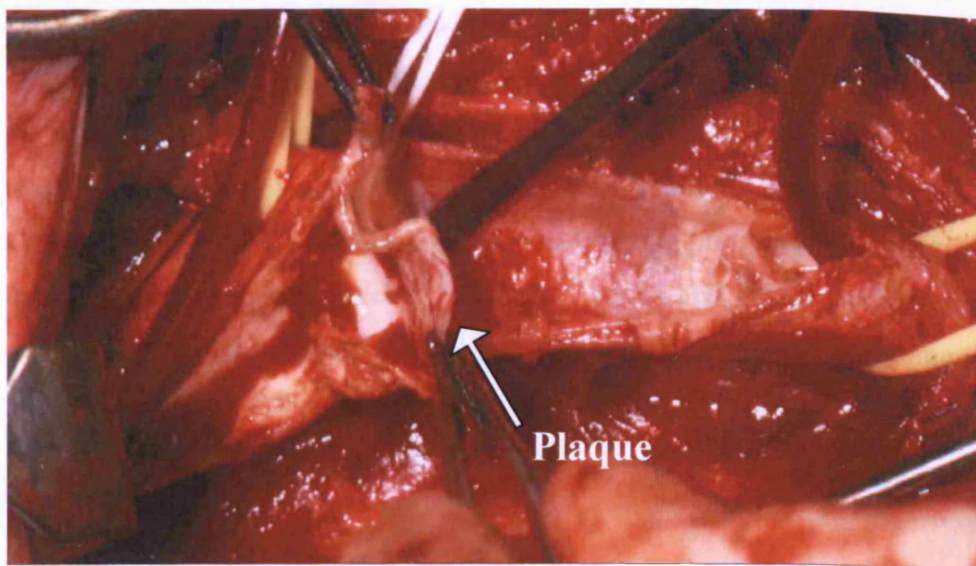
The figure shows the external (ECA) and internal (ICA) branches of the common carotid artery (CCA). Photograph courtesy of Professor AR Naylor.

Figure 1.5.3 Photograph of an atherosclerotic plaque in the carotid artery



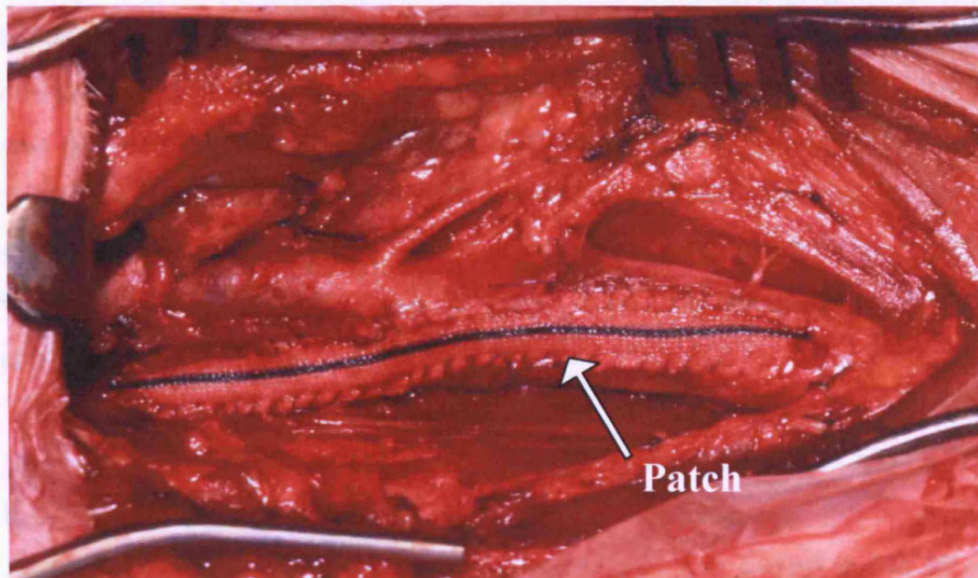
The figure shows an atherosclerotic plaque (marked with the white arrow) within the carotid artery. Photograph courtesy of Professor AR Naylor.

Figure 1.5.4 Atherosclerotic plaque being removed from the carotid artery



The figure shows an atherosclerotic plaque marked with the white arrow being removed from the carotid artery. Photograph courtesy of Professor AR Naylor.

Figure 1.5.5 Patch Closure of the Carotid Artery following CEA



The figure shows a completed Carotid Endarterectomy with Dacron patch closure marked with the white arrow. Photograph courtesy of Professor AR Naylor.

1.5.3 Patient Selection, Risk and Benefit of Carotid Endarterectomy

Controversy over the benefit of CEA and its role in stroke prevention led to several randomised trials. ECST and NASCET, two important and detailed trials have given specific information about the risk–benefit balance of CEA in symptomatic patients, compared with best medical management alone. Because the trials used different methods of measuring and defining the degree of carotid artery stenosis, the studies could not be directly compared. To amalgamate the results of these two trials plus the results of the VA studies, the Carotid Endarterectomy Trialists Collaboration (CETC) re-measured stenoses using the NASCET method. An overview of the results is given in table 1.5.1. ACST and ACAS studied outcomes of CEA in patients with asymptomatic carotid stenosis with results summarised in table 1.5.2. The evidence from these trials guides patient selection for surgery and is reviewed in the following paragraphs.

European Carotid Surgery Trial (ECST)

Began in 1981, ECST included patients with carotid stenosis who had suffered a TIA or stroke within the preceding six months and in whom the neurologist and surgeon were substantially uncertain of best treatment course. The trialists classified stenoses as mild (0-29%), moderate (30-69%) and severe (70-99%), based on angiographic evidence comparing the residual lumen diameter with an

estimated carotid bulb diameter. The final results of the study, published in 1998 included 3024 patients – 1811 (60%) randomised to surgery in the presence of best medical treatment and 1213 (40%) to medical therapy. The mean follow up period was 6.1 years. There was no significant benefit for surgery over best medical therapy in patients with mild or moderate stenosis (0-69%). However in symptomatic patients with a severe stenosis there was an absolute risk reduction of 9.6% (from 21.9% 3 year risk with no surgery to 12.3% with surgery). The relative risk reduction was 56%.

North American Symptomatic Carotid Endarterectomy Trial (NASCET)

In 1991, the interim results of, NASCET were published in the New England Journal of Medicine. This randomised control study was carried out in 50 centres across North America. Stenoses were graded as 30-69% or 70-99% based on the residual lumen compared with the normal ICA lumen above the lesion.

In effect, a stenosis of 50% in the NASCET trial equates to 64% in ECST and 70% NASCET to 85% ECST. Patients must have had related symptoms in the 180 days prior to surgery. No benefit from surgery was seen in the group with 30-69% stenoses. 659 patients with more severe narrowing were randomised. Of the 331 patients assigned to best medical therapy, 26% went on to suffer a stroke in the 2 year follow up period. Of the 328 patients treated surgically, 9 % had a stroke, giving an absolute risk reduction of $17 \pm 3.5\%$ ($p < 0.001$). The risk of death or disabling stroke was 13.1% in the medical group and 2.5% in the surgical, giving a risk reduction of $10.6 \pm 2.6\%$ ($p < 0.001$).

By the time the final results were published in 1998, the study had randomised 1108 patients to CEA and 1118 to medical therapy alone. The mean follow up period was 5 years and data 99.7% complete. Their primary outcome was any stroke. In the patients with 50-69% stenosis the 5 year stroke risk was 15.7% with surgery and 22.2% with medical management ($p = 0.045$). This is equivalent to a number needed to treat of 15. For those patients with stenoses of $< 50\%$, there was no significant difference between the two groups. For those with severe stenoses, the benefit of surgery remained at the eight year point.

Asymptomatic Carotid Atherosclerosis Study (ACAS)

This study of 1662 patients aged between 40 and 79 years with >60 % carotid artery stenosis showed that surgery conferred a benefit over best medical management – with the initial peri-operative period mortality of 2.3% for surgery and 0.4% for the medical arm moving in favour of surgery at 5 years, but the outcomes were predicted using Kaplan-Meier rather than actual. The predicted risk of stroke or death was 5.1% in the surgery group compared with 11% in the medical, giving an absolute risk reduction of 5.9% and relative risk reduction of 53% at 5 years ($p=0.0004$).

Asymptomatic Carotid Surgery Trial (ACST)

More recently, the results of ACST were published in the Lancet. This study, began in 1993 took 2120 asymptomatic patients and randomised them to immediate surgery and best medical management or best medical management and deferred CEA. 50% of those randomised to surgery had had their CEA within one month and 88% within one year. 4% of the control group per year became symptomatic and underwent surgery. The mean follow up was 3.4 years. The peri-operative risk of stroke or death from surgery was 2.8%. The 5 year stroke risk was 6.4% for the surgical group compared with 11% in the non-surgical group ($p<0.0001$). The study found that in patients over the age of 75years, 50 % will die of other causes within 5 years. The authors conclude that for patients with a stenosis of 70% or greater under the age of 75, a CEA performed immediately will halve the five year stroke risk from 12% to 6% provided that CEA is performed with a <3% risk of any stroke or death. It must be borne in mind that outside the stringent conditions of a clinical trial these results may not be reproducible unless strict selection criteria and high surgical standards are maintained.

Table 1.5.1 The CETC combined databases from ECST, NASCET and VA Studies when re-measured using the NASCET method

Stenosis (%)	Operative risk (%)	5 year risk of any stroke		ARR (%)	NNT	Strokes prevented per 1000 CEAs in 5 years
		Surgery	BMT			
<30	-	18.36	15.71	- 2.6	n/b	0
30-49	6.7	22.80	25.45	+ 2.6	38	26
50-69	8.4	20.00	27.77	+ 7.8	13	78
70-99	6.2	17.13	32.71	+ 15.6	6	156
string	5.4	22.40	22.3	- 0.1	n/b	0

BMT (best medical therapy alone); ARR (absolute risk reduction); RRR (relative risk reduction); NNT (number of CEAs performed to prevent one stroke). Reproduced from Naylor AR – An update on the randomised trials of intervention for symptomatic and asymptomatic carotid artery disease. Italian Journal of Vascular and Endovascular Surgery 2006

Table 1.5.2 Summary of results from ACST and ACAS

Any stroke at 5years (including peri-operative risk)								
	Stenosis (%)	30-day risk	CEA	BMT	ARR	RRR	NNT	Strokes prevented per 1000 CEAs at 5 years
ACST	60-99	2.8	6.4	11.8	+ 5.4	46	19	53
ACAS	60-99	2.3	12.4	17.5	+ 5.1	29	20	50
Ipsilateral stroke at 5years (including peri-operative risk)								
ACST	60-99	2.8	No data	No data	-	-	-	-
ACAS	60-99	2.3	5.1	11	+5.9	54	17	59

BMT (best medical therapy alone); ARR (absolute risk reduction); RRR (relative risk reduction); NNT (number of CEAs performed to prevent one stroke). Reproduced from Naylor AR – An update on the randomised trials of intervention for symptomatic and asymptomatic carotid artery disease. Italian Journal of Vascular and Endovascular Surgery 2006

1.5.4 Risks and complications of Carotid Endarterectomy

As with any surgical procedure, there are associated risks and complications. Indeed, CEA carries significant morbidity (1.5-25%) and mortality (1.5-8.7%) rates. (Matsumoto et al 1977, White et al 1981, Aldoori and Baird 1988, AbuRahma and Robinson 1988, McCrory et al, 1993, NASCET, ECST, ACAS, ACST). Complications may be wound-related, such as bleeding, haematoma formation, and dehiscence; cranial nerve damage (vagus, hypoglossal, accessory spinal or facial nerves), or the more significant cardiovascular complications: stroke, MI, seizures, hyperperfusion syndrome and death.

The ACAS peri-operative complication risk was low, reported as 2.3% overall of which 1.1% resulted from the angiogram. Ferguson 1999 reporting the NASCET surgical results declared a 1.8% 30 day risk of disabling stroke, 2.9% risk of disabling stroke or any cause mortality. For wound complications, the overall rate was 9.3%, 97% of which were mild or moderate. Nerve damage occurred in 8.6%, but was mild in 92% of cases, and affected the facial, vagus, spinal accessory or hypoglossal nerves. Nerve damage in a series of Leicester patients was 2.9-3.6% (Hayes et al, 2001).

Late complications include re-stenosis of the vessel, patch infection and aneurysm formation with rupture, which will not be discussed in any more detail in this thesis.

Stroke may be classified as intra-operative or post-operative, or by its aetiology – thrombotic, embolic or haemorrhagic. When monitoring a cohort of 658 patients undergoing CEA with EEG, Krul (1989) found that 6.4 % suffered a peri-operative stroke. Of these around a fifth were haemodynamic in origin, occurring in the watershed area. The remainder were thrombo-embolic occlusions in the middle or anterior cerebral artery territories. The causes of intra-operative stroke and methods of reducing its incidence will be described in the section on monitoring and quality control.

1.5.5 Controversies in Carotid Endarterectomy

Anaesthesia

CEA may be performed under general (GA) or loco-regional anaesthesia (LA). There is currently no Level 1 or Grade A evidence to prove that one anaesthetic technique is superior to the other. Surgeons preferring LA argue that the operation can be performed safely with the patient awake, and that the patient's conscious state is a superior marker of cerebral perfusion during CEA. It has also been suggested that the use of LA is associated with reduced peri-operative cardiovascular complications. Advocates of GA would suggest that the technique allows the surgeon time for a calm, controlled procedure, facilitates teaching of trainees, is more comfortable for the patient and is safe when used with appropriate monitoring and a skilled anaesthetist. Rerkasem et al reviewed the current evidence as part of the Cochrane Database. There have been seven randomised control trials giving a total of 554 operations and 41 non-randomised studies, eleven of which were prospective and another 29 were reported consecutive series. A meta-analysis of the non-randomised studies showed that the use of LA was associated with a significant decrease in the odds of death (in 35 studies), stroke (31 studies), stroke or death (26 studies), MI (22 studies) and pulmonary complications (7 studies) within the 30 day post-operative period. However, these are non-randomised studies, with the inherent associated bias.

A meta-analysis of the seven randomised studies revealed a non-significant trend towards reduced mortality within 30 days of surgery with the use of L.A. There was no difference in the reported stroke rate. There was also a decrease in post-operative haemorrhage with LA, but this was non-significant. However, constituent studies were relatively small, and have not secured a general consensus opinion. GALA (GA or LA for CEA), a large-scale multi-centre randomised control study comparing general or local anaesthesia for carotid endarterectomy, is currently underway, and aims to recruit 5000 patients. The results of this study should provide definitive evidence for best practice in the future.

Arteriotomy Closure

The arteriotomy may be closed primarily, with a vein patch (usually harvested from the long saphenous vein at the groin) or a synthetic patch (PTFE, Dacron).

Potential benefits of closing the arteriotomy with a patch include improved haemodynamics, increased calibre of the endarterectomised segment and decreased flow disturbance. However, the use of a patch increases operation time and is associated with complications related to patch type. (Carotid Artery Surgery. A problem-based approach. Edited by A Ross Naylor and William C Mackey). A meta-analysis by Counsell et al showed that both early and long-term outcomes were improved with the routine use of a patch over routine primary closure.

The different patch types have their merits and drawbacks. Autologous vein is usually harvested from the long saphenous at the groin (less risk of patch rupture than vein taken from the ankle) and is preferred by some surgeons as it is endothelialised tissue, with less thrombogenic potential and higher resistance to infection. Its use, however, is associated with a 0.5-1% risk of early rupture leading often to stroke or death. The vein may develop aneurysmal dilatation over time and there may be harvest-site complications such as infection and poor wound healing. Synthetic materials have the benefit of being immediately accessible without the problem of groin wound complications. They do however convey added risks of infection and potential increased thrombogenicity. A Cochrane review of the eight trials (1480 operations) comparing either vein with PTFE, vein with Dacron, or Dacron with PTFE has shown that the differences between the patch types are non-significant and that the risk of infection with synthetic patches is equivalent to the risk of rupture with vein. Bond et al in 2004 published a meta-analysis of 13 randomised controlled trials. Seven trials involving 1281 operations comparing primary closure with routine patching showed there was a decreased risk of stroke or death with patching both in the peri-operative period ($p=0.007$) and in the long-term ($p=0.004$). Patching also decreased the risk of arterial occlusion and recurrent stenosis. Of the eight studies comparing patch types, seven showed no difference in outcome at one year and one suggested that Dacron patches were associated with worse results than PTFE.

Shunting

Shunts (figure 1.5.8) may be used during the arteriotomy phase of surgery to maintain cerebral flow and protect against intra-operative haemodynamic stroke; these may be used selectively or routinely. There are no randomised trials proving the superiority of routine over selective shunting. However, there is a general

consensus that either strategy is preferable to never shunting. (Editorial Comment, Carotid Artery Surgery, A problem-based approach). When used routinely, it is estimated that 80% of shunts are unnecessary. However, proponents argue that in the absence of a perfect way of monitoring cerebral perfusion shunting is the most reliable practice. Routine use also implies well-practised technique to facilitate quick and safe insertion in the more difficult cases.

There has been no difference demonstrated between the different types of shunt available. (Wilkinson JM, 1997)

Figure 1.5.6 Placement of a shunt during CEA

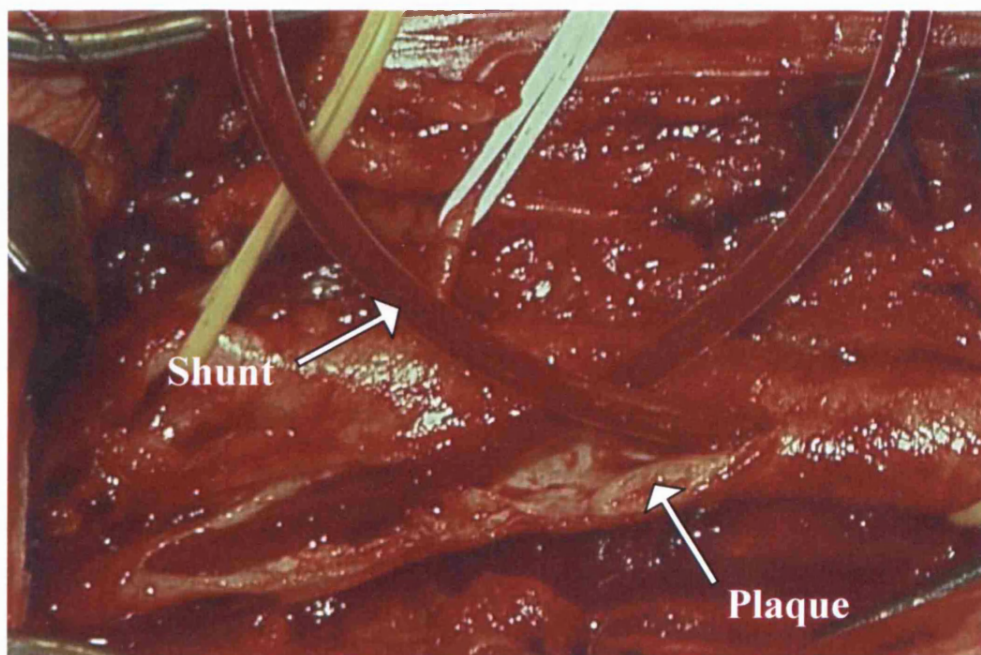


Figure shows shunt placement during carotid endarterectomy (the plaque and shunt are marked by white arrows). Photograph courtesy of Professor AR Naylor.

Anti-coagulation

There is little randomised trial evidence for anti-platelet and anti-coagulation regimes during carotid endarterectomy. Most surgeons continue Aspirin throughout the peri-operative period, but would exercise caution when Aspirin and Clopidogrel are prescribed in combination due to the increased risk of bleeding time and complications. (Payne et al, 2004, Dempsey et al, 2004)

In general, intravenous heparin is administered immediately prior to cross-clamping of the carotid arteries. This may be a standard dose (usually 5000 units) or may be tailored to patient size (for example 60-70 units per kilogram). Some units monitor

anti-coagulation by checking APTT intra-operatively, but most do not. There is evidence suggesting that non-reversal is associated with a higher incidence of neck haematoma (Treiman et al, 1990; Levison et al 1999) but a study by Mauney et al 1995 showed no difference. Moreover, this study showed that reversal of heparin's effect by giving protamine sulphate was associated with an increased risk of post-operative thrombotic stroke.

1.5.6 Monitoring and Detection of Technical Error

Once a patient has suffered an intra-operative stroke, there is little that can be done to alter outcome. Monitoring and detection of technical error are therefore of utmost importance in identifying patients at risk of impending stroke in order to prevent it.

Monitoring

Where a local anaesthetic technique is employed, the adequacy of cerebral perfusion is gauged by the patient's conscious level (awake testing). Within a few minutes of cross clamping the carotid, patients with inadequate cerebral perfusion via the Circle of Willis (from the contralateral carotid artery or vertebral arteries) will generally show signs such as loss of speech, motor function or consciousness. Under general anaesthetic the patient's exact neurological status is completely unknown. Many techniques have therefore been assessed, with the aim of detecting any hypo-perfusion of the brain early, to employ a shunt thereby reducing the risk of permanent neurological damage or intra-operative stroke.

One currently available technique is Trans-cranial Doppler monitoring (TCD). This low frequency (2MHz) ultrasound signal is focused on the middle cerebral artery (MCA) across the thinnest part of the skull – the temporal bone and gives an easy to interpret, real time display of blood flow velocity. This provides an “index” of cerebral blood flow, though does not account for changes in the diameter of the MCA.

Halsey et al (1989) described a mild risk of cerebral ischaemia when the measured middle cerebral artery velocity (MCAV) fell below 40% of the pre-clamp value and a severe risk if the MCAV dropped lower than 15%.

Although a relatively reliable method of monitoring, TCD is not possible in around 10% of people due to an inadequate “window” through the temporal bone. The technique also relies on the presence of a skilled technician to set up and interpret the signals during surgery. It has been suggested that for patients with recent cerebral infarct who cannot tolerate even minimally decreased cerebral blood flow this technique is not sensitive enough.

The electroencephalogram (EEG) monitors the electrical activity of the brain. In the presence of cortical ischaemia, the signals recorded tend to be of slower frequency and smaller amplitude. There is a reasonable correlation between the EEG changes and cerebral blood flow (Zampella et al, 1991), and its use in directing selective shunting leads to a reduction in peri-operative strokes (Plestis et al, 1997, Schneider et al, 2002). However, care must be taken when interpreting results, as they may be influenced by anaesthetic agents and physiological alterations (hypo- or hypercapnia, hypotension, hypothermia).

Somatosensory evoked potentials measures brain activity in response to stimulation of a peripheral nerve. Although changes are detectable in situations of reduced blood flow comparable to EEG (Prokop et al, 1996), the technique is of limited value when evaluating neurological defects. (Wober et al, 1998). The recordings are of sensory pathways only (not motor) and the technique may not be useful in patients with pre-operative neurological deficits. (Linstedt et al, 1998)

Using the same principle as the widely available finger and earlobe probes for pulse oximetry, Reflected Near-Infrared Light Spectrometry (or Trans-cerebral Oximetry) has been used to measure the oxygen saturation of blood in the cerebral circulation, (Kirkpatrick et al, 1995). Though it compares favourably with crude cerebral blood flow measurements, it is essentially an indirect measure of cerebral perfusion, with predominant recording from the superficial cerebral cortices (i.e. may miss hypoxia in the deeper brain structures such as internal capsule).

Other techniques for intra-operative monitoring of cerebral blood flow include recording stump pressure measurements – one of the first described techniques (Michel et al, 1966), but debate has arisen about its reliability and value (McKay et al, 1976). Calligaro and Dougherty (2005) studied 474 patients undergoing CEA

under LA and concluded that a carotid artery stump pressure of less than or equal to 40 mm Hg systolic may be considered as an equally reliable but more cost-effective method to predict the need for carotid shunting during CEA compared with EEG monitoring.

Intra-operative cerebral blood flow can be measured using intra-arterial injection of Xenon-133, an inert radioactive substance. Although this gives accurate blood flow information which correlates well with TCD monitoring (Halsey et al, 1986), it is rarely used, as it is expensive, relies on a technician and has potential hazards related to the use of radioactive material.

Quality Control

There are a number of potential complications that may occur during CEA, which if left unrecognised can lead to intra-operative stroke. These could be described as technical error or poor surgical technique and understandably, efforts have been made to find methods of detecting errors to facilitate correction.

Complications arising during the operation or in the early post-operative period are listed in table 1.5.3.

Intra-operative angiography can detect problems such as vessels stenosis but it can be difficult to obtain accurate images in the theatre setting with Valenti et al in 2003 reporting its accuracy to be less than colour duplex studies (Stendel et al, 1998). Both angiography and Duplex scanning however are expensive techniques, requiring trained staff and specialist equipment. Hand-held continuous wave Doppler can be used, but is less precise, subjective and practically difficult in some patients (Gaunt et al, 1994).

Table 1.5.3 Causes of peri-operative stroke that may be related to technical error

Intra-operative	Early Post-operative
Emboli during dissection Emboli on clamping Arterial spasm Intra-cranial disease Shunt Problems -Kinking Occlusion	Emboli / thrombosis from platelet thrombus formed on endarterectomised area Intimal flaps (from shunt insertion / intimal steps) Vessel kinks
Intra-cranial haemorrhage secondary to hypertension / hyperperfusion / rupture of intracranial aneurysm Intra-cranial thrombosis secondary to intracranial stenosis Distal carotid thrombosis	

Angioscopic examination prior to closure of the arteriotomy is fairly easy to perform and can identify intimal flaps, though may not detect arterial kinks or stenoses. (Mehigan and Alcott, 1986).

TCD monitoring during surgery and in the early post-operative period can detect lowered velocities in the middle cerebral artery and is a reliable method for the identification of particulate emboli and detecting shunt occlusion from kinking or thrombosis (Devuyst et al, 2001; Gaunt et al, 1994, 1998; Ghali et al 1997; Spencer 1997). Thus the TCD technique is useful both for monitoring and quality control.

1.6 Reducing the risk of peri-operative stroke: the Leicester experience

As discussed in this introduction, carotid endarterectomy is now a well recognised operation to reduce the risk of stroke in symptomatic and selected asymptomatic patients with significant carotid artery stenosis. Clearly the degree of benefit from surgery is directly related to the inherent risk of the procedure. If operative and peri-operative morbidity and mortality can be minimised, then the number of patients benefiting from surgery will increase. This is of particular importance in the asymptomatic group. One of the most devastating complications of CEA is stroke and we are therefore faced with the paradoxical situation where an operation aiming to prevent the condition can result in it.

In Leicester a quality-control programme was instituted in 1991 and has evolved over the subsequent years, virtually abolishing the risk of intra-operative stroke. This on-going work has laid the foundation for this thesis, and will be described in this section.

Gaunt's initial work (1994, 1996, 1998) aimed to compare angioscopy, hand-held continuous wave doppler (CWD), B-mode ultrasound (BMU) and trans-cranial doppler (TCD) modalities in the detection of intra-operative technical error, and their feasibility and usefulness at detecting the cause of the error. Of 100 consecutive patients undergoing CEA, 98 were assessed with angioscopy. Gaunt found that this was a sensitive method for identifying intimal flaps and intra-luminal thrombus prior to closure of the arteriotomy, thereby obviating the need for re-opening of the vessel. The equipment is operated by the surgeon, requiring no other specialist staff. Both CWD (91%) and BMU (76%) were less complete due to technical and practical difficulties with the equipment. They were also less sensitive at picking up intimal flaps and luminal thromboses. They did, however detect more arterial stenoses, but on follow up duplex scans, several were undetectable and the most significant of 30% had not progressed i.e. they were not clinically significant. The TCD also proved useful in detecting particulate emboli during the dissection phase in 23 patients and shunt malfunction in 15 patients. During the early post-operative period, six patients were found to have emboli. 50 % of these were mild and self-limiting, but in the other three the emboli heralded impending carotid thrombosis needing re-exploration. In all three, platelet thrombi were found on the recently endarterectomised zone. No technical errors had been detected with any modality during the operation.

Lennard followed on from these initial observations aiming to assess whether the introduction of a rigorous quality control programme could produce a consistent decrease in the intra-operative stroke rate, whilst assessing its feasibility and practicality. The study also assessed the incidence of sustained embolisation in the early post-operative period and trialled the use of Dextran 40 in reducing emboli and the progression to thrombotic stroke.

A prospective audit was performed of 133 consecutive patients. 91% of these had an accessible trans-cranial window allowing TCD monitoring and 94% underwent

completion angiography with a 5% technical error detection rate. During this time no patient suffered an intra-operative stroke suggesting that the quality control programme was working. During the post-operative monitoring, however, 5% of patients were found to have significant embolisation, but after receiving incremental Dextran 40, none progressed onto carotid thrombosis or stroke.

The standardised CEA technique and quality control methods used in Leicester will be described.

CEA is performed under general anaesthesia, with systemic heparinisation (5000 units of unfractionated heparin (UFH)), using loupe magnification and routine shunting (Pruitt-Inahara). Where TCD is possible, the patient is monitored during the operation and for 3 hours post-operatively. Completion angiography is performed immediately prior to patch closure, thereby enabling correction of any identified defects. Patients with high rates of embolisation detected in the post-operative monitoring period are treated with Dextran-40. (Naylor et al, 2000)

Despite minimising technical errors during surgery and dramatically reducing the risk of intra-operative stroke, Gaunt and Lennard both showed that a small percentage of patients went on to develop high rates of embolisation in the early post-operative period. Left untreated, approximately 50% of these patients were at risk of developing carotid thrombosis and stroke. When these patients were re-explored, Gaunt showed that there were platelet-rich thrombi adherent to the endarterectomy zone, despite anti-platelet medication (usually aspirin) and heparinisation during surgery.

Hayes hypothesised that the risk of thrombo-embolic events may be patient-related and a number of pieces of evidence from his work supported this. Firstly, in studies of patients undergoing staged, bilateral CEA, patients who were high rate embolisers following the first operation tended to be high embolisers following the second (Hayes 2001). Secondly, platelet aggregation studies have shown that platelets of patients with higher rates of embolisation following CEA have a significantly higher aggregatory response to ADP than those patients with lower levels of embolisation (Hayes 2003). Thirdly, in a randomised study of 274 patients comparing the use of a vein or Dacron patch to close the arteriotomy, the type of patch had no influence on the rate or magnitude of post-operative embolisation. (Hayes et al, 2001). Finally, flow cytometric studies and platelet aggregometry

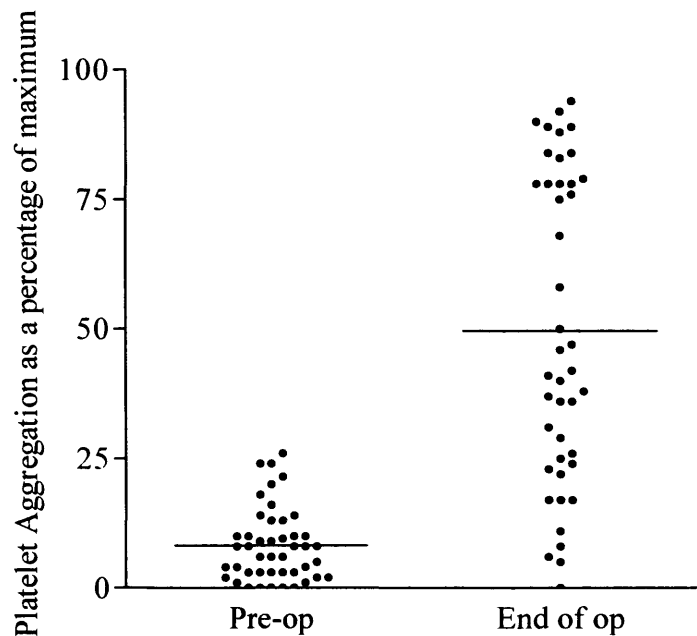
showed that the platelets of patients with high numbers of emboli pre- and post-operatively showed increased aggregatory response to stimulation with ADP.

This body of evidence suggests that in the absence of technical error, the risk of post-operative thrombotic stroke may be patient-mediated and platelet-dependant. Further work then looked at the platelet mechanisms behind thrombus formation and pharmacological means to reduce platelet aggregation without causing increased morbidity due to bleeding complications. Payne performed platelet aggregation studies on patients randomised to either placebo or a single dose of Clopidogrel (ADP-blocking anti-platelet drug) pre-operatively, in addition to the patient's usual daily dose of aspirin. (Payne et al, 2004). The study showed that a single dose of Clopidogrel (150mg) led to a >20 fold relative reduction in the post-operative emboli count with an 8.8% reduction in ADP-stimulated platelet activation. An increased skin closure time was noted, reflecting the increased time for haemostasis, but there was no increase in the bleeding complications (post-operative haematoma or return to theatre for bleeding).

An incidental and additional finding from this study was that despite an adequate dose of regular aspirin patients exhibited increased platelet aggregation (from 8.0 +/-7.1% to 42.0 +/- 29.7%, $p<0.0001$) in response to arachidonic acid in the post-operative period. (See figure 1.6.1)

This finding totally contradicted all convention regarding the mechanism of action of aspirin i.e. that it irreversibly acetylates cyclo-oxygenase, thereby blocking that enzyme pathway for the lifetime of each platelet.

Figure 1.6.1 The increased platelet aggregation seen during CEA (Payne et al, 2004)



The figure shows a significant rise in platelet aggregation from 8.2 +/- 7.1% pre-operatively to 49.7 +/- 29.7% at the end of carotid endarterectomy ($p < 0.0001$). Each dot represents a patient.

This phenomenon could be of crucial importance in explaining why a small proportion of patients are at higher risk of post-operative thrombotic stroke, despite having a technically adequate operation and being on an appropriate dose of Aspirin (Lennard et al, 1999). The finding that aspirin may be transiently ineffective at preventing platelet aggregation during CEA and that this may contribute to the risk of peri-operative thrombotic complications, forms the basis of the work for this MD thesis.

1.7 Description of studies undertaken

1.7.1 Overview

The work in this thesis builds on previous studies outlined in Chapter 1. Initially, a randomised control trial was initiated to compare the effects of Dextran as an irrigant solution during CEA with the standard Heparinised Saline. The main studies looked at platelet aggregation, particularly via the COX enzyme pathway during CEA, aiming to firstly identify the time point at which aggregation to arachidonic acid resumes; and secondly to identify the mechanism behind this. Thirdly, a pilot study comparing the effects of Unfractionated Heparin (standard) with Low Molecular Weight Heparin during CEA was undertaken.

1.7.2 Dextran Solution as an irrigation fluid during CEA

Intravenous Dextran 40 solution has been shown to reduce the risk of post-operative stroke in patients with high numbers of post-operative emboli. Intravenous Dextran therapy however, is associated with complications, occasionally precipitating heart failure and increasing bleeding. It was hypothesized that more targeted use of Dextran – applied locally to the operative field - would be as effective at preventing emboli, but have a lower incidence of systemic complications.

There have been anecdotal reports of the use of Dextran as a solution with which to irrigate the endarterectomy zone leading to a reduction of post-operative emboli and carotid thrombosis. If Dextran applied locally to the highly thrombogenic endarterectomy zone could reduce platelet adherence, it may significantly reduce the risk of post-operative thrombotic stroke and remove the need for intensive post-operative TCD monitoring, (which is currently impractical for many small units).

The hypothesis for this study was that local application of Dextran solution to the endarterectomised zone would reduce the number of emboli detected by Doppler monitoring of the MCA in the early post-operative period. A randomised trial comparing the effects of Dextran 40 in normal saline irrigation with conventional Heparinised Saline solution was undertaken. Post-operative emboli were quantified and compared.

1.7.3 Time Course Study

As part of the “control” studies in the earlier work on ADP receptor function, aggregation studies in response to arachidonic acid (AA) were performed before, during and after CEA. Very surprisingly, these studies showed a significant increase in aggregation in response to arachidonic acid (the substrate for the cyclo-oxygenase pathway) by the end of the operation. This contradicted all current evidence on the mechanism of action of aspirin (i.e. that it irreversibly acetylates the Ser-529 residue of cyclo-oxygenase rendering the enzyme inactive for the lifetime of each platelet).

This study was undertaken to further investigate this phenomenon. The principal aims were first to confirm that the increased aggregation noted in earlier studies was not simply an artefact and second, to determine the exact time-point at which the changes in platelet aggregation to AA were occurring. The platelet function of forty-one patients undergoing CEA was assessed at eight peri-operative time points. These time points were chosen to correspond with physiological or pharmacological changes that could potentially be underlying the increased platelet aggregation seen in response to arachidonic acid.

1.7.4 Mechanism behind the loss of effectiveness of Aspirin

Having demonstrated that the anti-platelet effect of aspirin was greatly reduced following the administration of unfractionated heparin during CEA, work was directed at identifying the mechanism(s) responsible. For this part of the study, blood tests were taken at three peri-operative time points – pre-operatively, 3 minutes following administration of UFH and 2 hours post-operatively. Samples from patients were studied, with addition of in vitro aspirin, heparin, COX-2 blocking agent, Thromboxane receptor blocker, ABCIXIMAB and excess AA. Tests were carried out using the PFA-100 and ELISA kits were used to measure TxB2 and 12-HETE.

1.7.5 A randomised control trial comparing UFH with LMWH

There is some evidence that LMWH causes less platelet activation than UFH. This study postulated that, unlike UFH, LMWH would not cause a reduction in aspirin's effect on platelet aggregation when AA was used as a substrate. Fifty patients were randomised to receive either UFH or LMWH. Samples were taken at three peri-operative time points – pre-operatively, 3 minutes following administration of heparin and 2 hours post-operatively. Aggregation to AA was tested and ELISA kits used for the measurement of TxB2 and 12-HETE.

Chapter 2 Time Course Study: When during CEA does Aspirin lose its anti-platelet effect?

2.1 Background and Objectives

Payne et al, 2004 studied platelets of patients undergoing CEA. These patients were enrolled in a randomised controlled trial to compare the effects of aspirin alone versus aspirin plus a single pre-operative dose of clopidogrel. Aggregation was tested in response to ADP, AA and TRAP, pre- and post-operatively. Very surprisingly, these studies showed a significant increase in aggregation in response to arachidonic acid (the substrate for the cyclo-oxygenase pathway) by the end of the operation. This finding contradicted all current evidence on the mechanism of action of aspirin i.e. that it irreversibly acetylates the Ser-529 residue of cyclo-oxygenase rendering the enzyme inactive for the lifetime of each platelet. (Roth et al, 1975).

A study was designed to elucidate the timing of the apparent loss of “aspirin’s activity”. The principal aims were first to repeat the aggregation studies to confirm that this was not an artefact and second, to determine the exact time-point at which the change in platelet aggregation to AA was occurring.

There are several distinct times during CEA when the patient platelet function could potentially be altered, beginning with the general anaesthetic which can affect platelet aggregation. However, most current evidence would tend to suggest that anaesthetic drugs exert an inhibitory, rather than pro-aggregatory effect on platelet aggregation (Hirakata et al, 1995; Mendez et al, 2003; Dordoni et al, 2004). This is followed by the initial stages of soft tissue dissection, with release of tissue factor and stress hormones in response to surgery. Tissue factor, a potent coagulation stimulant, is released during surgery and binds factor VII in plasma (in the presence of calcium) leading to increased factor X activity and generation of thrombin. Adrenaline and noradrenaline released in response to psychological or physiological stress can enhance platelet function, (Lalau et al, 1988; Larsson et al, 1994). Prior to clamping of the carotid artery in preparation for the endarterectomy phase of the operation, patients are given intravenous unfractionated heparin (UFH). Heparin is known to cause platelet activation, by way of an interaction with

platelet factor IV on the platelet surface (Dawes et al, 1982, Horne & Hutchison 1998; Newman & Chong, 2000), but has not previously been described as having a specific effect on the COX pathway, or the effects of aspirin. It is possible that platelet activation could be occurring during the clamping of the artery and/or following insertion of the synthetic shunt used to supply blood to the cerebral circulation during clamping. Finally, the additive effects of all the aforementioned factors may explain the increased platelet aggregation in response to arachidonic acid.

By sampling patients' blood at set peri-operative time points, this study aimed to investigate the effects of these various physiological and pharmacological changes on platelet aggregation, particularly the response of the aspirinated platelet to *ex vivo* stimulation with arachidonic acid.

Angioplasty Control Group

As described, Aspirin's effect on the platelet has previously been believed to be permanent for the lifetime of that platelet. It was considered, therefore, that the observed changes may be artefactual, related to some part of CEA. As a control group, patients undergoing peripheral artery angioplasty were studied. This group by definition have a diagnosis of peripheral vascular disease, and the majority will be prescribed aspirin. The angioplasty itself involves a similar degree of vascular endothelial disruption and the use of UFH, but without other factors that may bring about physiological changes, such as general anaesthesia or use of a shunt.

2.2 Methods

2.2.1 Patients

Ethical committee approval was granted and all patients gave their informed consent. A consecutive series of patients undergoing elective carotid endarterectomy were studied. Forty-one patients fitted the inclusion criteria for the study (see below).

A second group of eighteen patients taking aspirin and undergoing lower limb angioplasty were also studied. These patients were also (by definition) vasculopaths undergoing a procedure leading to a similar endothelial disruption, but with minimal soft tissue dissection, and under a local anaesthetic. The same inclusion / exclusion criteria applied to this group, as outlined below.

All patients were taking 150mg of aspirin for at least 4 weeks prior to angioplasty / surgery. Patients who had taken other anti-platelet drugs in the pre-operative period (Dipyridamole, Clopidogrel, NSAIDS) were excluded, as were those with abnormal platelet count or any haematological disease. If the patient's pre-operative aggregometry showed a significant response to AA, they were presumed to be either non-compliant or "aspirin-resistant" and excluded from the study.

2.2.2 Carotid Endarterectomy

All patients underwent CEA under normocarbic, normotensive general anaesthesia, performed by a consultant vascular surgeon or supervised vascular trainee. A bolus of 5000 units of unfractionated heparin was given prior to clamping of the carotid artery and a policy of routine shunting, completion angiography and peri-operative trans-cranial Doppler monitoring was employed in all patients. (Naylor et al, 2000)

2.2.3 Angioplasty

Lower limb angioplasty was performed in 18 patients with symptomatic iliac or superficial femoral artery atherosclerosis. The procedures were carried out under local anaesthetic, with Omnipaque contrast (Amersham, UK) and a bolus of 3000 units of unfractionated heparin given prior to the angioplasty.

2.2.4 Blood Collection

As described, it was hypothesised that the changes in platelet aggregation occurred at a specific time point during CEA – e.g. following induction of anaesthesia, soft tissue dissection, heparin administration, blood flowing through the synthetic shunt or the physiological stress of surgery. The following time points were therefore selected to correspond with changing events during surgery. For the CEA patients, the samples were taken at eight peri-operative time points.

- A. Pre-operative, on admission to hospital
- B. After induction of anaesthesia, but prior to skin incision
- C. Following skin incision and soft tissue dissection, but prior to heparinisation
- D. Three minutes after heparin was administered, prior to the insertion of the shunt
- E. Three minutes after shunt opening

- F. At the end of surgery, after flow restoration
- G. Four hours post-operatively
- H. 24 hours post-operatively, but prior to the next dose of Aspirin

Blood from the patients undergoing angioplasty was analysed at five similar time points.

- A. Pre-procedure
- B. Following the angiogram
- C. Three minutes after heparin was given
- D. Three minutes after the angioplasty
- E. Four hours post-procedure

Blood samples were taken into vacutainer tubes (Becton Dickinson, Oxford, UK) with a standardised technique (Harker & Zimmerman, 1983) to prevent artefactual platelet activation. The first 4 mls were taken for full blood count and subsequent samples into 4.5mls 0.9% citrated tubes. All samples were analysed within 2 hours of collection. The initial pre-procedure samples were taken with venepuncture, with subsequent samples being taken from the radial arterial line (CEA) or the femoral line (Angioplasty).

2.2.5 Platelet Aggregometry

There are several techniques available for measuring platelet activation and aggregation. A well-established and recognised technique is Born Aggregometry, which measures light transmission through a stirred platelet suspension. As platelet aggregation occurs, the platelet rich plasma becomes less turbid and therefore allows the passage of more light. A multi-step process is seen following addition of agonist – shape change, primary (reversible) aggregation and secondary activation. The aggregation studies described in this thesis were performed in a four-chamber aggregometer (PAP4C, Bio Data Corp., Horsham, USA (figure 2.2.1)) by Born's method (1962).

Born aggregometry is a reliable technique if some quality control measures are employed. (Breddin, 2005). Firstly, the blood samples must be collected in a standardised fashion (see 2.2.4) and processed quickly. The aggregometer must be set to the same parameters and the agonists used must be batch prepared, stored

appropriately and allowed to equilibrate to room temperature before use (see below).

In these studies, the citrated blood was centrifuged at 900 rpm for 20 minutes at 20°C to obtain platelet-rich plasma (PRP). A sample of this PRP was further spun at 10G for 10 minutes to obtain 500µl platelet-poor plasma (PPP) for calibration of the aggregometer. The aggregometer was allowed to warm to 37°C, and then an aliquot of 450µl of PRP was placed into a cuvette and incubated for 10 minutes. Stir bars were added, and speed set to 900rpm. Agonists were then added and maximum aggregation achieved in ten minutes recorded. Spontaneous aggregation was also tested. Samples were run in duplicate.

Aggregation was recorded in response to the agonists arachidonic acid (AA) (2.5 and 5mmol/L, Sigma, Poole, Dorset), adenosine diphosphate (ADP) (0.1 and 0.5 µmol/L, Sigma) and thrombin-receptor activating peptide (TRAP, 3 and 6 µmol/L, PNAC Laboratory, University of Leicester) for 10 minutes.

Figure 2.2.1 Platelet Aggregometer



The figure shows a four-chamber platelet aggregometer – this model is the PAP4 supplied by BIODATA© (photograph from BIODATA©)

2.2.6 Statistical Analyses

Mean values and standard deviations are used for normally distributed data, with median values being use for those data not normally distributed. Analysis was performed using the paired student t-test for normally distributed data and the Mann-Whitney or Wilcoxon matched pairs test for those variables not normally distributed. GraphPad Prism 4 software was used for the analyses.

2.3 Results for Patients undergoing Carotid Endarterectomy

2.3.1 Patient demographics

Of the forty-one patients, 28 were male. 25 of the 41 CEAs were performed on the right side. The mean age was 69.6 (+/- 7.7) years. The average patient weight was 79.1 (+/- 12.9) kg. As expected, there was a high incidence of associated diseases, such as diabetes mellitus (19.5%), hypertension (78.0%) and previous myocardial infarction (14.6%). A little over half of the patients had previously been smokers, and one fifth were current smokers. These results are summarised in table 2.3.1. Ten of the 41 patients were asymptomatic.

2.3.2 Aggregation Results (summarised in table 2.3.2)

Spontaneous aggregation (figure 2.3.1)

At baseline, spontaneous aggregation was 1.8 +/- 2.3%, and rose to 4.4 +/-3.0%, following induction of anaesthesia ($p<0.0001$). To allow for this, further p values shown are for comparisons with the first sample following anaesthesia. There was a minimal rise to 4.7% +/-3.6% (not statistically significant) after 15 minutes of soft tissue dissection. After the bolus of UFH, spontaneous aggregation rose to 13.6 +/-9.6%, which was highly significant ($p<0.0001$). This increased degree of aggregation persisted until restoration of flow, and at 4 hours post-op there was still a tendency toward greater aggregation (5.4+/-4.3%), though this was not significant ($p=0.2335$). By 24 hours post-operatively, the spontaneous aggregation had returned to base levels.

Table 2.3.1 Demographics of patients undergoing CEA

Variable	Results (n=41)
Male : Female	28 : 13
Age (years)	69.63 +/- 7.66
Weight (kg)	79.05 +/-12.88
Diabetes	8 (19.51%)
Hypertension	32 (78.04%)
Previous MI	6 (14.63%)
Current smoker	8 (19.51%)
Ex smoker	22 (53.66%)
Never smoked	8 (19.51%)
Right : left	25 : 16
Asymptomatic:Symptomatic	10 : 31

Aggregation to AA (figures 2.3.2 & 3)

Pre-operative aggregation to AA (5mmol/L) was low reflecting the aspirinated state of the platelets (3.94+/-2.20%). There was a small but significant rise in aggregation (10.1+/-7.1; $p<0.0001$) following induction of anaesthesia, which was accounted for by an increase in spontaneous aggregation (figure 2.3.3). Following administration of heparin, but before insertion of the shunt, there was a highly significant ten-fold increase in aggregation to AA (45.1+/-29.3%; $p<0.0001$) (figure 2.3.3, table 2.3.2). This rise persisted into the early post-operative period (15.1+/-13.3%; $p<0.0001$), and in some patients for up to 24 hours post-operatively (11.2+/-9.0%). In the majority of patients aggregation had returned to near pre-operative levels by 24 hours *without* administration of further aspirin. The results described are for the 5mmol/L concentration of arachidonic acid, but the same trend is observed for the lower concentration. (Figure 2.3.2)

Aggregation to ADP (figures 2.3.4 & 5)

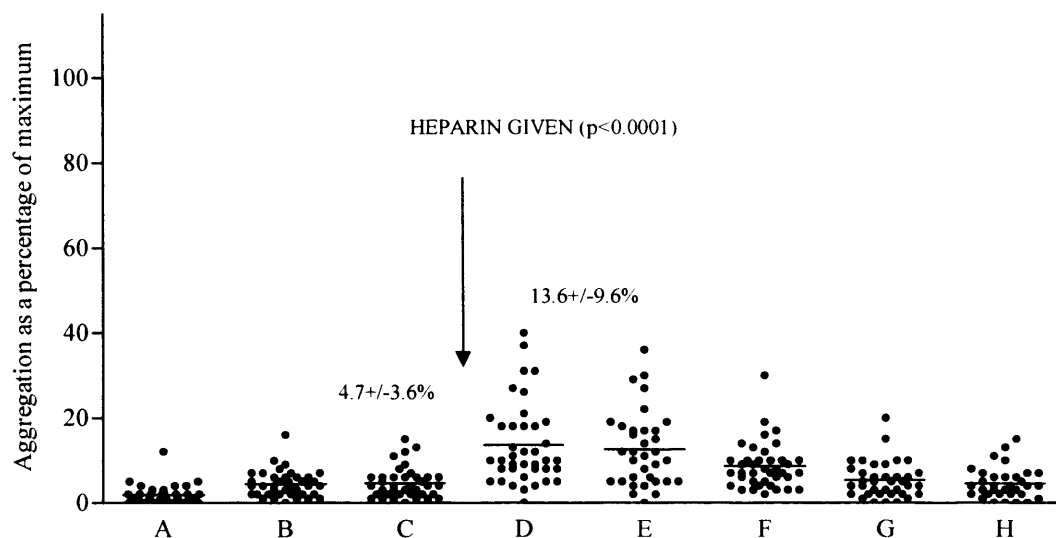
Similar rises in aggregatory response were seen in the samples stimulated with two concentrations of ADP, with aggregation to ADP (0.1micromol/L) rising from 42.1 +/- 21.5% after induction of anaesthesia to 57.1 +/-19.0% following UFH

administration ($p<0.0001$), figure 2.3.4. Aggregation to the higher dose of ADP followed a similar trend ($63.4 \pm 15.2\%$ to $75.9 \pm 13.2\%$). (Figure 2.3.5)

Aggregation to TRAP (figures 2.3.6. & 7)

Aggregation to TRAP ($3\mu\text{mol/L}$), figure 2.3.5, rose from $37.6 \pm 30.0\%$ to $53.6 \pm 27.4\%$ ($p=0.0011$) between induction of anaesthesia, and 2 minutes following the heparin. With addition of TRAP ($6\mu\text{mol/L}$), figure 2.3.6, aggregation rose from $65.4 \pm 23.9\%$ to $75.8 \pm 17.9\%$

Figure 2.3.1 Spontaneous Aggregation in patients undergoing CEA



The figure shows spontaneous aggregation as a percentage of maximum at time points A to H. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin)

Figure 2.3.2 Aggregation in response to 2.5mmol/L AA

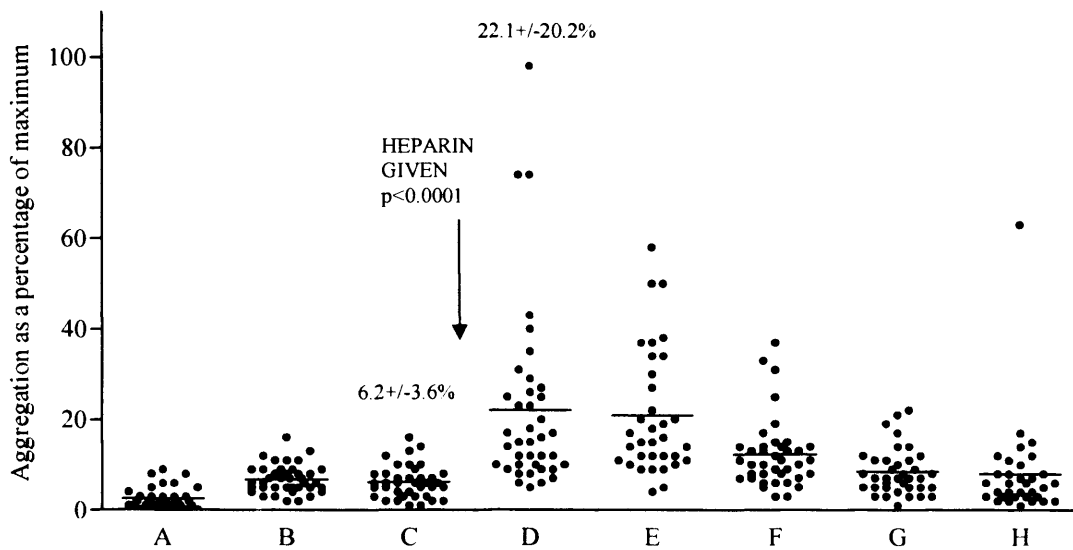
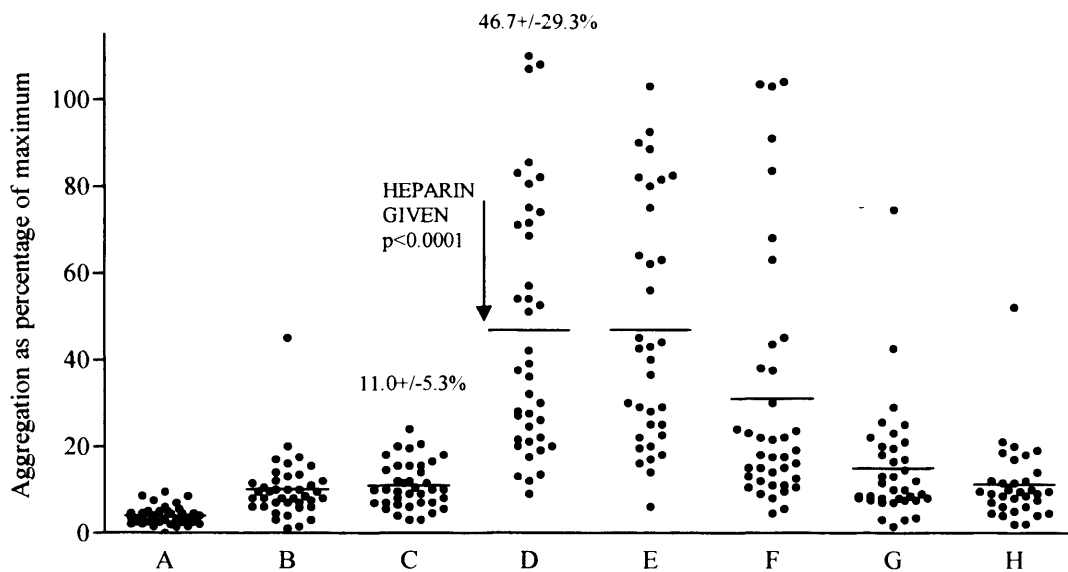


Figure 2.3.3 Aggregation in response to 5mmol/L AA



The figures show aggregation in response to AA 2.5 and 5mmol/L, as a percentage of maximum at time points A to H in patients undergoing CEA. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin)

Figure 2.3.4 Aggregation to 0.1 μ mol/L ADP

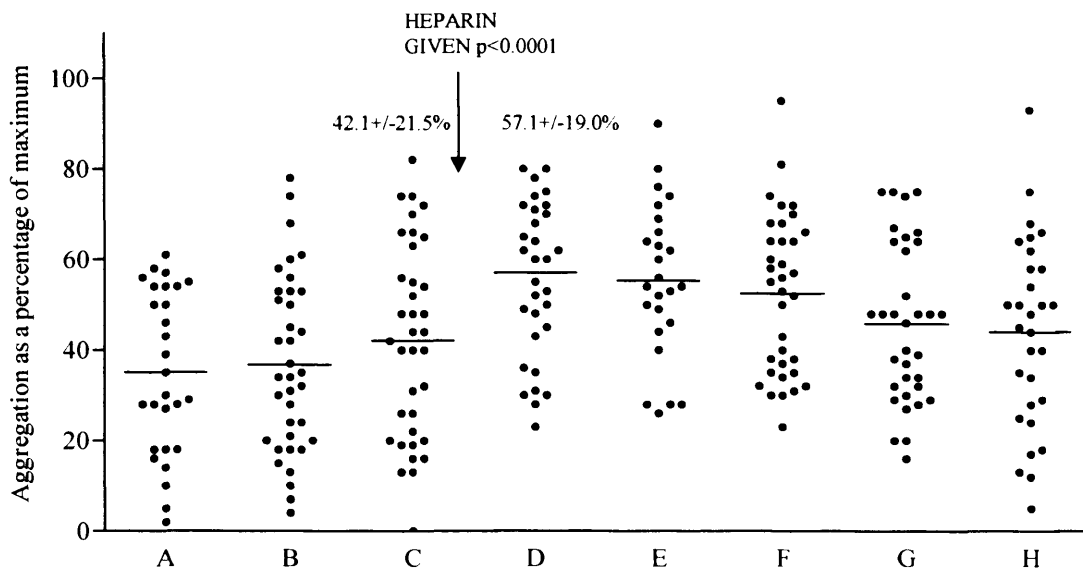
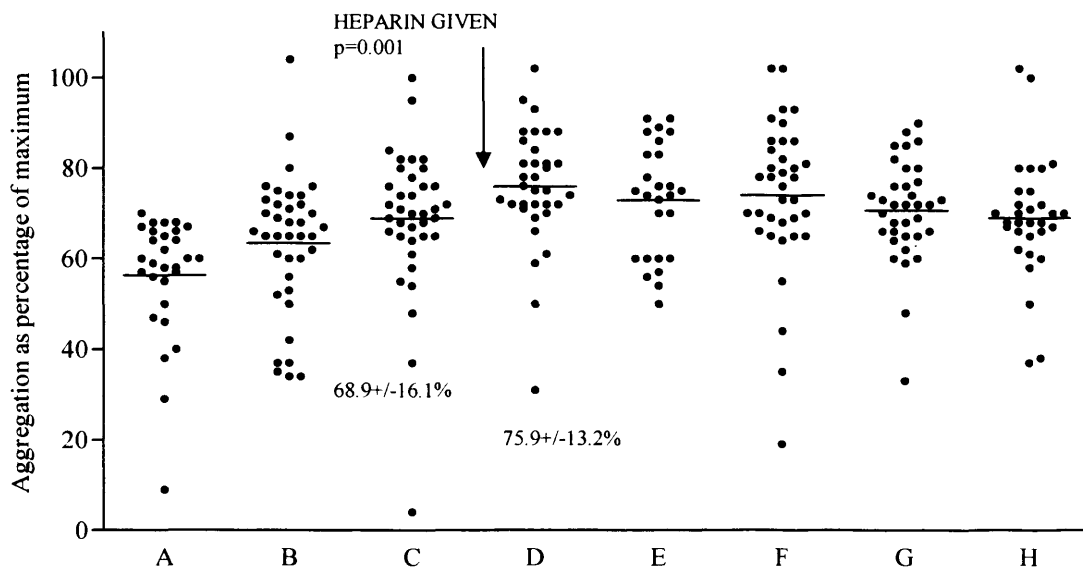


Figure 2.3.5 Aggregation to 0.5 μ mol/L ADP



The figures show aggregation in response to ADP 0.1 and 0.5 μ mol/L, as a percentage of maximum at time points A to H in patients undergoing CEA. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin).

Figure 2.3.6 Aggregation to 3 μ mol/L TRAP in patients undergoing CEA

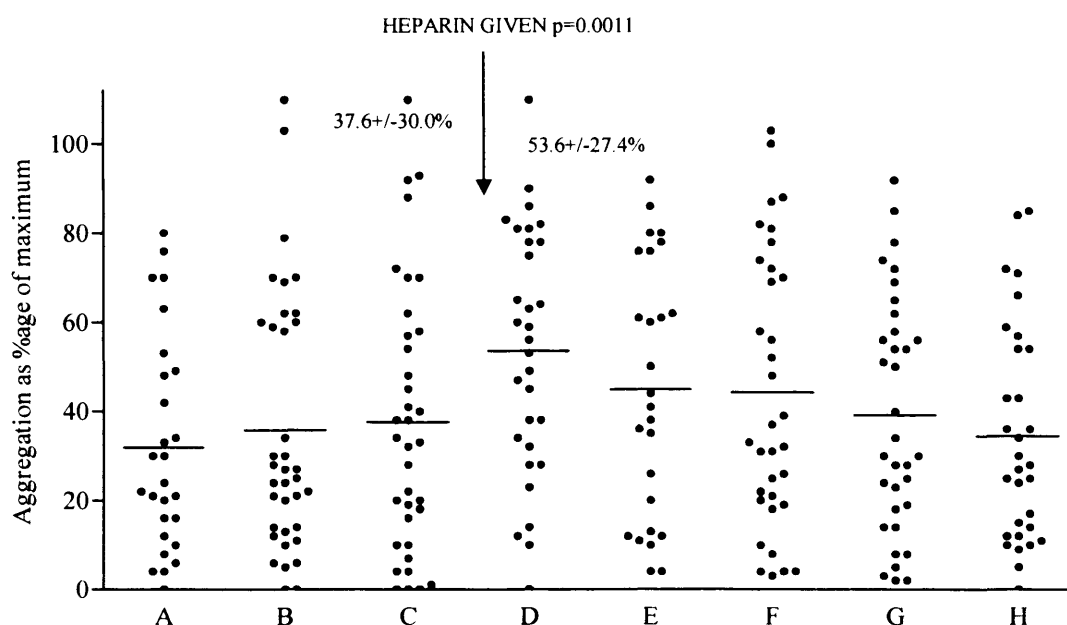
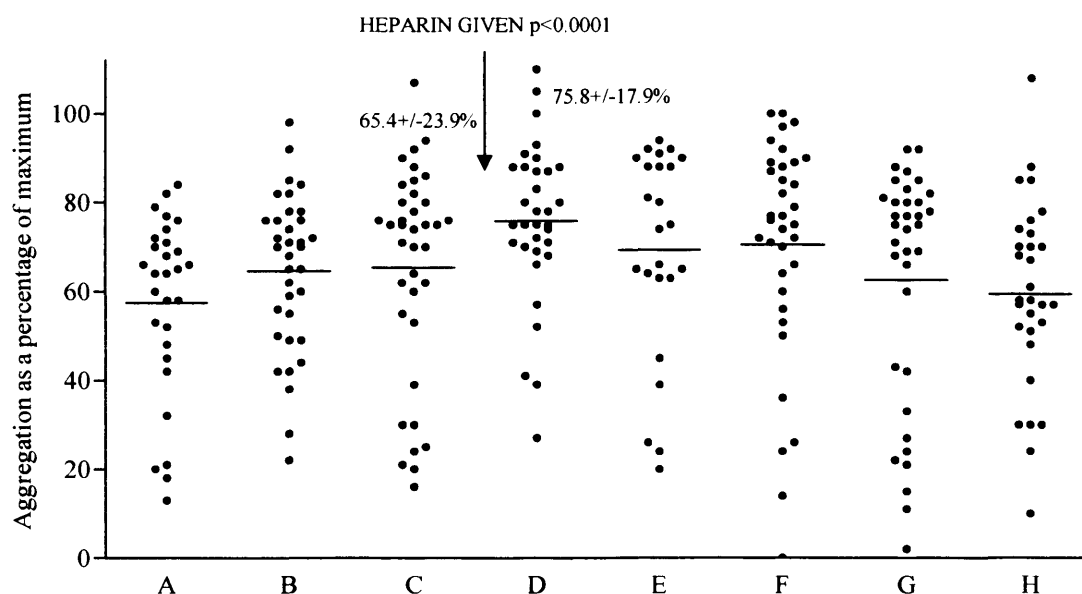


Figure 2.3.7 Aggregation to 6 μ mol/L TRAP in patients undergoing CEA



The figures show aggregation in response to TRAP 3 and 6 μ mol/L, as a percentage of maximum at time points A to H in patients undergoing CEA. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin).

Table 2.3.2 Percentage aggregation in response to stirring, AA (2.5 and 5 mmol/l), ADP (0.1 and 0.5µmol/L) and TRAP (3 and 6µmol/L) at time points A to H in patients undergoing CEA

	Percentage Aggregation In Response To Different Agonists							
	Pre-heparin			Post-heparin			Post-op	
	A	B	C	D	E	F	G	H
SPONT-ANEIOUS	1.8 +/- 2.3	4.4+/- 3.0	4.7+/- 3.6	13.6+/-9.6	12.6+/-8.8	8.6+/-5.4	5.4+/- 4.3	4.6+/- 3.7
<i>p-value*</i>	<0.0001		0.7322	<0.0001	<0.0001	<0.0001	0.234	0.7514
AA 2.5 mmol /L	2.6 +/- 2.4	6.8+/- 3.1	6.2+/- 3.6	22.1+/- 20.2	20.9+/- 13.8	12.3+/-7.7	8.5+/- 5.2	8.0+/- 10.7
<i>p-value*</i>	<0.0001		0.4104	<0.0001	<0.0001	0.0001	0.117	0.4230
AA 5 mmol/L	3.9 +/- 2.2	10.1+ /-7.1	11.0+/- 5.3	46.7+/- 29.3	46.8+/- 28.0	31.0+/- 29.4	14.9+ /-13.2	11.2+/- 9.0
<i>p-value*</i>	<0.0001		0.2915	<0.0001	<0.0001	<0.0001	0.465	0.439
ADP 0.1µmol/L	35.1 +/- 18.1	36.8+ /-19.3	42.1+/- 21.5	57.1+/- 19.0	55.4+/- 17.1	52.5+/- 17.5	45.8+ /-17.5	44.0+/- 20.8
<i>p-value*</i>	0.4663		0.0123	<0.0001	<0.0001	<0.0001	<0.00 01	0.0074
ADP 0.4 µmol/L	56.3 +/- 13.5	63.4+ /-15.2	68.9+/- 16.1	75.9+/- 13.2	73.0+/- 12.5	74.0+/- 16.9	70.7+ /-11.3	68.9+/- 13.4
<i>p-value*</i>	0.0148		0.0209	0.001	0.0716	0.0019	0.014	0.0121
TRAP 3 µmol/L	31.9 +/- 24.0	35.7+ /-28.8	37.6+/- 30.0	53.6+/- 27.4	44.9+/- 28.7	44.3+/- 30.6	39.1+ /-26.3	34.5+/- 24.3
<i>p-value*</i>	0.5430		0.6100	0.0011	0.0870	0.0492	0.181	0.1836
TRAP 6 µmol/L	57.5 +/- 20.1	64.6+ /-17.7	65.4+/- 23.9	75.8+/- 17.9	69.3+/- 23.2	70.5+/- 25.0	62.6+ /-26.5	59.4+/- 21.2
<i>p-value*</i>	0.0918		0.3631	<0.0001	0.1289	0.0185	0.805	0.6747

** p-values are for the relevant time points compared with time point B - taken after induction of anaesthesia, but before skin incision, using paired t-test, mean +/- standard deviation (as data was normally distributed)*

The time points are A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin

Aggregation with AA: relationship to sex, diabetes, smoking, weight and symptoms

There was no correlation between increasing age and platelet aggregation to AA following heparin administration.

There was no difference between platelet aggregation to AA following heparin administration in males (median = 42.0, range = 6.0-110.0) and females (median = 22.5, range = 12.0-83.0), $p=0.223$.

There was no difference noted between patients with or without diabetes, nor seen between smokers, ex-smokers and non-smokers.

Increasing weight was associated with higher rates of platelet aggregation, with a linear relationship, and patients weighing less than 65kg having a mean aggregation of $22.0 \pm 5.9\%$, and those weighing more than 85kg having a mean aggregation of $61.45 \pm 32.77\%$, $p=0.0133$. See figure 3.3.8.

The symptomatic patients may have been expected to have higher rates of aggregation (more reactive platelets) than the asymptomatic. However, the results showed an opposite trend, with the asymptomatic group showing a median aggregation of 78% (range 19 to 110), and the symptomatic group having a median value of 21.5% (range 6 to 107%). This approaches statistical significance with $p=0.005$, but should be interpreted with caution as the sub-groups analysed are small.

Figure 2.3.8 Platelet aggregation to 5mmol/L AA by patient weight

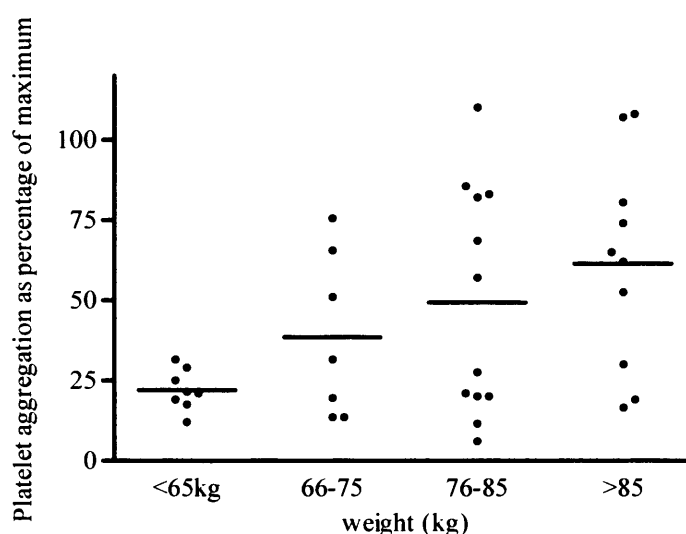


Figure shows platelet aggregation to 5mmol/L of AA, following UFH given during CEA tends to increase with increasing patient weight

2.3.3 Blood cell counts

The samples at each time point were used to measure platelet count, haemoglobin, haematocrit and white cell count. Platelet counts were also performed on the PRP samples.

Platelet count in whole blood

The mean platelet count pre-operatively was $264.7 \pm 76.11 \times 10^9/L$, which dropped to $221.3 \pm 67.55 \times 10^9/L$ following induction of anaesthesia, $p=0.463$. There was no significant change in the platelet counts in whole blood for the remainder of the studied points (figure 3.3.9).

Haemoglobin Concentration

The mean haemoglobin concentration in the pre-operative blood samples was $14.13 \pm 2.398 g/dL$. This dropped to $12.78 \pm 3.154 g/dL$ following induction of anaesthesia, though this was not statistically significant ($p=0.0608$). Thereafter there was no significant change (figure 3.3.10).

Haematocrit

The haematocrit of the samples dropped following induction of anaesthesia to 39.04 ± 9.494 from 44.38 ± 7.653 ($p=0.0554$). By 24 hours post-operatively, the haematocrit had dropped to 35.36 ± 6.853 ($p=0.0002$) (figure 3.3.11).

White Cell Count

The white cell count initially drops from $7.59 \pm 1.60 \times 10^9/L$ to $6.08 \pm 1.29 \times 10^9/L$ ($p<0.0001$), but by 4 hours post-operatively is raised to $10.36 \pm 2.56 \times 10^9/L$ ($p<0.0001$). This rise persists to 24 hours post-operatively, ($9.77 \pm 1.79 \times 10^9/L$) (figure 3.3.12).

Platelet count in PRP

The platelet count in the spun PRP was $254.5 \pm 101.4 \times 10^9/L$ in the pre-operative sample. It falls to $233.4 \pm 92.3 \times 10^9/L$ following the induction of anaesthesia, but this is not statistically significant ($p=0.1142$). However, the count does significantly fall following the intravenous heparin is given to $142.5 \pm 82.86 \times 10^9/L$. $p=0.0005$ (figure 3.3.13).

Figure 2.3.9 Platelet counts in whole blood during CEA

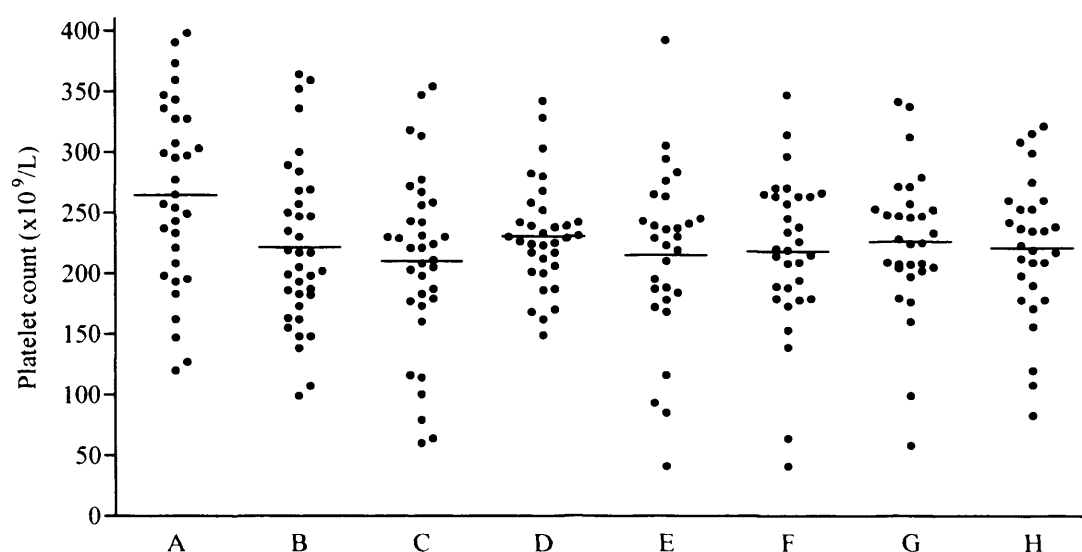
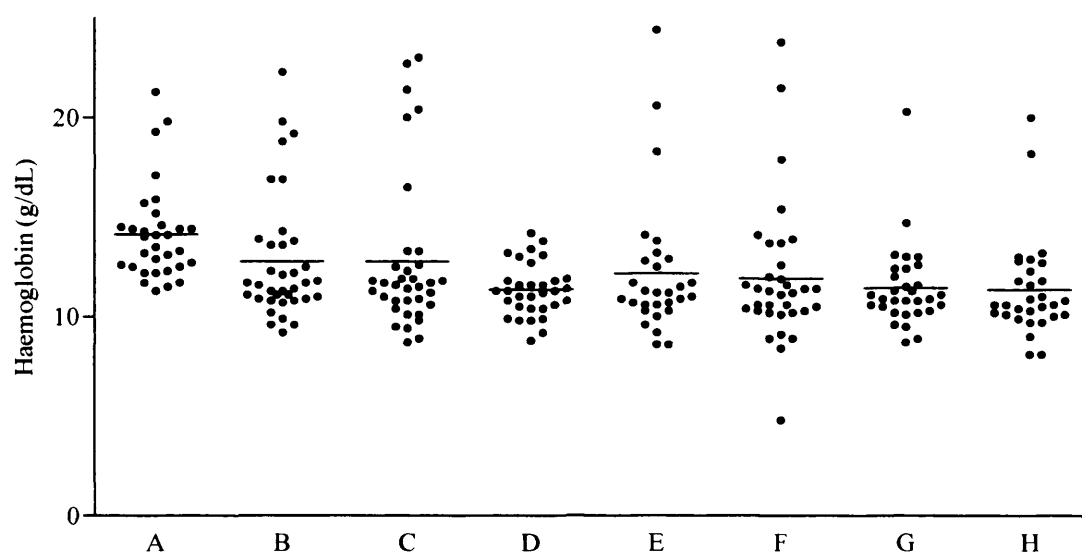


Figure 2.3.10 Haemoglobin concentrations during CEA



The figures show platelet count (x10⁹/L) and Haemoglobin concentrations (g/dL) at time points A to H. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin).

Figure 2.3.11 Haematocrit changes during CEA

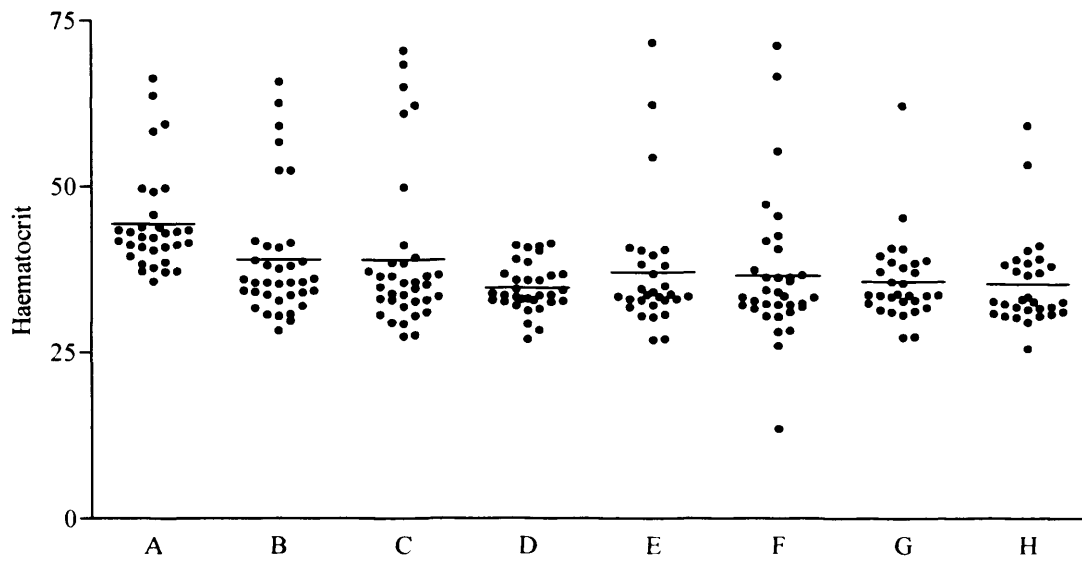
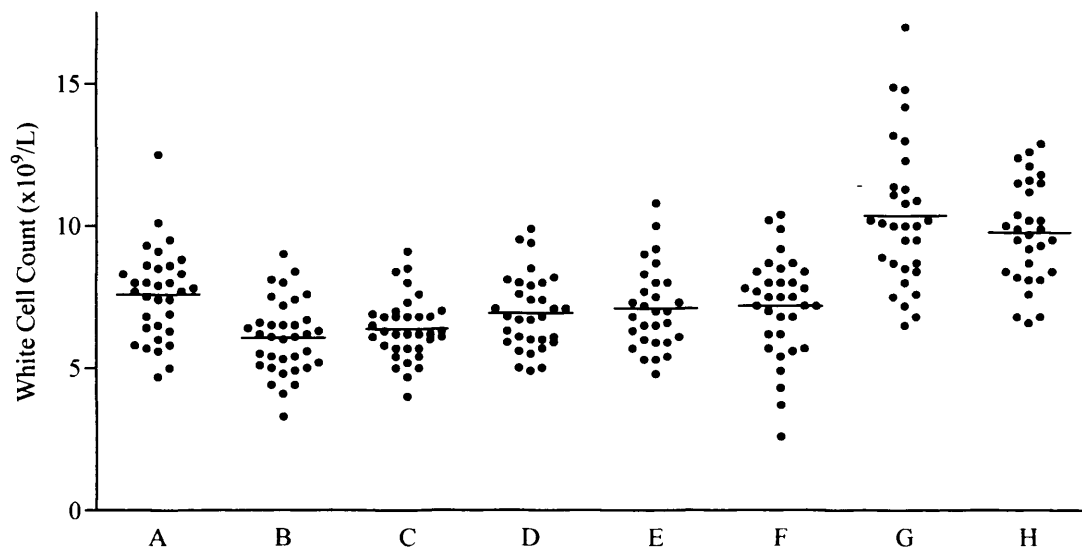
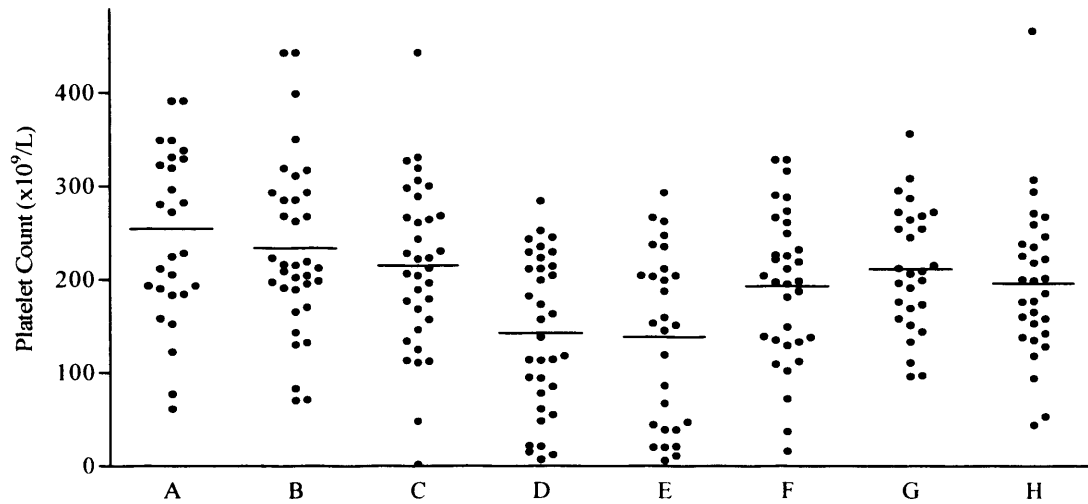


Figure 2.3.12 White cell counts in whole blood during CEA



The figures show Haematocrit and White Cell Counts ($\times 10^9/L$) at time points A to H. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin).

Figure 2.3.13 Platelet counts in PRP during CEA



The figure shows platelet counts ($\times 10^9/\text{L}$) in PRP at time points A to H. (A. Pre-operative, on admission to hospital; B. After induction of anaesthesia, but prior to skin incision; C. Following skin incision and soft tissue dissection, but prior to heparinisation; D. Three minutes after heparin was administered, prior to the insertion of the shunt; E. Three minutes after shunt opening; F. At the end of surgery, after flow restoration; G. Four hours post-operatively; H. 24 hours post-operatively, but prior to the next dose of aspirin).

2.4 Results for patients undergoing Angioplasty

2.4.1 Patient Demographics

Of the eighteen patients undergoing lower limb angioplasty, 14 were male. The median age of these patients was 68 years, and the median body weight was 72kg. As with the CEA group, there was a high incidence of co-morbidity; 5/18 patients had a history of diabetes mellitus, 14/18 with hypertension and 5/18 having previously suffered a myocardial infarction. Thirteen of the eighteen were ex-smokers and four continued to smoke. Presenting complaints included rest pain, ulceration and gangrene.

2.4.2 Aggregation – summarised in table 24.1

Spontaneous

The pre-procedure spontaneous aggregation was $2.7 \pm 2.8\%$ and did not rise significantly following injection of local anaesthetic and contrast medium ($3.2 \pm 2.3\%$), $p = 0.441$. Three minutes after a bolus of UFH, the mean aggregation rose to $9.8 \pm 8.1\%$ ($p = 0.0003$). Aggregation did not change significantly following angioplasty of the vessel(s) ($10.1 \pm 8.1\%$). By four hours post-procedure, spontaneous aggregation had returned to base levels ($3.1 \pm 2.0\%$, $p = 0.5855$). Figure 2.4.1

Aggregation to AA

The pre-procedure aggregation to AA (5mmol/L) was $5.6 \pm 4.5\%$ and rose to $15.6 \pm 18.7\%$ prior to the bolus of intravenous heparin ($p = 0.0542$). However, following the UFH, there was a marked rise to $33.8 \pm 24.2\%$ ($p = 0.0001$). This rise persisted following the angioplasty, and up to four hours post-procedure. Figure 2.4.3. A similar pattern was seen with the lower concentration of AA, shown in figure 2.4.2.

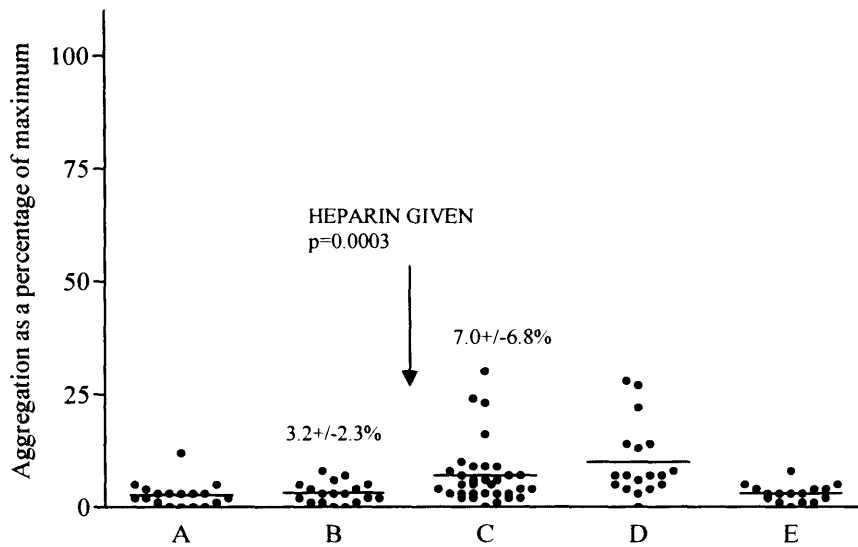
Aggregation to ADP

Aggregation in response to stimulation with ADP ($0.1 \mu\text{mol/L}$) rose from $38.1 \pm 10.6\%$ to $61.6 \pm 21.4\%$ following UFH during the procedure ($p < 0.0001$), figure 2.4.4. By four hours following the angioplasty, this had returned to baseline levels. The same pattern is seen for the higher dose of ADP. (See figure 2.4.5).

Aggregation to TRAP

Aggregation in response to stimulation with TRAP ($3\mu\text{mol/L}$) rose from $30.3\pm 17.7\%$ to $53.4\pm 21.2\%$ following administration of UFH during the procedure ($p=0.0011$), see figure 2.4.6. By four hours later, the aggregation levels had returned to baseline. Similar results were seen for the higher dose of TRAP, as seen in figure 2.4.7.

Figure 2.4.1 Spontaneous Aggregation during Angioplasty



The figure shows spontaneous as a percentage of maximum at time points A to E. (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. following the angioplasty; E. Four hours post-procedure).

Figure 2.4.2 Aggregation to 2.5mmol/L AA during Angioplasty

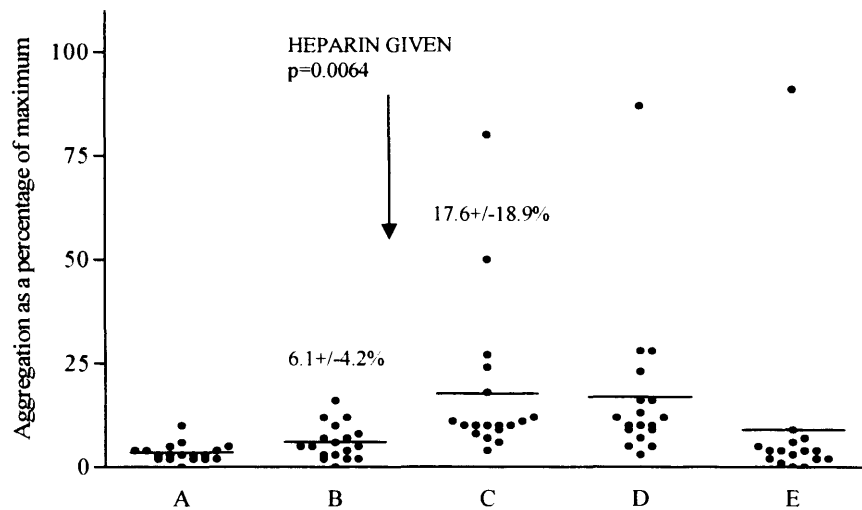
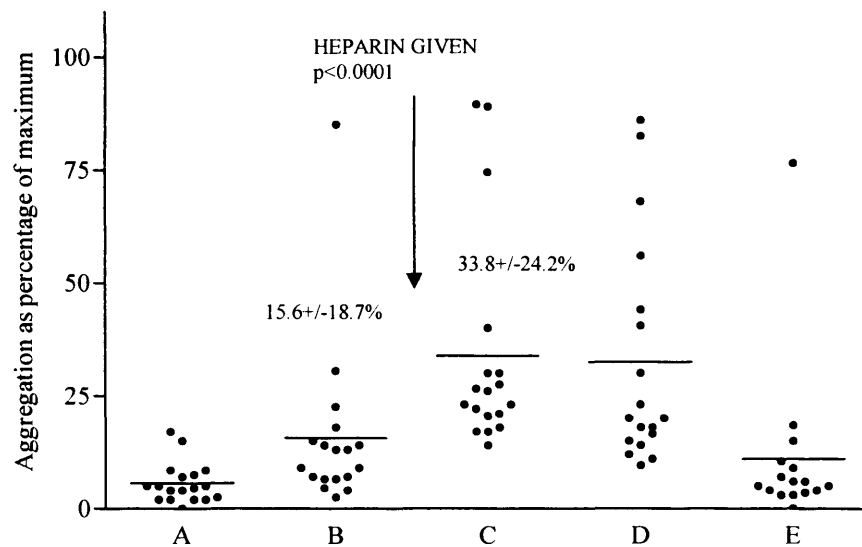


Figure 2.4.3 Aggregation to 5mmol/L AA during Angioplasty



The figures show aggregation in response to AA 2.5 and 5mmol/L, as a percentage of maximum at time points A to E. (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. following the angioplasty; E. Four hours post-procedure).

Figure 2.4.4 Aggregation to 0.1 $\mu\text{mol/L}$ ADP during Angioplasty

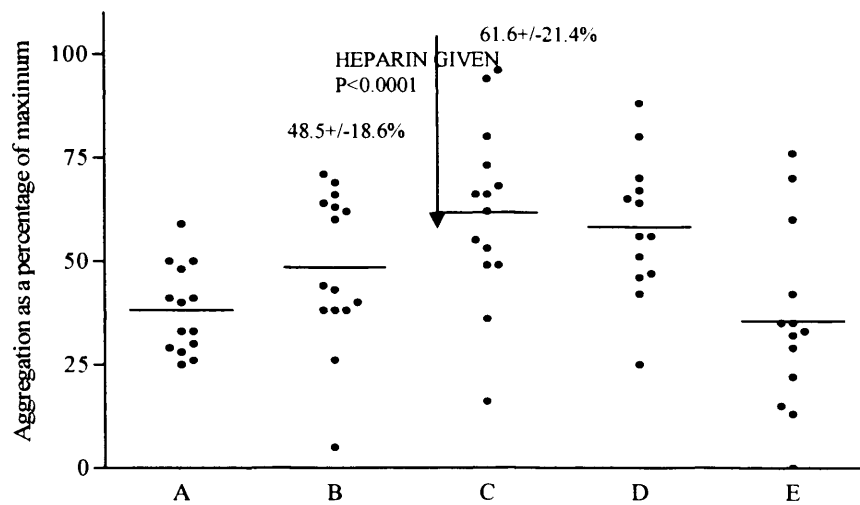
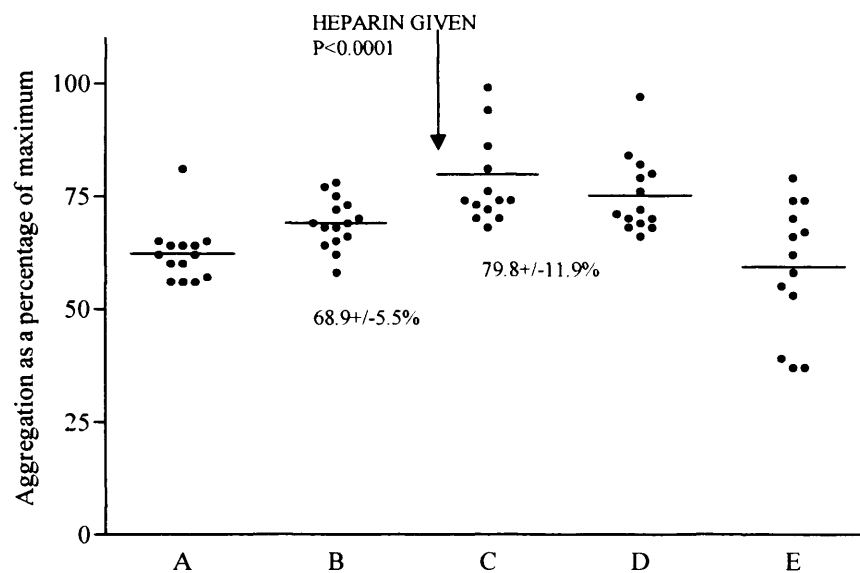


Figure 2.4.5 Aggregation to 0.5 $\mu\text{mol/L}$ ADP during Angioplasty



The figure shows aggregation in response to ADP 0.1 and 0.5 $\mu\text{mol/L}$, as a percentage of maximum at time points A to E. (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. following the angioplasty; E. Four hours post-procedure).

Figure 2.4.6 Aggregation to 3 $\mu\text{mol/L}$ TRAP during Angioplasty

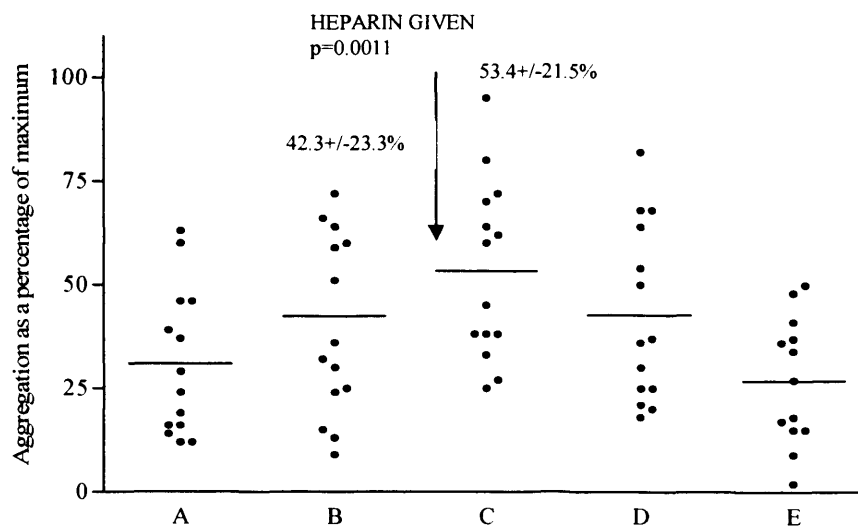
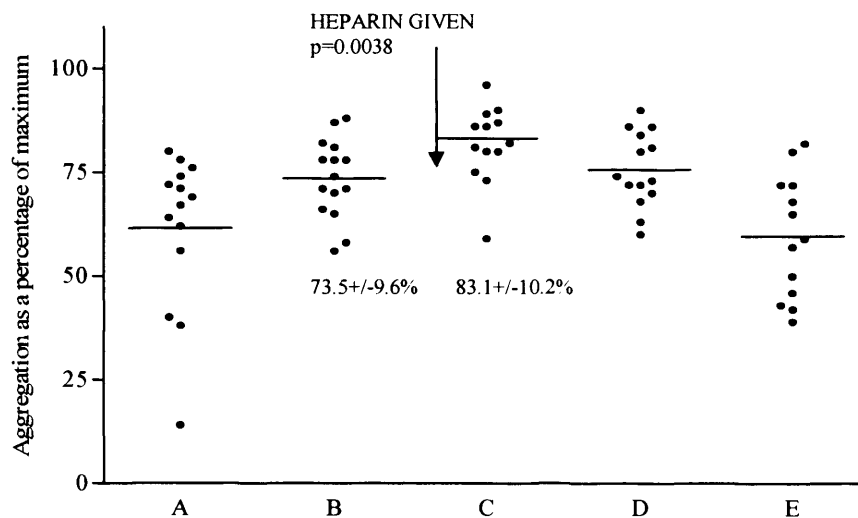


Figure 2.4.7 Aggregation to 6 $\mu\text{mol/L}$ TRAP during Angioplasty



The figure shows aggregation in response to TRAP 3 and 6 $\mu\text{mol/L}$, as a percentage of maximum at time points A to E. (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. following the angioplasty; E. Four hours post-procedure).

Table 2.4.1 Percentage aggregation in response to stirring, AA (2.5 and 5 mmol/l), ADP (0.1 and 0.5µmol/L) and TRAP (3 and 6µmol/L) at time points A to E in patients undergoing angioplasty

	Percentage Aggregation In Response To Different Agonists				
	Time points (Pre-heparin)		Time points (Post-heparin)		
	A	B	C	D	E
SPONTANEOUS	2.7+/-2.8	3.2+/-2.3	7.0+/-6.8	10.1+/-8.1	3.1+/-2.0
<i>p-value*</i>		0.4441	0.0003	0.0003	0.5855
AA (2.5mmol /L)	3.5+/-2.1	6.1+/-4.2	17.6+/-18.9	16.8+/-19.0	9.0+/-22.0
<i>p-value*</i>		0.0392	0.0064	0.0103	0.3629
AA (5mmol/L)	5.6+/-4.5	15.6+/-18.7	33.8+/-24.2	32.4+/-24.9	11.0+/-18.1
<i>p-value*</i>		0.0542	<0.0001	0.0003	0.2930
ADP (0.1µmol/L)	38.1+/-10.6	48.5+/-18.6	61.6+/-21.4	58.2+/-16.7	35.5+/-22.2
<i>p-value*</i>		0.1325	<0.0001	0.0004	0.9764
ADP (0.5µmol/L)	62.3+/-6.4	68.9+/-5.5	79.8+/-11.9	75.1+/-8.5	59.3+/-14.5
<i>p-value*</i>		0.0026	<0.0001	<0.0001	0.8136
TRAP (3µmol/L)	30.9+/-17.7	42.3+/-23.3	53.4+/-21.5	42.7+/-21.3	26.9+/-15.3
<i>p-value*</i>		0.1221	0.0011	0.0861	0.9418
TRAP (6µmol/L)	61.5+/-18.8	73.5+/-9.6	83.1+/-10.2	75.6+/-9.1	59.6+/-14.8
<i>p-value*</i>		0.0502	0.0038	0.0334	0.8251

* compared with pre-procedure, using paired t-test

Time points are A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. following the angioplasty; E. Four hours post-procedure

2.4.3 Blood cell counts

As for the CEA samples, platelet count, haemoglobin, haematocrit and white cell count were measured at every time point. Platelet counts were also performed on the PRP samples.

Platelet count in whole blood

The mean platelet count pre-procedure was $274.5 \pm 101.4 \times 10^9/L$, which dropped to $210.2 \pm 80.31 \times 10^9/L$ following injection of local anaesthesia, $p=0.0026$. There was no significant change in the platelet counts in whole blood for the remainder of the studied points. Figure 2.4.8

Haemoglobin Concentration

The mean haemoglobin concentration in the pre-operative blood samples was 14.1 ± 2.3 g/dL. There was no change in haemoglobin concentration at the time points tested. See figure 2.4.9.

White Cell Count

The white cell count initially is $8.25 \pm 2.3 \times 10^9/L$ and does not change significantly at any of the time points tested as shown in figure 2.4.10.

Platelet count in PRP

The platelet count in the spun PRP was $258.2 \pm 76.68 \times 10^9/L$ in the pre-operative sample. There was no significant change until following the administration of heparin when it falls to $179.8 \pm 82.81 \times 10^9/L$ ($p=0.0146$). By four hours post-op, the count is returning to baseline levels ($222.7 \pm 66.0 \times 10^9/L$). See figure 2.4.11.

Figure 2.4.8 Platelet counts ($\times 10^9/\text{L}$) in whole blood during angioplasty

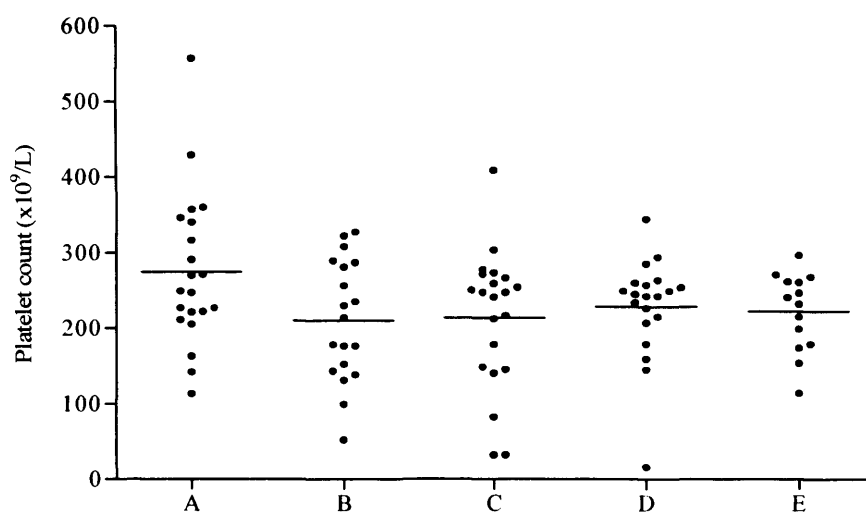
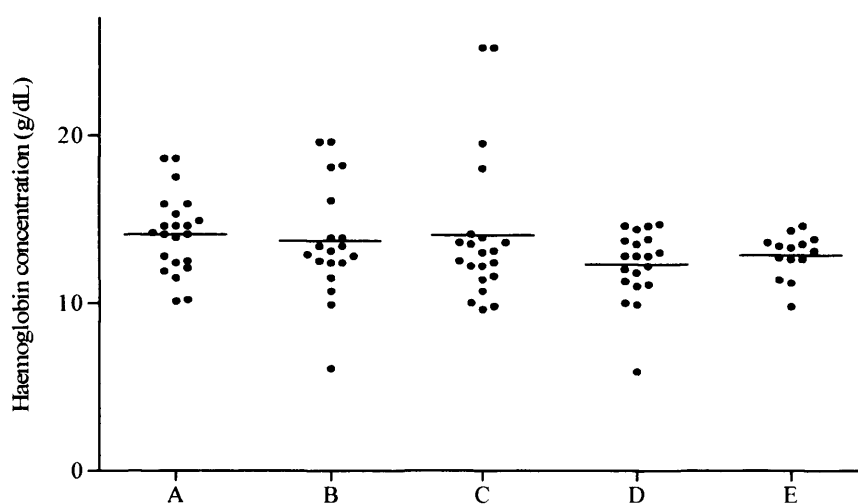


Figure 2.4.9 Haemoglobin (g/dL) in whole blood during angioplasty



Figures show platelet counts ($\times 10^9/\text{L}$) and Haemoglobin (g/dL) concentrations at time points A to E (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. Following the angioplasty; E. Four hours post-procedure).

Figure 2.4.10 White cell counts ($\times 10^9/\text{L}$) in whole blood during angioplasty

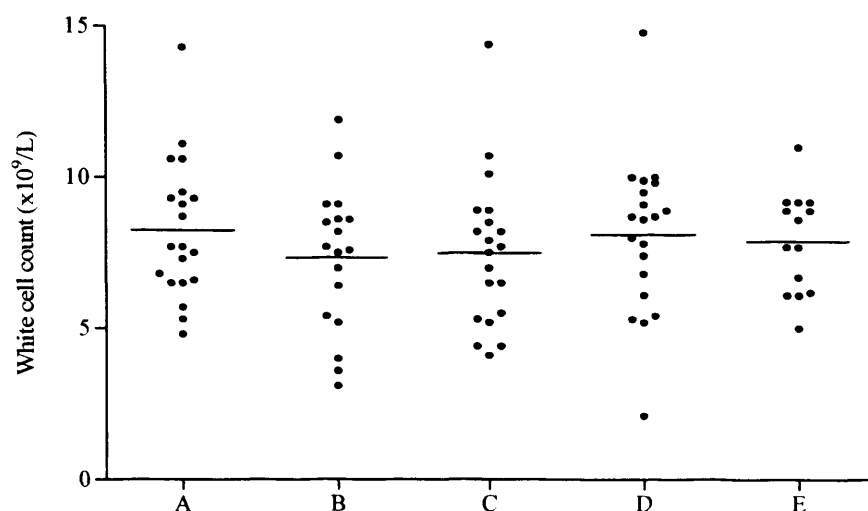
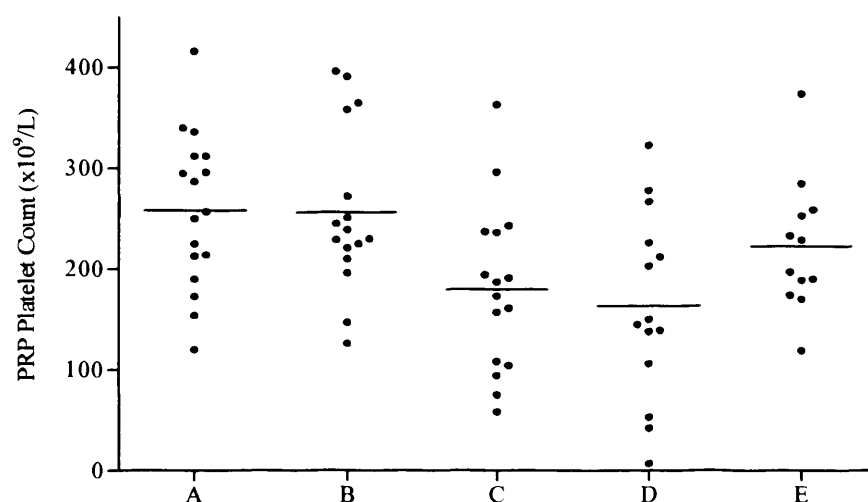


Figure 2.4.11 Platelet counts ($\times 10^9/\text{L}$) in PRP during angioplasty



Figures show white cell and platelet counts ($\times 10^9/\text{L}$) and at time points A to E (A. Pre-procedure, on admission to hospital; B. Following the angiogram, but prior to heparin; C. Three minutes after administration of heparin; D. Following the angioplasty; E. Four hours post-procedure).

2.5 Discussion

The main finding of this study contradicts classically held teaching regarding aspirin's effect on the platelet. A transient, but diminished efficacy of aspirin could explain why a small number of patients are at increased risk of thrombo-embolic complications (e.g. stroke and myocardial infarction) in the immediate post-operative period following cardiovascular procedures.

Following induction of anaesthesia in the patients undergoing CEA there was a small but significant rise in spontaneous platelet aggregation ($p < 0.0001$), aggregation in response to both 2.5 and 5mmol/L of AA and to the higher doses of ADP ($p = 0.0148$) and TRAP ($p = 0.0918$) when compared with baseline pre-operative levels. This conflicts with most of the current evidence which suggests either no effect (Hirakata et al, 1995; Aoki et al, 1998; Parolari et al, 1999) or an inhibitory effect on aggregation, particularly with halothane (Gibbs 1991; Mendez et al, 2003; Dordoni et al, 2004). Frohlich et al in 1998, however did show that several of the volatile anaesthetic agents can induce expression of p-selectin and GPIb on the platelet surface indicating platelet activation. The increased aggregation seen therefore, may be due to platelet activation by anaesthetic drugs, or may be due to the increased psychological stress experienced by the patients in the immediate pre-operative period whilst in the anaesthetic room (Levine et al, 1985; Larsson et al, 1994).

The major increase in aggregation was seen to occur directly after administration of heparin in both the CEA and angioplasty groups suggesting that the mechanism by which this occurs is related to a direct or indirect effect of heparin on the platelet. There are several possible explanations for the increased aggregation seen.

First, there may be a release of new platelets that have escaped the effects of aspirin. Surgery can induce the release of new platelets from the bone marrow (Cooper and Ingram, 1991) and as few as 10% non-aspirinated platelets can be enough to overcome the effects of aspirin. (Rao et al, 1981). However, there is evidence that aspirin affects megakaryocytes also (Di Minno et al, 1983) so that any new platelets released would be likely to be aspirinated. The change also occurs quickly, within three minutes of the administration of UFH, so would seem unlikely that there is enough time for the release of new platelets. There have been no reports of UFH stimulating the release of new platelets. If new non-aspirinated

platelets were responsible for the loss of aspirin's anti-platelet effect, one would expect this to persist until further aspirin is given. In these series, however, the aggregation in response to AA spontaneously returned to pre-operative levels within 24 hours and prior to the next dose of aspirin.

Secondly, the principal effects are seen immediately after heparin administration. This suggests that directly, or indirectly, heparin is having some effect on the platelet. In *in vitro* studies, heparin has been shown to both directly stimulate platelet aggregation (Chong and Ismail, 1989; Cheng et al, 1992; Xiao and Theroux, 1998) and potentiate aggregation induced by other agonists (Mohammad et al, 1981; Xiao and Theroux, 1998). In vivo, heparin enhances aggregation caused by ADP (Berglund and Wallentin 1991; Knight et al, 1998) and causes platelet activation with increased thromboxane metabolite excretion (Landolfi et al 1994). These pro-aggregatory effects were also seen in patients on aspirin (Michaelidis et al, 1985; Gasperetti et al, 1993; Reininger et al, 1994), but the specific response to AA was not tested in any previous studies.

Heparin is known to induce release of lipoprotein lipase and hepatic lipase (Perrson et al, 1998), which cause liberation of arachidonate. Muriithi et al, 2002 suggested that the effect of UFH on the platelet involved the endothelium with the release of lipases and platelet factor 4, rather than a simple direct effect on the platelet itself. This increase in circulating AA may somehow override aspirin's effect, or be utilised by an alternative enzyme pathway. The lipoxygenase enzyme for example, metabolises AA to 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE). Although their actions are poorly understood HETE may potentiate the activation of platelets (Buchanan et al, 1986; Santos et al, 1993; Calzada et al, 2001). Fletcher-Cieutat et al (1985) suggested that there was interaction between the COX and lipoxygenase pathways in platelets.

There has been recent interest in the possibility of COX-2 being present in the platelet, and accounting for "aspirin resistance" (Weber et al, 1999; Reiter et al, 2001; Rocca et al, 2002). Other sources of thromboxane – COX-2, monocytes and macrophages may be involved. The phenomenon of "aspirin resistance" has been described using laboratory tests (Weber et al, 2002) and seems to have some correlation with poor clinical outcome (Grottemeyer et al, 1993; Gum et al, 2003).

However, this transient form has not previously been reported and may be occurring via a different mechanism. The possible causes of aspirin resistance have been summarised by Hankey and Eikelboom, 2006 and include lack of patient compliance. This is unlikely and in the cohort described here, those patients with >20% platelet aggregation (so non-compliant or “aspirin-resistant”) on their baseline pre-operative tests were excluded. An inadequate dose of aspirin was also considered a possible mechanism, but higher doses in clinical trials have not correlated with decreased events, but do significantly increase the risk of side effects. Interestingly, the correlation with increasing weight and increasing platelet aggregation shown in the patients undergoing CEA may reflect a need for heavier patients to have higher doses of aspirin to adequately block the COX enzyme of their platelets. An alternative explanation is that heavier patients have more fat, therefore a different lipase metabolism, interacting with the heparin.

2.6 Suggestions for further work

The theories, which may provide an explanation for the observed changes during CEA and angioplasty following the intravenous administration of heparin, were used to design the studies described in the next chapter. They aim to elucidate the mechanism behind the increased aggregation and also to compare the effects of UFH and LMWH, and are described in Chapters 5 and 6.

2.7 Conclusion

The anti-platelet effect of aspirin is significantly reduced following administration of unfractionated heparin during CEA. This may explain why some patients are at risk of thrombo-embolic complications following carotid surgery.

Chapter 3 The Mechanism by which Heparin reduces Aspirin's effect on platelet aggregation

3.1 Introduction

The time course studies described in Chapter 2 showed that the loss of aspirin's ability to prevent platelet aggregation in response to arachidonic acid occurred within three minutes of the administration of heparin, and was not induced by any of the other peri-operative events. The mechanism by which heparin exerts this effect is less clear, leaving a number of hypotheses to be explored.

The first and perhaps most simple explanation is that the loss of aspirin's blockade of the cyclo-oxygenase enzyme is due to the direct effect of heparin on the platelet or some other cell/factor present in whole blood. Indeed, it is known that heparin can bind to platelets – either directly or via an interaction with immunoglobulin (IgG) and platelet factor 4 (Horne & Hutchison, 1998; Amiral J et al, 2000) leading to platelet aggregation, increased spontaneous aggregation, and in some patients, the clinical syndromes of Heparin-Induced Thrombocytopenia (HIT, types I and II) (Fabris et al, 2000; Newman & Chong, 2000; Chong, 2003). There has not been any previous evidence to suggest that these forms of heparin-induced platelet aggregation can reduce aspirin's effect on COX. If this first theory is correct, then the effects of heparin on platelet aggregation stimulated by AA (and blocked by aspirin) should be reproducible by the addition of heparin to the samples *in vitro*. To test this hypothesis, UFH was added to whole blood and PRP samples in concentrations based on those achieved following administration of intravenous heparin *in vivo*.

Secondly, there may be induction of new “un-aspirinated” cyclo-oxygenase, or its isoform COX-2. However, the effect of heparin is seen quickly (within three minutes of administration) implying that there would have to be release or activation of pre-stored enzyme, rather than synthesis of new enzyme. This may come from other cell types or platelet pre-cursors / new platelets. Evidence suggests that megakaryocytes and new pre-circulation platelets in the bone marrow are also subject to the effects of aspirin, but this may be reversible (Patrono et al, 1985; van Pampus et al, 1993), so are unlikely to be able to release functional

COX. The (stored) cyclo-oxygenase in the platelets is aspirinated (demonstrated by the pre-operative samples' lack of aggregation to arachidonic acid), and the presence of COX-2 in platelets is still a subject of debate, with some evidence for (Weber et al 1999 & 2002, Evagelos 2001) and against (Patrignani et al, 1999; Reiter et al, 2001; Rocca et al, 2002). However, if COX -1 or -2 is the active enzyme (either new or re-activated), addition of COX-1 and/or COX-2 blocking agents *in vitro* should lead to a return to the baseline (inhibited) aggregation levels. Therefore COX-1 and COX-2 blockers were added to the post-heparin samples prior to addition of AA in the aggregometer.

Thirdly, it is possible that heparin, through the release of lipases, causes an increase in the circulating, or local levels of arachidonic acid, thereby simply “flooding” the COX enzyme with substrate and overwhelming aspirin’s effect. This theory also seems unlikely as the binding of COX by aspirin is irreversible and non-competitive (Loll P et al 1995). However, to test this hypothesis, increasing concentrations of AA were added to the aspirinated (pre-heparin) samples.

A fourth hypothesis to consider is the conversion of Arachidonate by an alternative enzyme pathway, leading to production of a pro-aggregatory substance. It has been shown that 12-HETE, the end product of the action of 12-lipoxygenase on arachidonic acid, can support platelet aggregation and adhesion (Buchanan et al, 1986; Cazada et al, 1997). ELISA measurements of 12-HETE were performed with the aim of identifying if the lipoxygenase pathway was active in the conversion of AA to products that initiated platelet aggregation.

The possibility that this phenomenon was an “artefact”, and that the platelets were not undergoing true aggregation, but undergoing agglutination also had to be considered. To exclude this, a GPIIb/IIIa antagonist (ABCIXIMAB) was used *in vitro* and as this receptor represents the final common pathway in platelet-platelet aggregation (Ruggeri 1997), the addition of ABCIXIMAB to the platelet suspension would abolish true aggregation, but not agglutination of the platelets.

In this mechanistic section, a second method of measuring platelet aggregation was trialled, namely the PFA-100®. The PFA-100® is an instrument and test cartridge system which simulates platelet adhesion and aggregation *in vitro*. It has been

shown to detect the changes in platelet function caused by Aspirin (Harrison P et al, 1999). The system is based on work described by Kratzer and Born (1985). Each test cartridge consists of a reservoir, a capillary and a membrane with an aperture. Anti-coagulated whole blood is aspirated from the sample reservoir through the membrane aperture under shear-flow conditions. The time taken (closure time (CT)) to occlude apertures in membranes coated with collagen-ADP (COL-ADP) or collagen-epinephrine (COL-EPI) is measured. The COL/EPI test cartridge is the primary cartridge used to detect platelet dysfunction, then the COL/ADP to indicate if the abnormal result may have been caused by ASA. CT must be tested on samples HCT 35-50% and platelet count 150,000/microlitre to 500,000. Normal range is 124 seconds (mean) 85-165 COL/EPI and 92 seconds (71-118) Col/ADP. Coefficient of variation is 0.6% for COL/EPI and 4% for COL/ADP. Several papers have suggested the PFA-100 as a useful *in vitro* measurement of bleeding time. (Kratzer et al, 1985; Harrison et al, 1999). It has recently been used to determine aspirin "resistance" or non-responsiveness (Gum et al, 2001; Anderson et al, 2002; Christiaens et al, 2002).

To determine whether COX (new or reactivated) was the enzyme responsible for the return of platelet aggregation in response to AA, ELISA measurements of thromboxane B2 were taken. As this is the stable end-product of the COX pathway, a rise in the production of TxB2 following heparin would be reasonable evidence that a recovery of COX activity was responsible for the increased platelet aggregation seen.

3.2 Materials and Methods

The patients involved in all of these studies were subject to the same consenting procedure, following ethics committee approval, as outlined in Chapter 2, the time course study. The exclusion criteria were the same, as were the obtaining and handling of blood samples prior to their use. Blood samples were taken pre-operatively, three minutes following heparinisation and two hours post-operatively. Platelet aggregometry was performed as described in the previous chapter, with the method described by Born.

The details of each experiment will be described individually in the following sections. For the sources, making up instructions and storage details of Aspirin, NS-398 and ABCIXIMAB, see individual sections.

3.2.1 *In vitro* Studies

Heparin

Unfractionated Heparin was diluted to 1 unit per 10 μ l in normal saline and added to whole blood in concentrations approximating those used in the in vivo setting. The samples were incubated for three minutes before centrifugation and aggregation studies. Spontaneous aggregation was studied in 42 patients and aggregation in response to AA (5mmol/L) in 38. UFH was also added to PRP samples with and without AA (n=6).

Aspirin

Aspirin was added to the whole blood of seventeen patients in concentrations that preliminary in vitro studies in normal subjects were high enough to abolish platelet aggregation (approximately 0.75mM). Samples were incubated for 3 minutes prior to centrifugation and aggregometry.

COX-2 blocker

NS-398 (Calbiochem) was used. It was impossible to perform initial experiments to determine the concentration of COX-2 blocker to abolish platelet aggregation, as this enzyme is not usually responsible for the aggregation under normal conditions; however a concentration of less than 3 micro-molar is specific for COX-2. This concentration was therefore used. The fifteen samples were incubated at room temperature for 3 minutes, prior to centrifuging of the whole blood and aggregometry.

ABCIXIMAB

ABCIXIMAB, the GPIIb/IIIa receptor antagonist is the FAb fragment of a chimeric monoclonal antibody 7E3. An equivalent dose to that used in clinical practice was used (3.5 μ g /ml). The eleven samples were incubated for 3 minutes prior to aggregometry. In initial experiments this was enough to inhibit aggregation in response to AA in normal subjects.

High dose AA

A double concentration (10mmol/L) of arachidonic acid was used for eleven patients, in the presence and absence of ABCIXIMAB.

3.2.2 PFA-100

The PFA-100 was used to analyse platelet function. Blood samples were collected into 4.5mls of citrate by the technique previously described, in order to minimise artefactual platelet activation. The samples were processed within 2 hours of being taken. Nine patients who exhibited high rises in aggregation were tested with this technique.

3.2.3 ELISAs

Thromboxane B2

Thromboxane B2 was measured using the “Thromboxane B2 Enzyme immunoassay (EIA) Biotrak System” from Amersham Biosciences (Code RPN 220). The kits were stored at 2-8°C until used. The assay is based on the competition between unlabelled thromboxane B2 and a fixed quantity of peroxidase labelled thromboxane B2 for a limited number of binding sites on a thromboxane B2 specific antibody. With fixed amounts of antibody and peroxidase labelled thromboxane B2, the amount of peroxidase labelled ligand bound by the antibody will be inversely proportional to the concentration of added unlabelled ligand. Working standards were prepared fresh for each kit and samples were run in duplicate. Blood samples were collected pre-operatively and 3 minutes following the *in vivo* administration of 5000 units of UFH, into citrated tubes, centrifuged and the plasma frozen immediately. The samples were then allowed to equilibrate to room temperature before use. Thromboxane concentrations were measured in the samples taken pre-operatively and following UFH (before (n=26) and after stirring (n=31)), and following *in vitro* stimulation with 5mmol/L AA (n=29). Initial samples were measured at a range of dilutions to ensure concentrations fell within the range of the kits. For the samples not stimulated with AA, the concentrations of TxB2 fell within the test range in neat (undiluted) samples. Following AA, the TxB2 concentrations were much higher, and samples were therefore diluted with assay buffer to 1:100.

12-HETE

Measurements of 12-HETE were made using the 12(S)-Hydroxyeicosatetraenoic acid [12(S)-HETE] Immunoassay supplied by R&D Systems (Code DE2900). The kits were stored at $\leq -20^{\circ}\text{C}$. The assay is based on the competitive binding technique in which 12(S)-HETE present in a sample competes with a fixed amount of alkaline phosphatase-labelled 12(S)-HETE for sites on a rabbit polyclonal antibody. During the incubation, the antibody becomes bound to the goat anti-rabbit antibody coated onto the micro-plate. Following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. The absorbance is read at 405nm, with the intensity of the colour being inversely proportional to the concentration of 12(S)-HETE in the sample. Samples were collected pre-operatively and 3 minutes following the *in vivo* administration of 5000 units of UFH, into 4.5ml citrated tubes, then centrifuged for 15 minutes at 1000g and frozen until use. The samples were allowed to equilibrate to room temperature before use. Initial concentration experiments were performed to determine dilutions needed for concentrations to fall within the ELISA range. Samples were diluted to 1:400 in assay buffer. 12-HETE measurements were made in 30 patients following *in vitro* stimulation with 5 mmol/L AA, in the pre-operative and post-heparin samples.

3.3 Results

Results are given as median with inter-quartile ranges (IQR) as most of the data are not normally distributed, and sample sizes were too small to use confidence intervals. Comparisons (p-values) are made using the Wilcoxon matched pairs test.

3.3.1 *In vitro* Studies

In vitro UFH ($n=42$)

The results are outlined in table 3.3.1, and are illustrated in the figures 3.3.1 (Spontaneous aggregation) and 3.3.2 (aggregation in response to AA). Spontaneous aggregation in the pre-operative sample increased from 3.0% (1.5-6.0) to 5.5% (2.0-11.5) following the *in vitro* addition of heparin. The increase was statistically significant ($p=0.0006$). This rise in aggregation to 7.0% (4.0-9.5) was no different from the rise seen in the sample taken following *in vivo* administration of heparin ($p=0.9428$). However, the rise in aggregation seen in the samples stimulated with arachidonic acid was different in the *in vivo* and *in vitro* heparin groups. With a rise

from 11.5% (6.5-18.5) pre-operative, to 44.5% (18.0-77.0) following *in vivo* UFH and 18.0% (11.5-38.5) *in vitro* heparin. The difference between the aggregation following *in vitro* and *in vivo* UFH was statistically significant ($p<0.001$), with *in vitro* addition of UFH causing much less of a rise in the aggregation.

For the samples comparing the addition of UFH to whole blood or PRP ($n=6$), there was no significant difference between the two groups, with spontaneous aggregation or aggregation stimulated by AA (Figures 3.3.3 and 3.3.4, Tables 3.3.2 and 3.3.3).

In vitro COX-1 blocker (aspirin) and COX-2 blocker (NS-398) (n=17)

Addition of either aspirin or NS-398 (selective COX-2 antagonist) to the blood samples prior to centrifuging or to the PRP samples did not affect the increased aggregation seen following *in vivo* heparin. See table 3.3.4 and figure 3.3.5.

In vitro ABCIXIMAB (n=11)

Following addition of ABCIXIMAB to the post-heparin sample, the platelet aggregation levels were reduced to base-line spontaneous aggregation levels (from 40% (32.0-88.0) to 11% (7.0-15.0), $p=0.0004$). See Table 3.3.5 and Figure 3.3.6.

Excess AA (n=10)

Arachidonic acid at 10 μ mol/L (double the usual concentration) was added to the pre-operative PRP sample in the aggregometer. The high dose AA caused near maximal platelet aggregation. However, when Abciximab was added to these samples, the “aggregation” was not blocked, unlike the aggregation seen following *in vivo* heparin. See table 3.3.6 and figure 3.3.7.

Figure 3.3.1 Spontaneous Aggregation

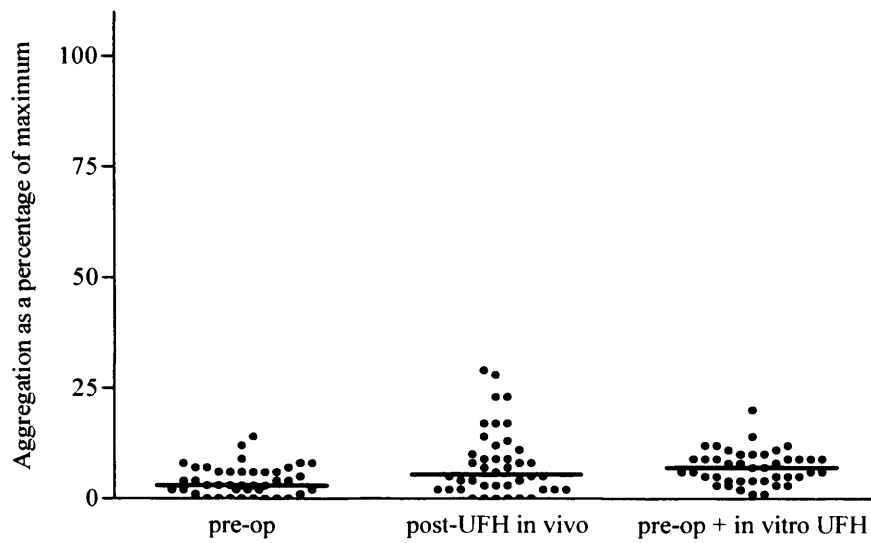
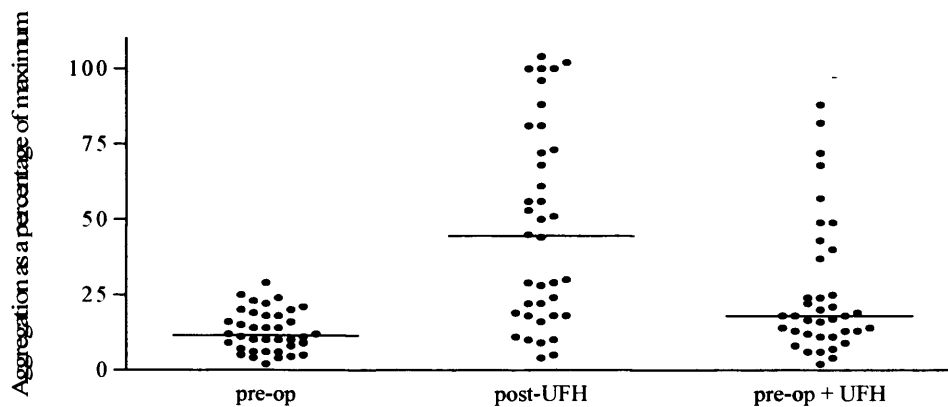


Figure 3.3.2 Aggregation in response to 5 mmol/L AA



Figures show spontaneous platelet aggregation as a percentage of maximum and aggregation in response to 5 mmol/L of AA. The results shown are for samples taken pre-operatively and following the administration of in vivo UFH, and the pre-operative sample with the addition of in vitro UFH.

Table 3.3.1 Spontaneous Aggregation and Aggregation in response to 5mmol/L AA with the addition of *in vitro* UFH to whole blood

		Pre-operative	3 minutes following UFH <i>in vivo</i>	Pre-operative + <i>in vitro</i> UFH
Median % (IQR)	Spontaneous	3.0 (1.5-6.0)	5.5 (2.0-11.5)	7.0 (4.0-9.5)
	p values	0.0006		
			0.9428	
	5 mmol/L AA	11.5 (6.5-18.5)	44.5 (18.0-77.0)	18.0 (11.5-38.5)
	p values	<0.0001		
			<0.0001	

Figure 3.3.3 Spontaneous Aggregation following UFH

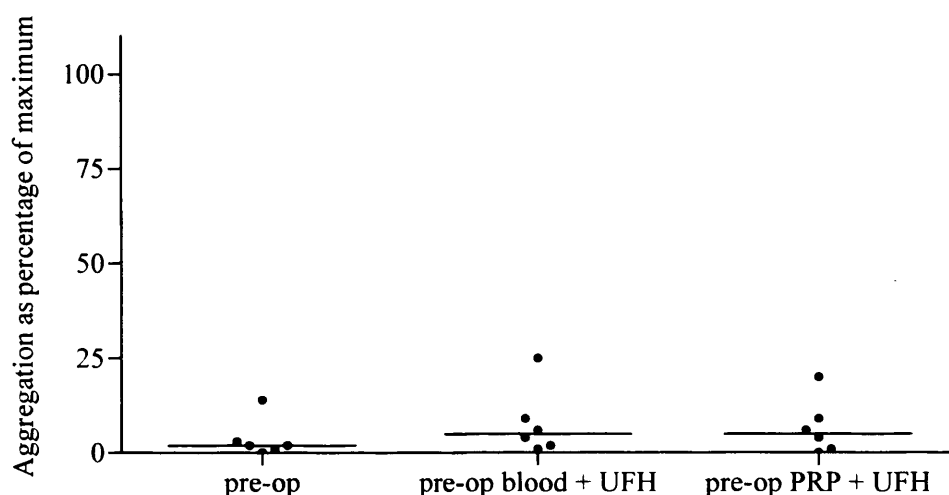


Figure shows spontaneous aggregation as a percentage of maximum in samples taken pre-operatively with the addition of UFH *in vitro* to either whole blood or PRP.

Table 3.3.2 Spontaneous Aggregation in samples - pre-operative, pre-operative blood + UFH *in vitro* and pre-operative PRP + UFH *in vitro*

	Pre-operative	blood + UFH <i>in vitro</i>	PRP + UFH <i>in vitro</i>
Median % (IQR)	2.0 (0.5-8.5)	5.0 (1.5-17.0)	5.0 (0.5-14.50)
P value	0.0625		
		0.3125	

Figure 3.3.4 Aggregation in response to 5mmol/L AA following *in vitro* UFH

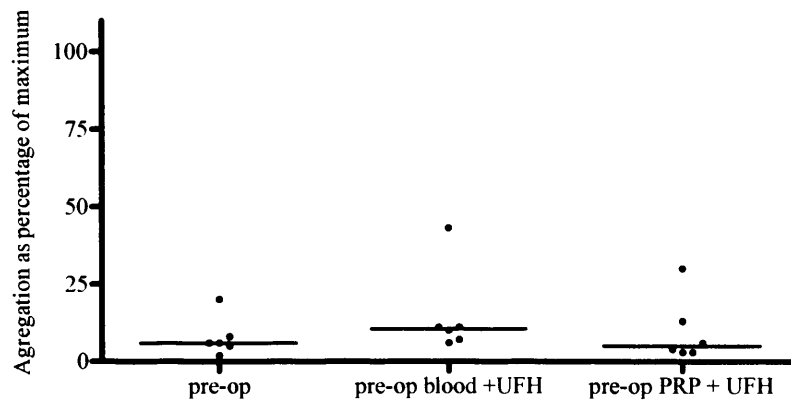


Figure shows aggregation as a percentage of maximum in samples taken pre-operatively and stimulated with 5mmol/L of AA, with the addition of UFH *in vitro* to either whole blood or PRP.

Table 3.3.3 Aggregation in response to 5µmol/L AA in samples taken pre-operatively (pre-op) and following the addition of *in vitro* UFH to pre-operative blood and pre-operative PRP

		Pre-op + AA	Pre-op Blood+UFH+AA	Pre-op PRP+UFH+AA
Median % (IQR)		6.0 (3.5-14.0)	10.5 (6.5-27.0)	5.0 (3.0-21.5)
p-value		0.0313		
			0.0625	

Figure 3.3.5 Aggregation in response to 5mmol/L AA following addition of in vitro aspirin or NS-398 (COX-2 blocker)

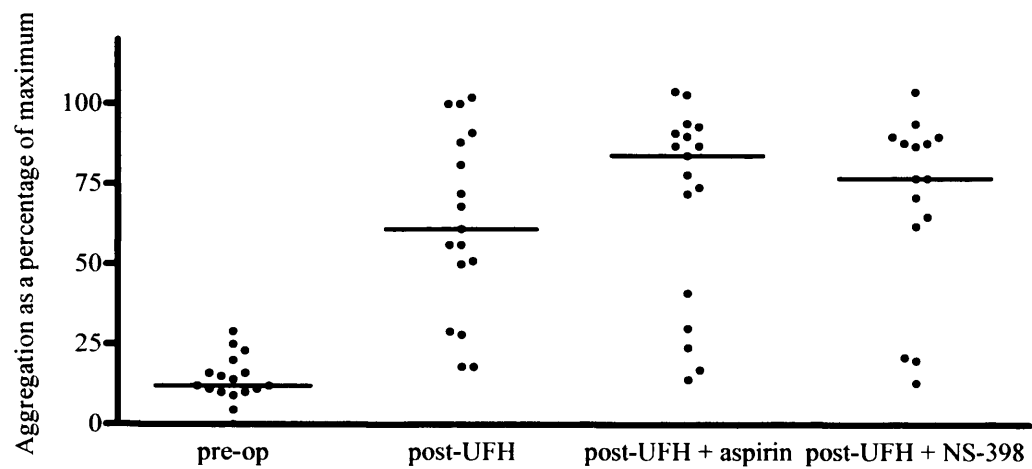


Figure shows aggregation in response to 5mmol/L AA in samples taken pre-operatively and 3 minutes following the administration of UFH, in the presence of Aspirin or NS-398 (COX-2 blocker)

Table 3.3.4 Aggregation to 5mmol/L AA following addition of *in vitro* Aspirin or NS-398 (COX-2 blocker) to samples taken following the *in vivo* administration of UFH

	Pre-op	3 minutes following UFH <i>in vivo</i>	Post-UFH + <i>in vitro</i> aspirin	Post-UFH + <i>in vitro</i> NS-398
Median % (IQR)	12.0 (10.0-18.0)	61.0 (39.5-89.5)	84.0 (35.5-92.0)	77.0 (62.0-90.0)
p values	0.0003			
		0.3620		
		0.5614		

Figure 3.3.6 Aggregation in response to 5mmol/L AA with *in vitro* ABCIXIMAB

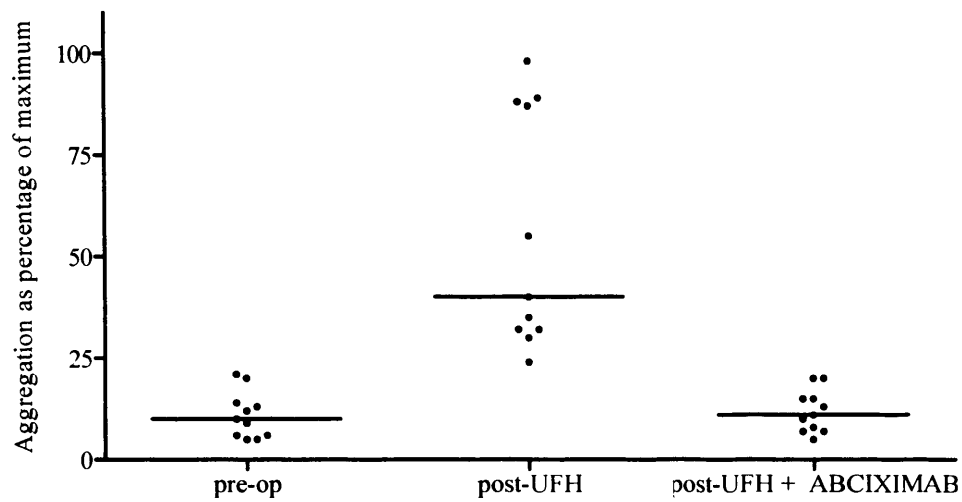


Figure shows aggregation in response to 5mmol/L AA in samples taken pre-operatively and 3 minutes following administration of UFH in the presence and absence of ABCIXIMAB

Table 3.3.5 Aggregation in response to AA in the presence of ABCIXIMAB

	Pre-op	Post in vivo UFH	Post-UFH + <i>in vitro</i> Abciximab
Median % (IQR)	10.0 (6.0-14.0)	40.0 (32.0-88.0)	11 (7.0-15.0)
p-value		0.0010	

Figure 3.3.7 Aggregation in response to high dose AA in the presence and absence of ABCIXIMAB

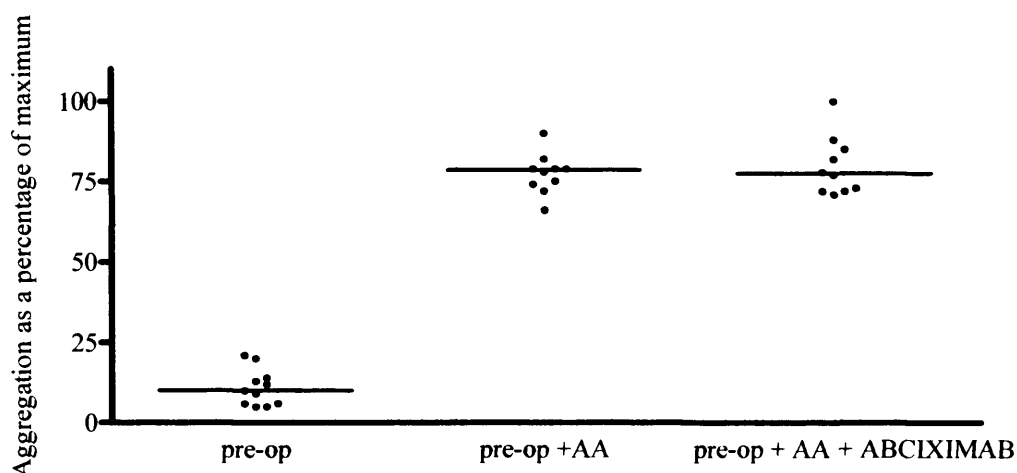


Figure shows aggregation in response to 10 mmol/L AA in samples taken pre-operatively and 3 minutes following administration of UFH in the presence and absence of ABCIXIMAB

Table 3.3.6 Aggregation in response to high dose AA in the presence and absence of ABCIXIMAB

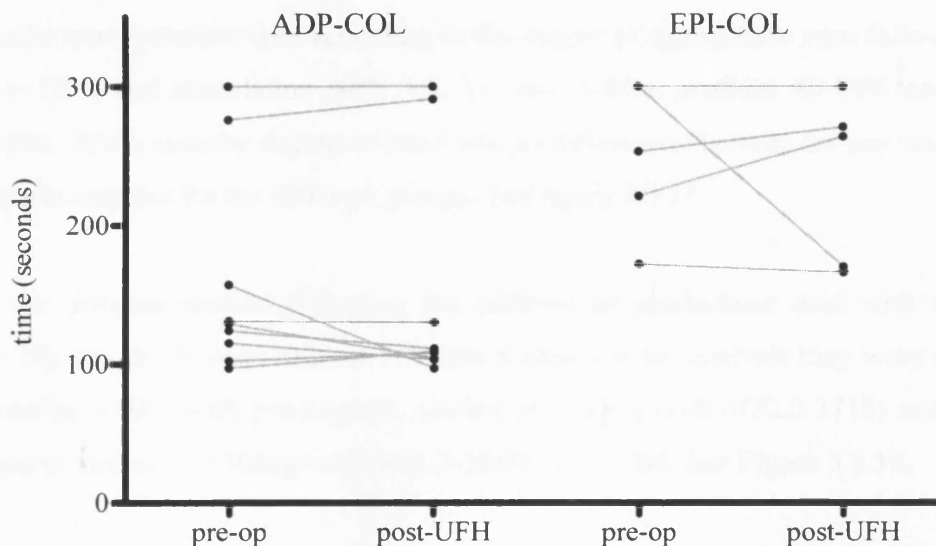
	Pre-operative	Pre-operative + high dose AA	Pre-operative + high dose Abciximab
median % (IQR)	10.0 (6.0-14.0)	78.5 (73.0-80.5)	77.5 (72.0-86.5)
p values	0.0039		
		0.8203	

3.3.2 PFA-100 (n=9)

The PFA-100 was used to assess a smaller sub-group of patients with high levels of platelet aggregation following *in vivo* heparin. The PFA-100 in this case could not detect any difference between the two time points. The median closure times for the ADP cartridge were 129.0 seconds (108.5-216.5) prior to heparin compared with 109.0 seconds (103.5-210.5) following heparin, $p=0.3828$. The median closure times for the epinephrine cartridge were 300.0 seconds (237.0-300.0) prior to heparin and 300.0 seconds (217.0-300.0) following heparin, as the PFA machine stops reading after 300 seconds.

See Figure 3.3.8.

Figure 3.3.8 PFA-100 results



The figure shows closure times for the collagen-ADP (COL-ADP) and collagen-epinephrine (COL-EPI) cartridges in patients who showed high levels of aggregation to 5mmol/L AA following administration of UFH *in vivo*

3.3.3 ELISAs

Thromboxane B2

The thromboxane B2 concentrations were measured in unstimulated (not stirred) samples taken pre-operatively and following *in vivo* administration of heparin (n=26). There was a rise from 1.028pg/well (0.3384-2.699) to 5.169pg/well (1.575-6.835), $p<0.0001$. See figure 3.3.9

In the samples from the same time points, but following stirring (n=31), the thromboxane B2 concentrations rose from 2.304pg/well (1.620-4.090) to 7.530pg/well (4.900-15.41), $p<0.0001$. See figure 3.3.9

Although most patients showed some increased aggregation in response to AA, there were some that tended to be “high aggregators” and some low. The TxB2 results were therefore split according to the degree of aggregation seen following *in vivo* UFH and stimulation with AA, i.e. low <40%, medium 40-80% and high >80%. When split for degree of there was no difference between the pre- and post-heparin samples for the different groups. See figure 3.3.11

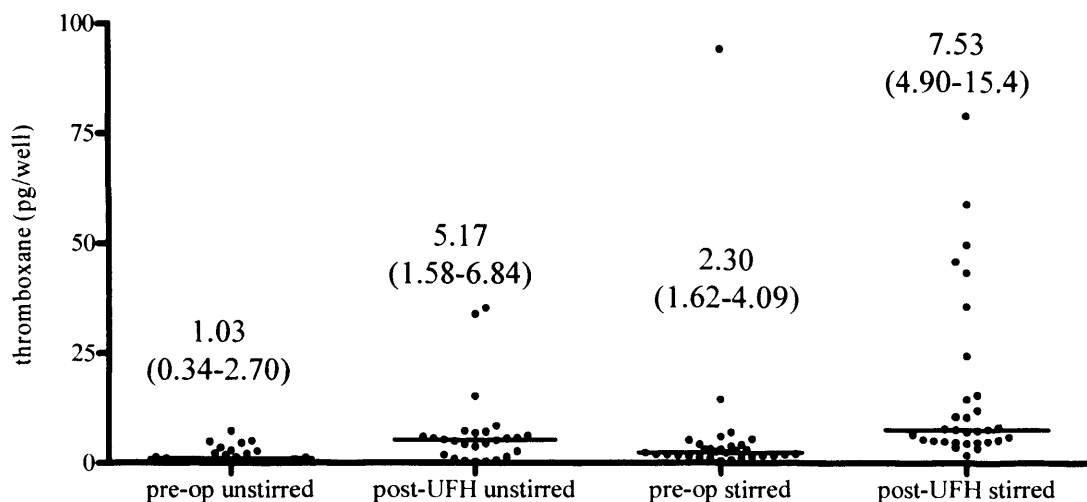
In the samples studied following the addition of arachidonic acid with stirring (n=29), the levels were at least 100-fold higher, (so for analysis they were diluted down to 1:100) with pre-heparin median of 1784pg/well (732.0-3716) and post-heparin median of 1306pg/well (608.7-2616), $p=0.5704$. See Figure 3.3.10.

12-HETE (n=30)

Following the addition of 5mmol/L arachidonic acid, the 12-HETE concentration rose from a median of 7.894µg/ml (1.848-13.31) to 10.21µg/ml (5.412-34.47) ($p=0.0485$). See figure 3.3.12

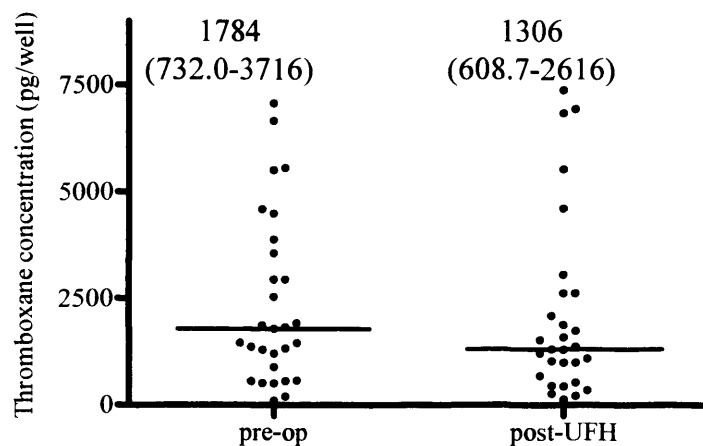
When these data are viewed split for the degree of aggregation, there is a trend towards increasing concentrations of 12-HETE as aggregation increases in the post-heparin samples, with $p=0.0137$ when comparing the high aggregators for pre- and post-heparin 12-HETE concentrations as shown in figure 3.3.12.

Figure 3.3.9 Thromboxane B2 concentrations



The figure shows Thromboxane B2 concentrations (pg/well) in stirred and unstirred samples taken pre-operatively and 3 minutes following in vivo UFH

Figure 3.3.10 Thromboxane B2 concentrations following addition of 5mmol/L AA



The figure shows Thromboxane B2 concentrations (pg/well) in samples stimulated with 5mmol/L AA, taken pre-operatively and 3 minutes following in vivo UFH

Figure 3.3.11 Thromboxane B2 concentrations when samples are split for degree of platelet aggregation

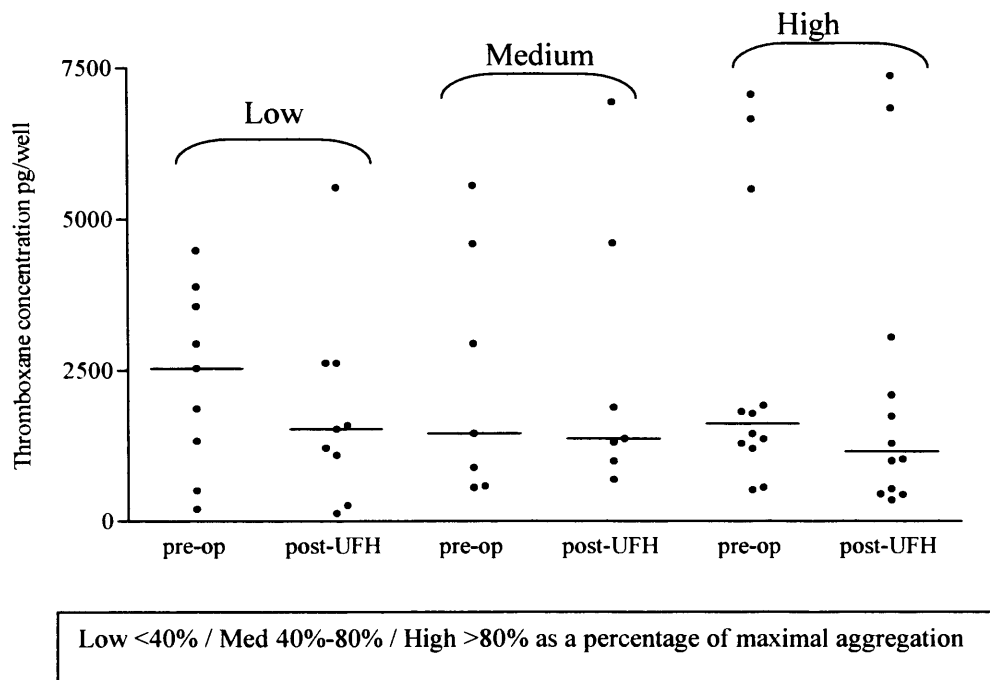
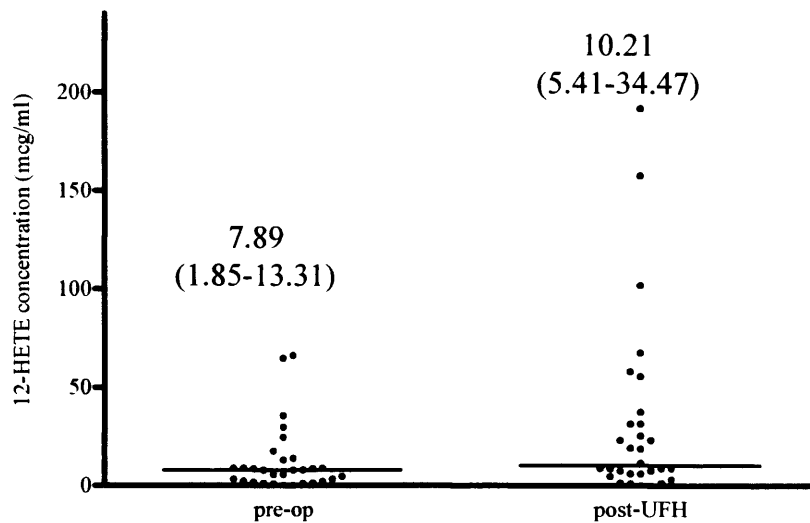


Figure shows thromboxane B2 concentrations in the samples stimulated with 5 mmol/L AA taken pre-operatively and 3 minutes following administration of in vivo UFH (5000 units). The results are split by the degree of platelet aggregation exhibited, where low <40%, High >80% and medium between the two.

Figure 3.3.12 12-HETE concentration in samples following stimulation with 5mmol/L AA



The figure shows 12-HETE concentrations (mcg/well) in samples stimulated with 5mmol/L AA, taken pre-operatively and 3 minutes following in vivo UFH

Figure 3.3.13 12-HETE concentrations when samples are split for degree of platelet aggregation

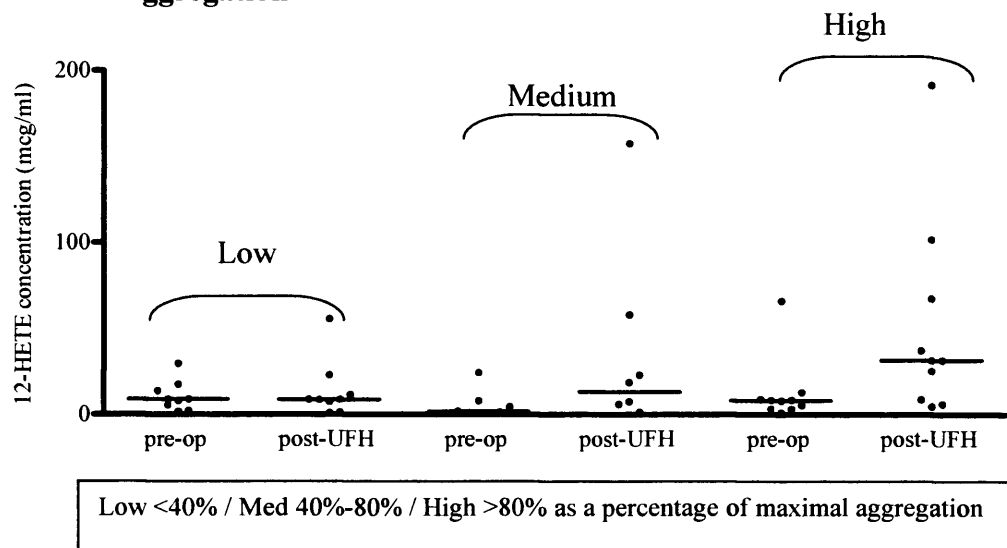


Figure shows 12-HETE concentrations in the samples stimulated with 5 mmol/L AA taken pre-operatively and 3 minutes following administration of in vivo UFH (5000 units). The results are split by the degree of platelet aggregation exhibited, where low <40%, High >80% and medium between the two.

3.4 Discussion

The studies described in this chapter were aimed at determining the underlying mechanism for the apparent loss of aspirin's anti-platelet actions which occurs following administration of UFH during carotid endarterectomy. One of the theories postulated included heparin having a direct effect on the platelet. The initial work looking at the addition of *in vitro* heparin to the samples showed that there was a rise in spontaneous aggregation following *in vitro* UFH and that this was the same as that seen following *in vivo* UFH. This would concur with other platelet studies that report a direct action of heparin on the platelet, as described in the introductory section to this chapter.

However, the rise in aggregation in response to AA could not be reproduced simply by the addition of UFH *in vitro*. The increased aggregation was twice as high following *in vivo* UFH than with *in vitro* UFH. This suggests that there are two mechanisms – the direct stimulatory effect of UFH on the platelet and something additional occurring *in vivo*. The *in vitro* effects are the same whether UFH is added to whole blood or PRP, excluding the possibility that it is a constituent of whole blood which is responsible for the additional effect of UFH on the platelet.

When a COX-1 blocker (aspirin) was added to the post-UFH (either whole blood or PRP) samples, there was no decrease in the aggregation seen, suggesting that the “new” or “recovered” aggregation may not be a simple re-activation or release of COX-1, and indeed, another enzyme pathway not blocked by aspirin may be responsible for the post-heparin aggregation seen on stimulation with AA.

Similarly, addition of the COX-2 blocker NS-398 did not decrease aggregation when added to whole blood or PRP. Again this would support the theory that COX-2 is not the enzyme behind the recovered aggregation.

With the aggregation in response to AA not being preventable by the *in vitro* addition of COX-1 or COX-2 inhibitors, it was considered that there true aggregation may not be occurring, with agglutination of the platelets giving false aggregometry results. The GPIIb/IIIa blocker ABCIXIMAB was therefore added to the samples. The aggregation seen to AA in the post-heparin samples was completely blocked by ABCIXIMAB; therefore this aggregation was occurring by

virtue of GPIIb/IIIa, the final common receptor pathway for platelet aggregation. This strongly implies that the post-heparin aggregation was true aggregation, not an artefact.

One theory postulated that as IV heparin causes a release of hepatic and lipoprotein lipase (Olivecrona et al, 1993; Persson, 1998), this would lead to an increased circulating arachidonate *in vivo*. In theory, then, addition of extra AA *in vitro* should mimic the aggregation seen. Initially, results seemed to confirm this, with a double dose of AA (10mmol/L) leading to maximal platelet aggregation as recorded by Born Aggregometry. However, this amount of AA could cause agglutination of the platelets by purely physical not chemical means. To assess this, AA 80 was added in the presence of ABCIXIMAB. In fact the “aggregation” could not be blocked with Abciximab, showing both that it is unlikely that an increase in circulating AA is causing a decrease in aspirin’s anti-platelet effect and also that this could not be reproduced in the *in vitro* environment.

The PFA-100 whole blood method of assessing platelet function has been used recently to both define and diagnose aspirin resistance (Harrison et al, 1999; Kratzer and Born, 1985; Kratzer et al, 1985)

The samples are tested on two cartridges, coated with ADP-collagen or Epinephrine-collagen. The epi-collagen cartridge has been reported (Gum et al, 2001; Anderson et al, 2002; Christiaens et al, 2002; Macchi et al, 2002) to show aspirin resistance as a shortened closure time (seconds). There was no difference seen between samples taken pre-operatively or following the administration of UFH for either cartridge, in patients with large differences in platelet aggregation between time 1 and 2. The machine stops recording when 300 seconds is reached – so the sample range in these patients may have fallen outside this. This may mean that the Born method of platelet aggregometry is a more sensitive method for measuring the diminished aspirin effect seen after UFH administration.

ELISA measurements of Thromboxane B2 have shown that aspirinated platelets are capable of generating small amounts of thromboxane both spontaneously and following physical stimulation (stirring). The addition of AA leads to a huge (>100-fold) rise in production. However, there does not appear to be a difference

between the samples before and after *in vivo* heparin. It is possible that there is some cross-over with the ELISA kit, and that in fact it is AA itself that is being measured. There was also no correlation seen between the degree of aggregation (“low aggregators” and high aggregators”) and the amount of TxB2 measured. A rise in 12-HETE concentration was seen following *in vivo* addition of heparin which approached statistical significance. When split for degree of aggregation, there seems to be a significant difference between the groups of low and high aggregators, supporting the theory that it is the activation of the lipoxygenase pathway and formation of 12-HETE that leads to the increased platelet aggregation seen.

3.5 Conclusions

The apparent recovery of the platelet to aggregate in response to AA despite adequate pre-operative aspirin cannot be reproduced by the addition of *in vitro* UFH or excess AA, is inhibited by neither COX-1 nor COX-2 inhibitors, and can be blocked by Abciximab, the final common receptor for platelet aggregation. This aggregation does not appear to be mediated by TxB2, but may be driven by the metabolism of AA via an alternative enzyme pathway (12-LOX) leading to the increased production of its other metabolite 12-HETE.

Chapter 4 A Pilot Randomised Controlled Trial comparing the effects of Unfractionated and Low Molecular Weight Heparin on platelet aggregation during CEA

4.1 Introduction

The time course study described in Chapter 2 looked at 41 patients undergoing CEA. This study showed a marked rise in platelet aggregation on stimulation with AA, from $3.94 \pm 2.20\%$ to $45.1 \pm 29.3\%$ ($p < 0.0001$) within three minutes of the administration of an intravenous bolus of UFH. Although the mechanism by which this occurs remains unknown, the studies described in chapter 3 suggest that the effect cannot be recreated simply by adding heparin to the samples *in vitro* / *ex vivo*. That is, there must be an additional process occurring *in vivo*.

It has been shown that UFH can induce spontaneous platelet aggregation (Chong & Ismail, 1989). This interesting study looked at platelets from a patient with Bernard-Soulier syndrome (no platelet Ib and IX receptors) and a patient with Glanzmann's thromboasthenia (no GPIIb/IIIa). Platelet aggregation was tested with an expanded scale Born Aggregometry. The study showed that there was immediate mild platelet aggregation on addition of heparin (0.5 to 1 U/ml), which peaked at 5 to 8 minutes and was non-reversible. The presence of small platelet aggregates was confirmed by electron microscopy. Variations in the response of the two patients (with Bernard-Soulier syndrome and Glanzmann's thromboasthenia) suggested that GPIIb/IIIa is required for heparin-induced platelet aggregation. The heparin-induced aggregation could be inhibited by protamine, EDTA, PGE1, but *not* aspirin, indomethacin or apyrase. It was also shown that AT III and fibronectin inhibited aggregation by binding to heparin in a dose dependant fashion and that anti GPIIb/IIIa monoclonal antibody strongly inhibited the heparin-induced aggregation. In addition, there was no increase seen in the TxB2 concentration and no secretion of α -granule and dense body constituents (no rise seen in B-thromboglobulin or radio-labelled serotonin). The aggregation could be blocked by inhibitors of metabolism implying that metabolic energy was required.

Current evidence suggests that LMWH causes less platelet activation / aggregation than UFH. A study by Landolfi et al (Thrombosis and Haemostasis 1994)

compared the effects of Unfractionated and Low Molecular Weight Heparins on platelet thromboxane biosynthesis *in vivo*, by giving heparin to patients undergoing cholecystectomy, then measuring urinary Tx_{B2} metabolites. They found that there was an increased metabolite concentration in the urine of those patients who had received UFH, but not those who had been given LMWH. In contrast to Chong et al, this production of urinary metabolites was inhibited by aspirin.

Muriithi, Belcher et al (2002) showed that lipolysis generates platelet dysfunction after *in vivo* heparin administration; however their study concluded that the release of hepatic and lipo-protein lipase following heparin administration *lowered* the aggregation seen in response to collagen (when measured with whole blood impedance Aggregometry).

Much of the research relating to heparin's interaction with platelets was born from the clinical interest in Heparin-Induced Thrombocytopenia (HIT). In 1995 Chong described two main mechanisms of HIT – 1 - non-immune – with a transient decrease in platelet count immediately after administration - due to direct interaction of heparin with platelet surface causing activation, reversible clumping, trapping and sequestration and 2 - based on production of antibodies, causing severe drop in platelet count several days after heparin. These are now two clinically recognisable entities - Type I – with a mild thrombocytopenia, occurring during the first few days, where the platelet count seldom falls below 100×10^9 and may return to normal even if heparin is continued and Type II – a severe thrombocytopenia with delayed onset, usually 4-14 days, but sooner if there has been previous exposure. Paradoxically, with type 2, there is usually a thrombotic clinical picture (occasionally disseminated intravascular coagulation). The diagnosis is made by a low platelet count occurring after heparin administration after exclusion of other causes (infection, auto-immune), with resolution of thrombocytopenia after cessation of heparin. It may be possible to demonstrate a heparin-dependant antibody by *in vitro* testing. Various laboratory tests have been described (platelet aggregation (HIT serum induced platelet aggregation in the presence of low/therapeutic concentration of heparin), platelet ¹⁴C-serotonin release (two-point ¹⁴C-serotonin release assay described by Sheridan), platelet factor 3 availability, complement fixation, passive haemagglutination tests, platelet-

associated IgG, serum bindable IgG to normal platelets by ELISA and flow cytometry).

Other studies have suggested a clinical benefit with the use of LMWH rather than UFH. A meta-analysis by Le Nguyen et al reported a possible improved outcome in acute coronary syndrome (unstable angina and non-Q-wave myocardial infarction). Zed has recommended that LMWH should replace UFH as the anti-thrombotic agent of first choice in the management of acute coronary syndromes.

When looking at platelet activation as a determinant of thrombotic and restenotic complications following intra-coronary stenting, Knight et al found a significant increase in platelet responsiveness to adenosine diphosphate in patients treated with conventional anticoagulants, as a probable consequence of treatment with heparin. They showed that LMWH stimulates platelets less than unfractionated heparin.

With this evidence in support of heparin's direct and indirect effects on the platelet, and the suggestions that LMWH is more efficacious, a pilot randomised study was designed to compare the effects of in vivo UFH with LMWH in patients undergoing CEA. As the rate / incidence of significant peri- and post-operative thrombotic complications and high embolisation rates are low (2-3%), the study was powered to detect a difference in platelet aggregation, not clinical outcome (emboli or stroke).

4.2 Materials and Methods

Ethical committee approval was granted for the study. Fifty consecutive patients undergoing elective CEA were recruited; all were given standard written patient information and gave their informed consent. The patients were randomised with a closed envelope technique to receive either 5000u UFH or an equivalent dose (2500u) of LMWH during standardised CEA (see Chapter 2). Samples were taken using a standardised technique (see Chapter 2) at three time points – pre-op, 3 minutes following heparin and 2 hours post-operatively. Platelet aggregation to arachidonic acid (AA) 5mmol/L was assessed by Born aggregometry, while Thromboxane-B2 (TxB2) and 12-HETE production (end-products of cyclo-oxygenase and lipo-oxygenase pathways respectively) were measured by ELISA. The materials – aggregometer, reagents, heparin and ELISA kits are as described in previous chapters. Fragmin™ was the LMWH used during the operation.

4.3 Results

4.3.1 Patient Demographics

The two groups of patients were very well matched for sex ratio (identical), age range (median 68 years in the UFH group, 69.5 years in the LMWH group) and weight (75kg in the group receiving UFH and 80kg in those receiving LMWH). The past medical and drug histories of the groups were also very similar. See Table 4.3.1

4.3.2 Blood Counts

In both groups, there was a small but significant rise in white cell count during and after surgery. (See tables 4.3.2 and 4.3.3). The whole blood platelet count, haemoglobin, and haematocrit did not alter significantly. PRP platelet count dropped significantly following administration of both unfractionated and low molecular weight heparin.

Table 4.3.1 Patient Demographics

		UFH (n=25)	LMWH (n=25)
Sex Ratio (Male: Female)		21:4	21:4
Median Age in years (range)		68 (52-86)	69.5 (52-81)
Median Weight in kg (range)		75 (62-111)	80 (60-108)
Operative side (Right: Left)		14:11	15:10
Past medical history	Diabetes	3	3
	Hypertension	18	18
	Past Myocardial Infarct	5	7
	Completed Stroke	9	9
Smoking History (Current: ex: never)		5:19:1	4:15:6
Drug History	Raised lipids	21	13
	Beta-blocker	5	8
	Ca-channel blocker	9	9
	Statin	19	14
	ACE Inhibitor	5	9
	Proton-pump inhibitor	4	3
Most recent symptom (TIA:CVA:amarosis fugax:none)		13:6:5:0	14:1:6:1

Table 4.3.2 Blood cell counts during CEA

	Time 1		Time 2		Time 3	
	LMWH	UFH	LMWH	UFH	LMWH	UFH
Platelet count (x10⁹/L)	202 (170-256)	205 (170-239)	212 (162-296)	210 (178.5-239)	190 (129-304)	195 (163.5-257.5)
Hb (g/dL)	11.5 (10.8-13.2)	12.2 (11.2-12.7)	11.4 (10.4-12.9)	11.3 (10.45-12.95)	11.4 (10.7-13.6)	11.9 (10.55-12.8)
WCC (x10⁹/L)	6.1 (5.3-8.3)	5.6 (4.9-6.4)	7.0 (5.6-9.3)	6.3 (5.35-6.9)	7.6 (6.3-10.1)	7.1 (6.35-8.0)
HCT	38.5 (36.0-42.2)	38.6 (35.0-39.9-54.6)	36.4 (34.6-41.0)	36.6 (32.9-40.1)	37.3 (35.6-43.9)	37.4 (33.6-39.2)
PRP Platelet counts (x10⁹/L)	152 (114-231)	147 (109-195)	101.5 (69.5-163)	100 (48-142)	142 (87-207)	108 (81-174.5)

Table 4.3.3 p-values for results shown in Table 4.3.2

		Platelet	Hb	WCC	HCT	PRP
LMWH	Pre-op to post-heparin	0.1704	0.1565	<0.0001	0.1401	0.0003
	Post-heparin to post-op	0.0283	0.0904	0.0380	0.0809	0.1514
	Pre-op to post-op	0.0911	0.2153	0.0005	0.1917	0.0135
UFH	Pre-op to post-heparin	0.0959	0.9138	0.0218	0.9172	0.0001
	Post-heparin to post-op	0.9822	0.1504	0.0115	0.1540	0.0145
	Pre-op to post-op	0.2627	0.5839	0.0003	0.4853	0.0291
LMWH vs UFH	Pre-op	0.8691	0.5753	0.056	0.4163	0.8864
	Post-heparin	0.6995	0.7163	0.2563	0.5626	0.5626
	Post-op	0.8171	0.8455	0.3064	0.4651	0.4808

4.3.3 Platelet Aggregation

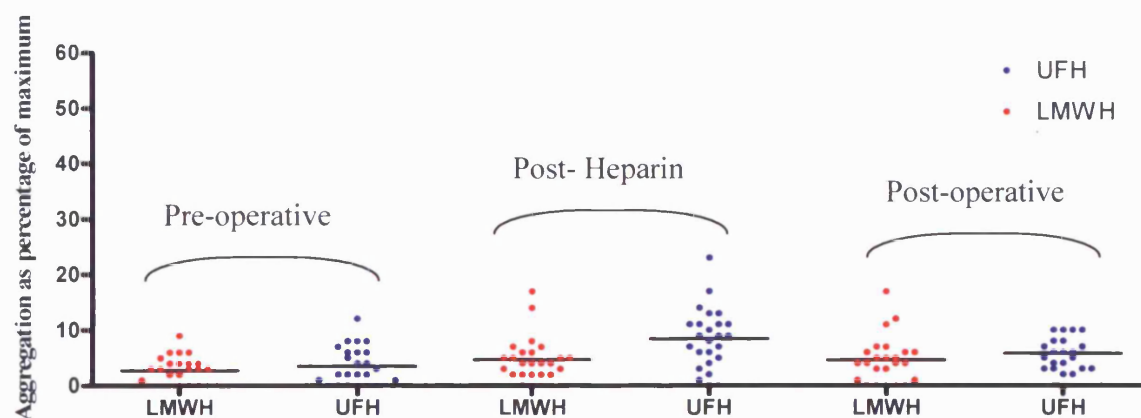
Spontaneous

The spontaneous aggregation was low in the pre-operative samples for both LMWH and UFH, with no difference between the groups. Both groups showed an increase in aggregation following the *in vivo* administration of heparin, which although low in both groups was more marked following UFH ($p=0.0062$). See Table 4.3.4 and Figure 4.3.1

Table 4.3.4 Spontaneous Aggregation of Platelets during CEA

		UFH	LMWH	p-value
Spontaneous Aggregation Median (range)	Pre-op	2 (0-12)	3 (0-9)	0.5530
	p-pre to post hep	0.0001	0.0269	
	Post-heparin	9 (0-23)	4 (0-17)	0.0062
	p- post hep to post op	0.0034	0.9556	
	Post-op	6 (2-10)	4 (0-17)	0.1515
	p-pre op to post-op	0.0159	0.0396	

Figure 4.3.1 Spontaneous Aggregation of Platelets during CEA



The figure shows spontaneous aggregation in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at three time points – pre-operatively, 3 minutes following the intravenous administration of heparin and 2 hours post-operatively.

Aggregation to 5mmol/L AA

Again, in the pre-heparin samples, the groups were almost identical in their response to stimulation with AA. Both groups showed increased aggregation following heparin. However, in the samples taken following heparin, there was a much more marked response to the UFH than LMWH (55% range 12-108% for UFH, 22% range 5-96%). This difference was highly significant ($p=0.004$). By two hours post-operatively, the platelets from the patients who received LMWH returned to their baseline response to AA, whereas the UFH group continued to exhibit an increased aggregatory response (median 11% versus 18%, $p=0.01$). See table 4.3.5 and figure 4.3.2.

4.3.4 Thromboxane B2 measurements

TxB2 concentrations in unstimulated samples (no stirring)

Pre-operatively (pre-heparin) there was no difference in the TxB2 concentrations between the two groups. Following administration of heparin, there was a significant increase in TxB2 in both groups (see table 4.3.6 and figure 4.3.3). There was no difference between the two groups following heparin and spontaneous stirring.

TxB2 concentrations following stimulation with stirring

The same pattern was seen in the samples when analysed following stirring (spontaneous aggregation). There was no difference between the two groups either pre- or post-heparin. Both groups showed a significant rise in TxB2 concentration following the addition of heparin. See table 4.3.7 and figure 4.3.4.

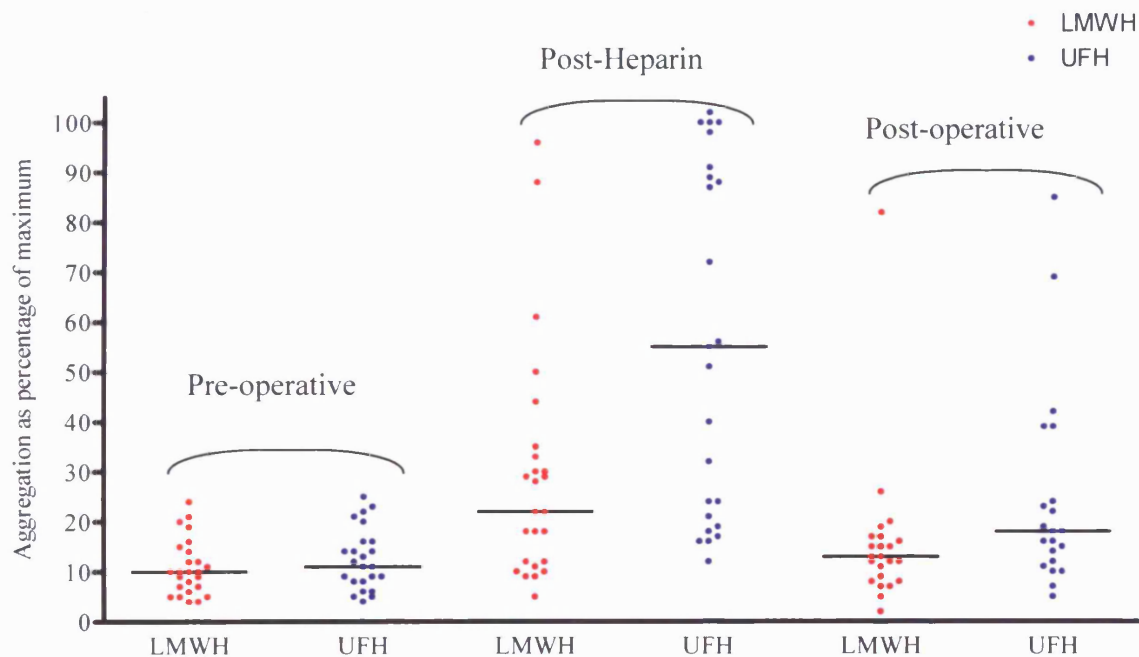
TxB2 concentrations following stimulation with AA 5mmol/L

Following addition of AA 5mmol/L, there was no significant rise in the TxB2 concentration in the post-heparin samples. No difference was detected between the two groups. See table 4.3.8 and figure 4.3.5.

Table 4.3.5 Aggregation in response to AA 5mmol/L

		UFH	LMWH	P-value
Aggregation to AA 40 Median (range)	Pre-op	11 (0-25)	10 (4-24)	0.5031
	p-pre to post hep	P<0.0001	0.0002	
	Post-heparin	55 (12- 108)	22 (5-96)	0.0044
	p- post hep to post op	0.0001	0.0423	
	Post-op	18 (5-85)	13 (2-82)	0.0445
	p-pre op to post-op	0.0111	0.1594	

Figure 4.3.2 Aggregation in response to AA 5mmol/L

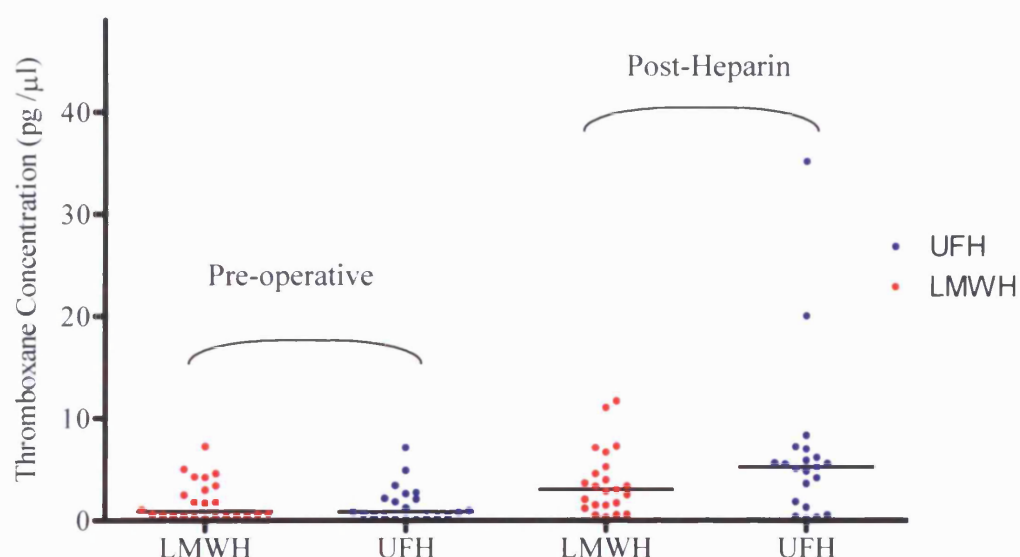


The figure shows aggregation in response to 5mmol/L AA in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at three time points – pre-operatively, 3 minutes following the intravenous administration of heparin and 2 hours post-operatively.

Table 4.3.6 TxB2 concentrations (pg/μl) in unstimulated samples (unstirred)

	LMWH		UFH	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median	0.91	2.96	0.88	5.17
(range)	(0.23 - 7.27)	(0.37 - 11.70)	(0.12 - 7.15)	(0.17 - 35.16)
Std. Deviation	1.95	3.24	1.81	7.38
p-value	p<0.0001 pre- v post-heparin LMWH		p=0.0003 pre- v post-heparin UFH	
p-value	p=0. 4193 LMWH v UFH pre-heparin		p=0.3580 LMWH v UFH post-heparin	

Figure 4.3.3 TxB2 concentrations in unstimulated samples (no stirring)

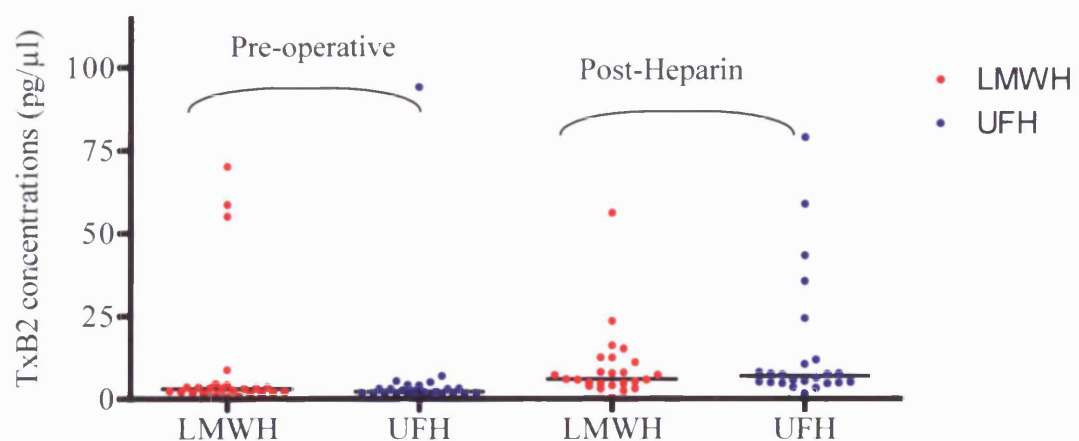


The figure shows thromboxane concentrations in unstimulated samples in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at two time points – pre-operatively, and 3 minutes following the intravenous administration of heparin.

Table 4.3.7 TxB2 concentrations (pg/μl) following stirring

	LMWH		UFH	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median (range)	2.33 (0.62-8.76)	5.76 (2.53 - 23.55)	2.28 (0.47 - 6.95)	6.06 (1.71 - 35.48)
Std. Deviation	1.76	5.22	1.72	7.89
p-value	p<0.0001 pre- v post-heparin LMWH		p=0.0004 pre- v post-heparin UFH	
p-value	p=0.7642 LMWH v UFH pre-heparin		p=0.8392 LMWH v UFH post-heparin	

Figure 4.3.4 TxB2 concentrations (pg/μl) following stirring

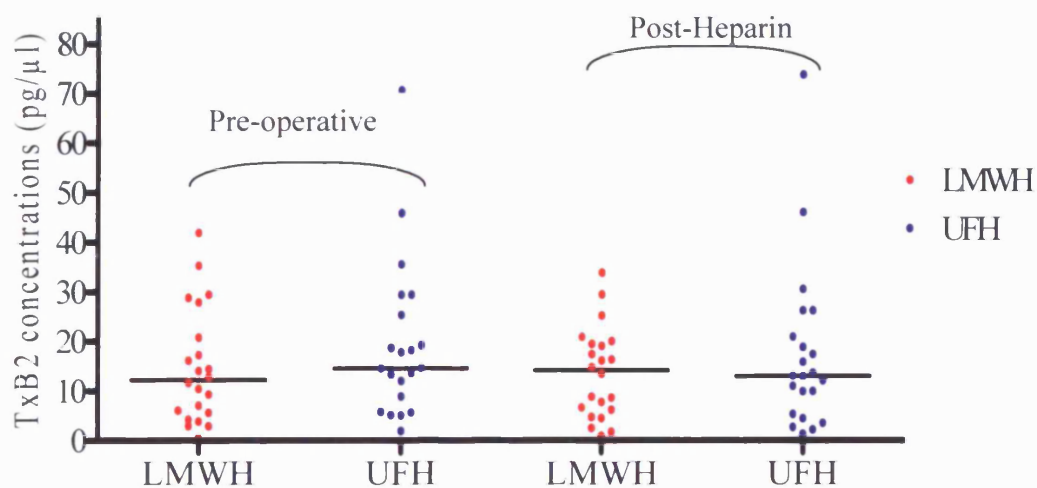


The figure shows thromboxane concentrations in stirred samples, in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at two time points – pre-operatively, and 3 minutes following the intravenous administration of heparin.

Table 4.3.8 TxB2 concentrations (pg/μl) following AA 5mmol/L

	LMWH		UFH	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median (range)	12.79 (2.97- 29.46)	13.44 (1.72- 33.82)	14.52 (5.07 - 70.66)	12.90 (2.63- 73.75)
Std. Deviation	8.63	9.11	15.97	16.72
p-value	p=0.9780 pre- v post-heparin LMWH		p=0.5621 pre- v post-heparin UFH	
p-value	p= 0.4278 LMWH v UFH pre-heparin		p=0.8799 LMWH v UFH post-heparin	

Figure 4.3.5 TxB2 concentrations (pg/μl) following AA 5mmol/L



The figure shows thromboxane concentrations in samples following aggregation stimulated by 5mmol/L AA, in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at two time points – pre-operatively, and 3 minutes following the intravenous administration of heparin.

4.3.5 12-HETE measurements in samples stirred with AA (5mmol/L)

Pre-heparin there was no difference between the concentrations of 12-HETE for the two groups. In the LMWH, there was no significant difference following the in vivo addition of heparin. However, following UFH, there was a significant rise in the concentration of 12-HETE, with the LMWH group having a much lower concentration of 12-HETE. See table 4.3.9 and figure 4.3.6.

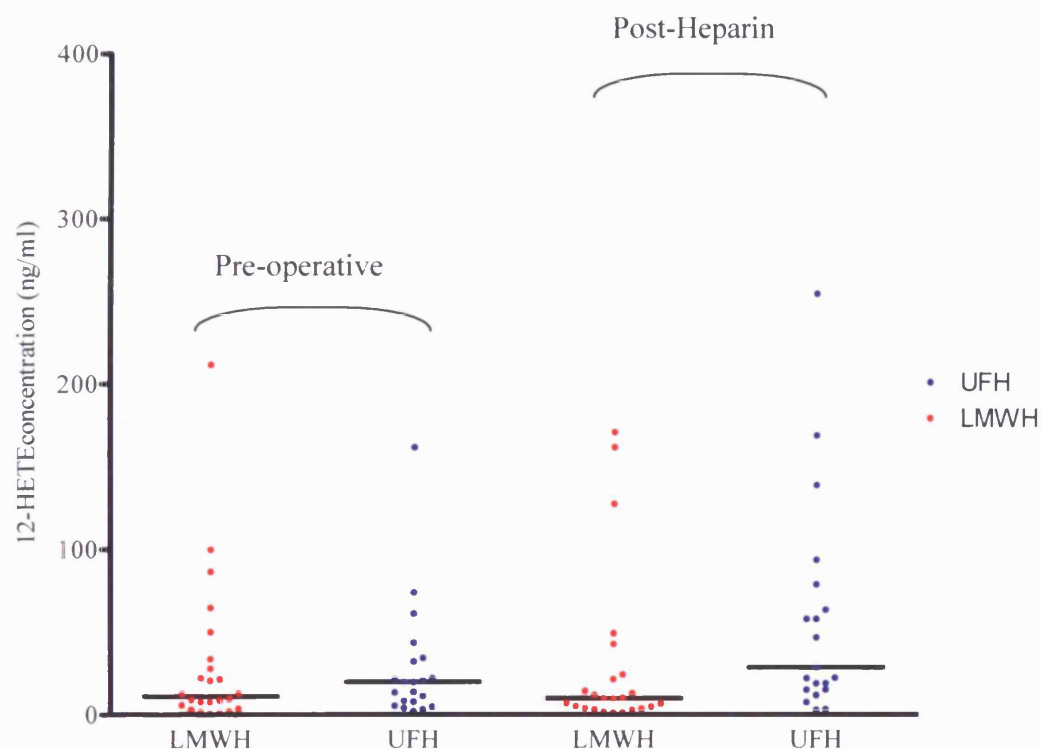
4.3.6 Results comparing aggregation with 12-HETE and TxB2 rise

When the whole group was analysed according to the degree of aggregation, regardless of the type of heparin that had been administered, there were some interesting results. The table 4.3.10 and figure 4.3.8 show the 12-HETE results and table 4.3.11 and figures 4.3.8 summarise those for TxB2. In the group whose aggregation did not significantly rise following heparin (aggregation <50%) there was no rise in either the TxB2 or 12-HETE concentrations. The group that showed a more significant rise in aggregation (>50%) also showed a significant rise in 12-HETE, but no corresponding rise in TxB2.

Table 4.3.9 12-HETE concentrations (ng/ml) in stirred samples with AA 5mmol/l

	LMWH		UFH	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median (range)	11.1 (0.8 – 211.5)	11.2 (1.8- 170.8)	16.8 (2.1- 161.6)	37.8 (2.9- 393.9)
Std. Deviation	50.9	53.0	36.1	98.7
p-value	p=0.5319 pre- v post-heparin LMWH		p=0.0536 pre- v post-heparin UFH	
p-value	p= 0.6750 LMWH v UFH pre-heparin		p=0.0295 LMWH v UFH post-heparin	

Figure 4.3.6 12-HETE concentrations (ng/ml) in stirred samples with AA 5mmol/l



The figure shows 12-HETE concentrations in samples stimulated with 5mmol/L AA, in patients receiving Low Molecular Weight Heparin (LMWH) with red dots and Unfractionated Heparin (UFH) with blue dots at two time points – pre-operatively, and 3 minutes following the intravenous administration of heparin.

Figure 4.3.7 12-HETE concentrations (ng/ml) when split for degree of platelet aggregation (< or >50%)

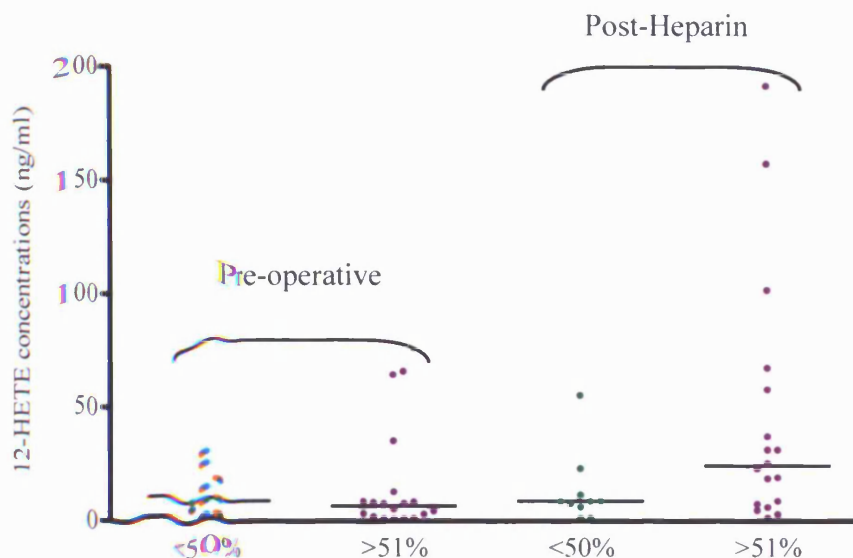
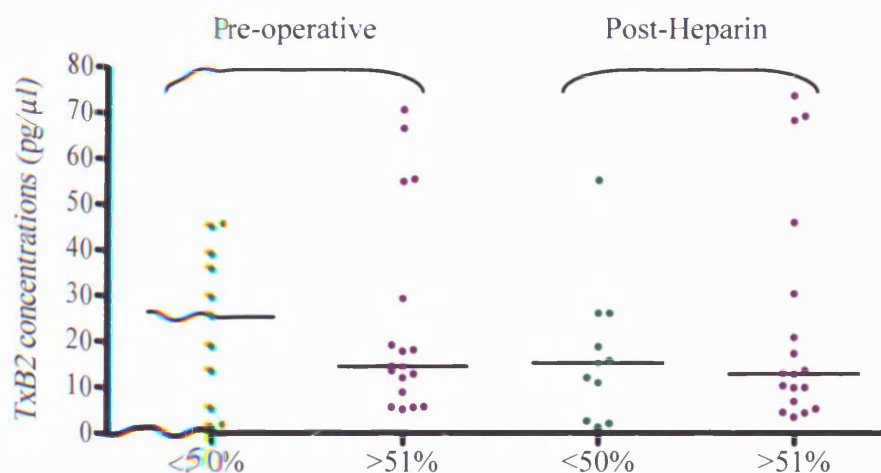


Figure 4.3.8 TxB2 concentrations (pg/ μ l) when split for degree of platelet aggregation (< or >50%)



Figures show 12-HETE and TxB2 concentrations following stimulation of samples with 5mmol/L AA, split for degree of aggregation where low <50% (green dots) and high >51% (purple dots). The samples are pre-operative and 3 minutes following intravenous administration of heparin (either LMWH or UFH).

Table 4.3.10 12-HETE concentrations (ng/ml) when split for degree of platelet aggregation (< or >50%)

	Low Aggregation (<50%)		High Aggregation (>50%)	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median (range)	8.89 (1.69-29.55)	8.72 (1.15- 55.52)	6.63 (0.84- 66.12)	24.27 (1.44- 191.8)
Standard Deviation	9.284	16.06	20.44	54.37
P values(pre- v post-heparin)	p=0.5566		p=0.0163	

Table 4.3.11 TxB2 concentrations (pg/μl) when split for degree of platelet aggregation (< or >50%)

	Low Aggregation (<50%)		High Aggregation (>50%)	
	Pre-heparin	Post-heparin	Pre-heparin	Post-heparin
Median (range)	25.32 (1.00 -45.85)	15.27 (1.31 - 55.22)	14.54 (5.16 - 70.66)	12.98 (3.51- 73.75)
Standard Deviation	16.75	15.40	21.98	24.05
P values (pre- post-heparin)	p=0.2061		p=0.4793	

4.4 Discussion

This study has shown that there is significantly less platelet aggregation when LMWH is used during CEA. Although this study was not powered to assess clinical endpoints, it is conceivable that patients with higher aggregatory potential following administration of heparin (via reversal or bypassing of the COX / aspirin pathway) are at increased risk from thrombo-embolic complications following CEA and indeed, other vascular operations and procedures. The rise in 12-HETE, which mirrors the rise in aggregation, is suggestive of a link between the two perhaps via the induction of lipoxxygenase by heparin. A further study is planned, with a larger cohort of patients to assess clinical end-points (emboli in the middle cerebral artery as measured with TCD monitoring).

4.5 Conclusions

UFH when given during CEA causes increased platelet aggregation by reducing the anti-platelet effect of aspirin. LMWH has a similar but much less marked effect and may be safer at preventing peri-operative thrombo-embolic events.

Chapter 5 Summary and Conclusions

A number of well-designed randomised control trials have proved that carotid endarterectomy can reduce the risk of stroke in symptomatic and selected asymptomatic patients with significant carotid artery stenosis. The overall degree of benefit is dependant on low peri-operative morbidity and mortality, particularly the thrombo-embolic complications of stroke and myocardial infarction.

There is a large body of research addressing the issues around stroke prevention and CEA, with optimisation of medical therapies, operative techniques, intra-operative monitoring, detection of technical error and post-operative management to reduce the risk of complications.

It has been shown that the risk of intra-operative stroke can be virtually abolished by a policy of quality control. Post-operative thrombotic stroke however is still a problem, despite absence of technical error and optimal medical therapy. The evidence points toward a patient / platelet dependant risk; patients with high numbers of emboli detected in the middle cerebral artery with TCD monitoring having increased platelet responsiveness. Once identified, these patients can be treated with intravenous Dextran 40 to reduce emboli and prevent the progression on to carotid artery thrombosis and stroke.

Aspirin's anti-platelet effect is reduced during CEA. When does this occur and how long does the effect last?

A trial comparing a single dose of the anti-platelet drug Clopidogrel with aspirin versus aspirin alone prior to CEA showed incidentally that the anti-platelet effect of aspirin seemed to diminish during the operation. This was a completely unexpected finding; a phenomenon never previously described. Initially thought to be an artefact, the results were consistent. This increased platelet aggregation could significantly contribute to the pro-thrombotic peri-operative state and the risk of thrombo-embolic complications.

A study was designed to test the hypothesis that a particular event during surgery triggered the change in platelet responsiveness to arachidonic acid. Samples were taken at eight peri-operative time points, each corresponding to an event. A second

group of patients with peripheral vascular disease were also studied at similar time points during lower limb angioplasty.

In both study groups, a consistent decrease in aspirin's anti-platelet effect following the administration of unfractionated heparin was seen. The effect, although transient, persisted into the early post-operative period and in some patients was still detectable 24 hours later. This contradicted the previous belief that aspirin's effect was irreversible for the life of the platelet, due to covalent bonding to the COX enzyme. This effect is important, not just for patients undergoing CEA, but also may contribute to risk of thrombo-embolic complications in patients undergoing other vascular interventional procedures (surgery, angioplasty, stenting).

How does Unfractionated Heparin reduce the effectiveness of Aspirin as an anti-platelet drug?

The time course studies showed that the loss of aspirin's ability to prevent platelet aggregation occurred within three minutes of the administration of heparin, and was not induced by any of the other peri-operative events. The next study aimed to elucidate the mechanism behind the loss of aspirin's effectiveness. It was postulated that this may occur by a direct effect of heparin on the platelet or some other cell/factor present in whole blood, by induction of new "un-aspirinated" cyclo-oxygenase (or its isoform COX-2), or by causing an increase in the circulating, or local levels of arachidonic acid and "flooding" the COX enzyme with substrate, overwhelming aspirin's effect. It is also possible that Arachidonate was converted via alternative enzyme pathway, leading to production of a pro-aggregatory substance, such as 12-HETE, the end product of the action of 12-lipoxygenase on arachidonic acid. The possibility that this phenomenon was an "artefact" caused by platelet clumping rather than true aggregation was also considered. The PFA-100 was used in addition to Born aggregometry to test platelet aggregation.

The results of these experiments showed that the platelets were truly aggregating not clumping, that the simple addition of heparin to either whole blood or PRP could not reproduce the effect. ELISA measurements of TxB2 and 12-HETE

suggested that the aggregation was not mediated by the COX pathway, but may be due to activation of the lipoxygenase enzyme.

Due to the relatively low incidence of patients with high numbers of emboli, no correlation between increased aggregation and emboli numbers / outcome has been seen.

Does Low Molecular Weight Heparin also reduce Aspirin's effectiveness as an anti-platelet drug during CEA?

Current evidence would suggest that LMWH causes less platelet activation / aggregation than UFH, and that its use over UFH may be associated with improved clinical outcomes.

This study randomised patients to receive either LMWH or UFH prior to cross clamping of the arteries during CEA. Platelet aggregation in response to AA was tested pre- and post-heparin.

Although the LMWH did seem to cause a similar reduction in aspirin's efficacy in preventing platelet aggregation stimulated by AA, significantly less aggregation was seen in the LMWH group. Again a rise in 12-HETE mirroring the rise in aggregation was seen, and is suggestive of a link between the two, perhaps via the induction of lipoxygenase by heparin.

Conclusions and Suggestions for Future Research

This body of work has shown that UFH given during CEA and angioplasty causes a loss of aspirin's anti-platelet effect. The effect may be minimised by the use of LMWH.

Although the proportion of patients who develop post-operative thrombotic stroke is relatively small, the consequences for the individual can be devastating. A pro-aggregatory state may contribute to the formation of platelet-rich thrombi on the endarterectomy site, leading to carotid thrombosis and stroke. By furthering our understanding of platelet aggregation in the peri-operative period, in particular the previously unrecognised phenomenon of heparin-related changes to the aspirinated platelet, it may be possible to identify the patients at risk. This may contribute to a reduction in the incidence of post-operative thrombotic stroke. The results of this work may also be applicable to patients undergoing other vascular interventional procedures such as peripheral artery angioplasty, coronary artery angioplasty or stenting or other vascular surgery.

Further studies have been funded and are currently underway with a larger cohort of patients to assess clinical end-points (emboli in the middle cerebral artery as measured with TCD monitoring). The initial results of this would suggest that there is an association between high levels of aggregation and emboli rates (Work presented and winning the Sol Cohen Prize at the annual meeting of the Vascular Surgery Society 2006). The mechanism behind the heparin-platelet interaction is also being studied further.

References

Aberg M, Hedner U, Bergentz SE

The Anti-thrombotic effect of Dextran

Scand J Haematol Supp; 34:61-8, 1979

AbuRahma AF, Robinson P

Indications and complications of carotid endarterectomy as performed by four different surgical specialty groups.

J Cardiovasc Surg (Torino). 1988 May-Jun;29(3):277-82

Aldoori MI, Baird RN

Local Neurological complications during carotid endarterectomy.

J cardiovasc Surg (Torino). 1988 Jul-Aug;29(4):432-6

Amiral J, Pouplard C, Vissac AM, Walenga JM, Jeske W, Gruel Y

Affinity purification of heparin-dependent antibodies to platelet factor 4 developed in heparin-induced thrombocytopenia: biological characteristics and effects on platelet activation.

Br J Haematol 2000;109(2):336-41

Andersen K, Hurlen M, Arnesen H, Seljeflot I

Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease

Thromb Res 2002;108(1):37-42

Antiplatelet trialists' Collaboration.

Collaborative overview of randomised trials of anti-platelet therapy: I. Prevention of death, myocardial infarction, and stroke by prolonged anti-platelet therapy in various categories of patients

BMJ 1994; 308:81-106

Antithrombotic Trialists' Collaboration.

Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients.

BMJ 2002;324:71-86

Bamford J, Dennis M, Sandercock P, Burn J, Warlow C

A prospective study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project; 1981-6. 2. Incidence, case fatality rates and overall outcome at one year of cerebral infarction, primary intracerebral and subarachnoid haemorrhage

J Neurol Neurosurg Psychiatry 1990;53:16-27

Barnett HJ, Taylor DW, Eliasziw M, Fox AJ, Fergusson GG, Haynes RB, Rankin RN, Clagett GP, Hachinski VC, Sackett DL, Thorpe KE, Meldrum HE, Spence JD

Benefit of CEA in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial

NEngJMed 1998 Nov12;339(20):1415-25

Berglund U, Wallentin L

Influence on platelet function by heparin in men with unstable coronary artery disease

Thromb Haemost 1991;66 (6):648-651,

Bergqvist D

Dextran and Haemostasis

Acta Chir Scand 1982; 148:633-40

Bond R, Rerkasem K, AbuRahma AF, Naylor AR, Rothwell PM

Arteriotomy Closure for Carotid Endarterectomy

Cochrane Review Database

Bond R, Rerkasem K, Naylor AR, Aburahma AF, Rothwell PM

Systematic review of randomised controlled trials of patch angioplasty versus primary closure and different types of patch material during carotid endarterectomy

J Vasc Surg 2004 Dec; 40 (6);1126-35

Born GVR

Quantitative investigations into the aggregation of blood platelets.

J Physiol 1962;162:167-71

Bousser MG, Eschwege E, Haguenau M, Lefaucconnier JM, Thibult N, Touboul D, Touboul PJ

“AICLA” controlled trial of aspirin and dipyridamole in the secondary prevention of athero-thrombotic cerebral ischaemia.

Stroke 1983;14:5-14

Breddin HK

Can platelet aggregometry be standardized?

Platelets 2005 May;16(3&4):151-8

Brown RD, Whisnant JP, Sicks JD, O’Fallon, Wiebers DO

Stroke incidence, prevalence and survival: secular trends in Rochester, Minnesota, through 1989

Stroke. 1996 Mar;27 (3) :373-80

Buchanan MR, Butt RW, Hirsh J, Markham BA, Nazir DJ

Role of lipoxygenase metabolism in platelet function: effect of aspirin and salicylate

Prostaglandins leukot med 1986 Feb;21(2):157-68

Calligaro KD, Dougherty MJ

Correlation of carotid artery stump pressure and neurologic changes during 474 carotid endarterectomies performed in awake patients.

J Vasc Surg. 2005 Oct;42(4):684-9

Calzada C, Vericel E, Lagarde M

Low concentrations of lipid hydroperoxides prime human platelet aggregation specifically via cyclo-oxygenase activation

Biochemical Journal 1997;315:495-500

Calzada C, Vericel E, Mitel B, Coulon L, Lagarde M

12(S)-Hydroperoxy-eicosatetraenoic acid increases arachidonic acid availability in collagen-primed platelets

Journal of Lipid research 2001;42:1467-73

CAPRIE Steering Committee

A randomised blinded trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE)

Lancet 1996;348:1329-39

Cazenave JP & Gachet C

A Review. Anti-platelet drugs- do they affect megakaryocytes?

Bailleres Clinical Haematology 1997 Feb;10(1):163-80

Cheng, Chen J, Karlberg K, Sylven C

Heparin enhances platelet aggregation irrespective of anticoagulation with citrate or with hirudin

Thromb Res 1992;67(2):253-62

Chong BH, Ismail F

The mechanism of heparin-induced platelet aggregation

Eur.J.Haematology 1989; 43:245-251

Chong BH

Heparin-induced thrombocytopenia

J Thromb Haemost 2003;1(7):1471-8

Christiaens L, Macchi L, Herpin D, Coisne D, Duplantier C, Allal J, Mauco G, Brizard A

Resistance to aspirin in vitro at rest and during exercise in patients with angiographically proven coronary artery disease

Thromb Res 2002;108(3):115-9

Cooper TM, Ingram DM

Platelet function in the peri-operative period

Aust NZJ Surg 1991 Jun;61(6):441-3

Coull AJ, Lovett JK, Rothwell PM on behalf of the Oxford Vascular Society

Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services.

BMJ 2004. Feb 7; 328(7435):326

Counsell CE, Salinas R, Naylor R, Warlow CP

A systematic review of the randomised trials of carotid patch angioplasty in carotid endarterectomy

European Journal of Endovascular Surgery 1197;13:345-54

Dawes J, Pumphrey CW, McLaren KM, Prowse CV, Pepper DS

The in vivo release of human platelet factor 4 by heparin.

Thromb Res 1982;27(1):65-76

Defreyn G, Bernat A, Delebasse D, Maffrand JP

The effects of aspirin on megakaryocyte prostaglandin production

Exp Biol Med 1980; 163:24-29

Demers LM, Budin R, Shaikh B

Pharmacology of Ticlopidine: a review

Semin Thromb Haemost 1989;15:159-166

Dempsey CM, Lim MS, Stacey SG

A Prospective audit of blood loss and blood transfusion in patients undergoing coronary artery bypass grafting after clopidogrel and aspirin

Crit Care Resusc 2004;6(4):248-52

Dennis M, Bamford J, Sandercock P, Warlow C

Prognosis of transient ischaemic attacks in the Oxfordshire Community Stroke Project

Stroke 1990 Jun;21(6):848-53

Devuyst G, Darbellay GA, Vesin JM, Kemeny V, Ritter M, Droste DW, Molina C, Serena J, Sztajzel R, Ruchat P, Lucchesi C, Dietler G, Ringelstein EB, Despland PA, Bogousslavsky J

Automatic classification of HITS into artifacts or solid or gaseous emboli by a wavelet representation combined with dual-gate TCD.

Stroke 2001; 32(12):2805-9

Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal P

European Stroke prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke.

Journal of Neurological Science 1996; 143:1-13

Diener HC, Darius H, Bertrand-Hardy JM, Humphreys M for the European Stroke Prevention Study 2.

Cardiac safety in the European Stroke Prevention Study 2 (ESPS2).

Int J Clin Pract. 2001;55:162-163

Diener HC, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, Leys D, Matias-Guiu J, Rupprecht HJ for the MATCH Investigators

Aspirin and Clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double blind, placebo-controlled trial

Lancet 2004.364:331-337

Di Minno G, Silver MJ, and Murphy S

Monitoring the entry of new platelets into the circulation after ingestion of aspirin.

Blood 61(6):1081-1085, 1983

Donné A

De l'origine des globules du sang, de leur mode de formation et leur fin.

C R Acad Sci 1842; 14:366

Dutch TIA study group

The Dutch TIA trial: protective effects of low-dose aspirin and atenolol in patients with transient ischaemic attacks or non-disabling stroke:

Stroke 1988;19:512-517

Eastcott HH, Pickering GW, Rob CG

Reconstruction of the internal carotid artery in a patient with intermittent attacks of hemiplegia.

Lancet 1954 Nov 13;267(6846):914-6

European Carotid Surgery Trialists Collaborative Group

MRC European Carotid Surgery Trial Interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis

Lancet 1991 May 25;337(8752):1235-43

European Carotid Surgery Trialists Collaborative Group

Randomised Trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Surgery Trial

Lancet 1998 May 9;351(9113):1379-87

The ESPS group

The European Stroke Prevention Study (ESPS). Principal end-points.

Lancet 1987 Dec12;2(8572):1351-4

Evangelos L

Detection of COX-2 in platelets

MSc (unpublished) 2001, *Leicester University*

Evers SM, Ament AJ, Blaauw G

Economic evaluation in stroke research: a systematic review.

Stroke 2001;31;5:1046-53

Fabris F, Luzzatto G, Stefani PM, Girolami B, Cella G, Girolami A

Heparin-induced thrombocytopenia.

Haematologica 2000;85(1):75-81

Farrell B, Godwin J, Richards S, Warlow C

The United Kingdom Transient Ischaemic Attack (UK-TIA) aspirin trial: final results.

J Neurol Neurosurg Psychiatry 1991;54:1044-1054

Ferguson GG, Eliasziw M, Barr HW, Clagett GP, Barnes RW, Wallace MC, Taylor DW, Haynes RB, Finan JW, Hachinski VC, Barnett HJ

The North American Symptomatic Carotid Endarterectomy Trial : surgical results in 1415 patients.

Stroke 1999;30(9):1751-58.

Fisher CM

Observations of the fundus oculi in transient monocular blindness

Neurology 1959 May;9(5):353-47

Gasperetti CM, Gonias SL, Gimple LW, Powers ER

Platelet activation during coronary angioplasty in humans.

Circulation 1993;88(6):2728-34

Fletcher-Cieutat M, Vanderhoek JY, Bryant RW, Bailey JM

Aspirin enhances the sensitivity of human platelet 12-lipoxygenase to inhibition by 15-HETE, an endogenous regulator

Prostaglandins Leukot Med 1985 May;18(2):255-9

Gaunt ME, Martin PJ, Smith JL, Rimmer T, Cherryman G, Ratliffe DA, Bell PR, Naylor AR

Clinical relevance of intra-operative embolisation detected by Transcranial Doppler ultrasonography during carotid endarterectomy: a prospective study of 100 patients.

Br J Surg 1994 Oct;81(10):1435-9

Gaunt ME, Smith JL, Ratliff DA, Bell PR, Naylor AR

A comparison of quality control methods applied to carotid endarterectomy.

Eur J Vasc Endovasc Surg 1996 Jan;11(1):4-11

Gaunt ME

Transcranial Doppler: preventing stroke during carotid endarterectomy.

Ann R Coll Surg Eng 1998; 80(6):377-87

Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, Panak E, Roberts RS, Sicurella J, Turpie AG

The Canadian American Ticlopidine Study (CATS) in thrombo-embolic stroke.

Lancet 1989;1:1215-20

Ghali R, Palazzo EG, Rodriguez DI, Zammit M, Loudenback DL, DeMuth RP, Spencer MP, Sauvage LR

Transcranial Doppler intra-operative monitoring during carotid endarterectomy: experience with regional or general anaesthesia, with and without shunting.

Ann Vasc Surg 1997; 11(1):9-13

Gorelick PB, Richardson D, Kelly M, Ruland S, Hung E, Harris Y, Kittner S, Leurgans S

for the African-American Anti-platelet Stroke Prevention Study Investigators. Aspirin and Ticlopidine for prevention of recurrent stroke in black patients: a randomized trial

JAMA 2003; 289: 2947-2957

Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, Levine SR, Mosca L, Sacco RL, Sherman DG, Wolf PA, del Zoppo GJ

Primary Prevention of Ischaemic Stroke: A Statement for Healthcare Professionals from the Stroke Council of the American Heart Association

Circulation 2001;103;163-82

Grotemeyer KH, Scharafinski HW, Husstedt IW

Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients.

Thromb Res 1993;71(5):397-403

Guiraud-Chaumeil B, Rascol A, David J, Boneu B, Clanet M, Bierme R

Prevention of recurrences of cerebral ischaemic vascular accidents by platelet anti-aggregants : results of a 3year controlled therapeutic trial (in French)

Rev Neurol (Paris). 1982;138:367-85

Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ

A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease.

J am Coll Cardiol 2003; 41(6):961-5

Halliday A, Mansfield A, Marco J, Peto C, Peto R, Potter J, Thomas D: MRC asymptomatic carotid surgery trial (ACST) collaboration group

Prevention of disabling and fatal strokes by successful CEA in patients without recent neurological symptoms: Randomised Controlled Trial

Lancet 2004 May 8; 363(9420):1491-502

Halsey JH, McDowell HA, Gelman S

Transcranial Doppler and rCBF compared in carotid endarterectomy.

Stroke. 1986 Nov-Dec;17(6):1206-8

Halsey JH Jr., McDowell HA Jr., Gelmon S, Morawetz RB

Blood flow velocity in the middle cerebral artery and regional cerebral blood flow during carotid endarterectomy

Stroke 1989;20:53-8

Hamberg M, Svensson J, Samuelsson B

Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxidases

Proc Natl Acad Sci USA 1975; 72:2994-8

Hankey GJ, Eikelboom JW

Aspirin resistance

Lancet 2006; 367(9510):606-18

Harrison P, Robinson MS, Mackie IJ, Joseph J, McDonald SJ, Liesno R, Savidge GF, Pasi J, Machin SJ

Performance of the platelet function analyser PFA-100 in testing abnormalities of primary haemostasis

Blood Coagul Fibrinolysis 1999 Jan; 10(1):25-31

Hass WK, Easton JD, Adams HP Jr, Pryse-Phillips W, Moloney BA, Anderson S, Kamm B

A Randomised trial comparing Ticlopidine hydrochloride with aspirin for the prevention of stroke in high risk patients: Ticlopidine Aspirin Stroke Study Group

N Engl J Med 1989;321:501-7

Hayes PD, Allroggen H, Steel S, Thompson MM, London NJ, Bell PR, Naylor AR

Randomized trial of vein versus Dacron patching during carotid endarterectomy: influence of patch type on postoperative embolization.

J Vasc Surg. 2001; 33(5)994-1000

Hayes PD, Payne D, Lloyd AJ, Bell PR, Naylor AR

Patients' thromboembolic potential between bilateral carotid endarterectomies remains stable over time.

Eur J Vasc Endovasc Surg 2001;22(6):496-8

Hayes PD, Box H, Tull S, Bell PRF, Goodall AH, Naylor AR

Patients' thrombo-embolic potential following carotid endarterectomy is related to the platelets' sensitivity to ADP

J Vasc Surg 2001; 38(6):1226-31

Heyman A, Wilkinson WE, Hurwitz BJ, Haynes CS, Utley CM, Rosati RA, Burch JG, Gore TB

Risk of ischaemic stroke in patients with TIA.

Neurology 1984 May;34(5):626-30.

Hobson RW, Weiss DG, Fields WS, Goldstone J, Moore WS, Towne JB, Wright CB

Efficacy of Carotid Endarterectomy for Asymptomatic Carotid Stenosis

NEJM 1993;328(4):221-227

Horne MK 3rd, Hutchison KJ

Simultaneous binding of heparin and platelet factor-4 to platelets: further insights into the mechanism of heparin-induced thrombocytopenia.

Am J Haemat 1998;58(1):24-30

Jack DB

One hundred years of aspirin.

Lancet 1997;350(9075):437-9

Jin J

Haemostasis, thrombosis and vascular biology

Blood 2002;99:1

Kabakibi A, Vamvakas EC, Cannistraro PA, Szczepiorkowski ZM, Laposata M

Collagen-induced whole blood platelet aggregation in patients undergoing surgical procedures associated with minimal to moderate blood loss.

Am J Clin Pathol 1998;109(4):392-398

Kannel WB, Wolf PA, McGee DL, Dawber TR, McNamara P, Castelli WP

Systolic blood pressure, arterial rigidity and risk of stroke. The Framingham Study

JAMA 1981 Mar 27;245(12):1225-9

Kirkpatrick PJ, Smielewski P, Whitfield PC, Czosnyka M, Menon D, Pickard JD

An observational study of near-infrared spectroscopy during carotid endarterectomy.

J Neurosurg. 1995 May;82(5):756-63

Knight CJ, Panesar M, Patrinely A, Chronos N, Wright C, Clarke D, Patel D, Fox K, and Goodall AH

Increased platelet responsiveness following coronary stenting. Heparin as a possible aetiological factor in stent thrombosis.

Eur Heart J 1998;19 (8):1239-1248

Kratzer MA, Bellucci S, Caen JP

Detection of abnormal platelet function with an in vitro model of primary haemostasis

Haemostasis 1985;15(6):363-70

Kratzer MA, Born GV

Simulation of primary haemostasis in vitro

Haemostasis 1985;15(6):357-62

Kuroda S, Houkin K, Abe H, Hoshi Y, Tamura M

Near-infrared monitoring of cerebral oxygenation state during carotid endarterectomy.

Surg Neurol. 1996 May;45(5):450-8

Lalau KC, Kinlough-Rathbone RL, Packham MA, Suzuki H, Mustard JF

Conditions affecting the responses of human platelets to epinephrine

Thromb Haemost 1988;60:209-16

Laman DM, Wieneke GH, van Duijn H, van Huffelen AC

High embolic rate early after carotid endarterectomy is associated with early cerebrovascular complications, especially in women.

J Vasc Surg 2002;11(1):9-13

Landolfi R, De Candia E, Rocca B, Ciabattini G, Antinori A, Masetti R, Patrono C

Effects of unfractionated and low molecular weight heparins on platelet thromboxane biosynthesis "in vivo"

Thromb Haemost 72(6):942-946, 1994

Larsson PT, Wallen NH, Hjemdahl P

Norepinephrine-induced human platelet activation *in vivo* is only partly counteracted by aspirin

Circulation 1994;89(5):1951-7

Lennard N, Smith J, Dumville J, Abbott R, Evans DH, London NJ, Bell PR, Naylor AR

Prevention of postoperative thrombotic stroke after carotid endarterectomy: the role of Transcranial Doppler ultrasound.

J Vasc Surg 1997;26(4):79-84

Lennard N, Smith JL, Hayes P, Evans DH, Abbott RJ, London NJ, Bell PR, Naylor AR

Transcranial Doppler directed Dextran therapy in the prevention of carotid thrombosis: three hour monitoring is as effective as six hours.

Eur J Vasc Endovasc 1999;17(4):301-5

Lennard N, Smith JL, Gaunt ME, Abbott RJ, London NJ, Bell PR, Naylor AR

A policy of quality control assessment helps to reduce the risk of intra-operative stroke during carotid endarterectomy.

Eur J Vasc Endovasc Surg 1999;17(3):234-40

Levi CR, Roberts AK, Fell G, Hoare MC, Royle JP, Chan A, Beiles BC, Last G, Bladin CF, Donnan GA

Transcranial Doppler microembolus detection in the identification of patients at high risk of perioperative stroke.

Eur J Vasc Endovasc Surg 1997;14(3):170-6

Levine SP, Towell BL, Suarez AM, Knieriem LK, Harris MM, George JN

Platelet activation and secretion associated with emotional stress

Circulation 1985;71(6):1129-34

Levison JA, Faust GR, Halpern VJ, Theodoris A, Nathan I, Kline RG, Cohen JR

Relationship of protamine dosing with post-operative complications of carotid endarterectomy

Ann Vasc Surg 1999;13:67-72

Linstedt U, Maier C, Petry A

Intraoperative monitoring with somatosensory evoked potentials in carotid artery surgery--
less reliable in patients with preoperative neurologic deficiency?

Acta Anaesthesiol Scand. 1998 Jan;42(1):13-6.

Loll PJ, Picot D, Garavito RM

The structural basis of aspirin activity inferred from the crystal structure of inactivated
prostaglandin H2 synthase

Nat Struct Biol 1995;2(8):637-43

**McKay RD, Sundt TM, Michenfelder JD, Gronert GA, Messick JM, Sharbrough
FW, Piepgras DG**

Internal carotid artery stump pressure and cerebral blood flow during carotid
endarterectomy: modification by halothane, enflurane, and innovar.

Anesthesiology. 1976 Oct;45(4):390-9.

**MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J,
Dyer A, Stamler J**

Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood
pressure: prospective observational studies corrected for the regression dilution bias.

Lancet 1990 Mar 31;335(8692):765-74

Magnus Aberg, Ulla Hedner, Sven-Erik Bergentz

The Anti-thrombotic effect of Dextran

*Scand J Haematol Suppl*1979; 34:61-8

Matsumoto GH, Cossman D, Callow AD

Hazards and safeguards during carotid endarterectomy. Technical considerations

*Am J Surg.*1977 Apr;133(4)458-62

**Mauney MC, Buchanan SA, Lawrence WA, Bishop A, Sinclair K, Danel TM, Tribble
CG, Kron IL**

Stroke rate is markedly reduced after carotid endarterectomy by avoidance of protamine

J Vasc Surg 1995;22:264-70

McCrory DC, Goldstein LB, Samsa GP, Oddone EZ, Landsman PB, Moore WS, Matchar DB

Predicting complications of carotid endarterectomy

Stroke 1993 Sep;24(9):1285-91

Mehigan JT, Alcott C

Video angioscopy as an alternative to intra-operative arteriography

Am J Surg 1986;152:139-45

Michel W, Hejhal L, First P

Zeitweilige shunts in der vaskularen chirurgie

Thoraxchirurgie 1966;14:35

Mikhailidis DP, Barradas MA, Jeremy JY, Gracey L, Wakeling A, Dandona P

Heparin-induced platelet aggregation in anorexia nervosa and in severe peripheral vascular disease

Eur J Clin Invest 1985;15 (6):313-9

Mohammad SF, Anderson WH, Smith JB, Chuang HY, Mason RG

Effects of heparin on platelet aggregation and release and thromboxane A2 production

Am J Pathol 1981;104(2):132-41,

Morita I, Murota S

Role of 12-lipoxygenase products of arachidonic acid on platelet aggregation

Adv Prostaglandin Thromboxane Leukot Res 1987 (17a):219-223

Muller I, Seyfarth M, Rudiger S

Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement

Heart 2001; 85:92-3

Muriithi EW, Belcher PR, Day SP, Mubarak CA, Caslake MJ, Wheatley DJ

Lipolysis generates platelet dysfunction after *in vivo* heparin administration

Clinical Science 2002;(103):433-40

NASCET collaborators

Beneficial effect of CEA in symptomatic patients with high grade carotid stenosis

NEngJMed 1991 Aug15;325(7):445-53

Naylor AR

An update on the randomised trials of interventions for symptomatic and asymptomatic carotid artery disease

Italian J Vasc Endovasc Surg 2006;13:111-20

Naylor AR

The Asymptomatic Carotid Surgery Trial: bigger study, better evidence

Br J Surg. 2004 Jul;91(7):787-9

Naylor AR, Hayes PD, Allroggen H, Lennard N, Gaunt ME, Thompson MM, London NJ, Bell PR

Reducing the risk of carotid surgery: a 7-year audit of the role of monitoring and quality control assessment.

J Vasc Surg 2000; 32(4):750-9

Newman PM, Chong BH

Heparin-induced thrombocytopenia: new evidence for the dynamic binding of purified anti-PF4-heparin antibodies to platelets and the resultant platelet activation.

Blood 2000; 96(1):182-7

Noorman F, Barrett-Bergshoeff MM, Bekkers M, Emels JJ, Rijken DC

Inhibition of mannose receptor-mediated Clearance of Tissue-type Plasminogen Activator (t-PA) by Dextran: a new explanation for its anti-thrombotic effect

Thromb Haemost 1997;78:1249-54

Olivecrona T, Bengtsson-Olivecrona G, Ostergaard P, Liu G, Chevreuil O, Hultin M

New aspects on heparin and lipoprotein metabolism

Haemostasis 1993 Mar;23 suppl 1:150-60

**Patrignani P, Sciulli MG, Manarini S, Santini G, Cerletti C,
Evangelista V**

COX-2 is not involved in thromboxane biosynthesis by activated human platelets

J Physiol Pharmacol 1999; 50(4):661-7

**Patrono C, Ciabattoni G, Patrignani P, Filabozzi P, Pinca E, Satta MA, van Dome D,
Cinotti GA, Pugliese F, Pierucci A, Simonetti BM**

Evidence for a renal origin of urinary thromboxane B₂ in health and disease.

Adv Prostaglandin Thromboxane Leukotriene Res 1983; 11:493-8

**Patrono C, Ciabattoni G, Patrignani P, Pugliese F, Filabozzi P, Catella F, Davi G,
Forni L**

Clinical pharmacology of platelet cyclo-oxygenase inhibition

Circulation 1985 Dec;72(6):1177-84

Patrono C, Ciabattoni G, Pugliese F, Pierucci A, Blair IA, FitzGerald GA

Estimated rate of thromboxane secretion into the circulation of normal humans

J Clin Invest 1986;77:590-4

Patrono C

Aspirin as an antiplatelet drug

New Eng J Med 1994;330(18):1287-94

Payne DA, Jones CI, Hayes PD, Bell PRF, Naylor AR, Goodall AH

Platelet inhibition by aspirin is diminished in patients during carotid surgery: a form of transient aspirin resistance.

Thromb Haemost 2004 Jul;92(1):89-96

**Payne DA, Jones CI, Hayes PD, Thompson MM, London NJ, Bell PR, Goodall AH,
Naylor AR**

Beneficial effects of Clopidogrel combined with aspirin in reducing cerebral emboli in patients undergoing carotid endarterectomy

Circulation 2004 Mar 30;109(12):1476-81

Perrson E

Lipoprotein lipase, hepatic lipase and plasma lipolytic activity. Effects of heparin and a low molecular weight heparin fragment (Fragmin).

Acta Med Scand Suppl 1998;724:1-56

Plestis KA, Loubser P, Mizrahi EM, Kantis G, Jiang ZD, Howell JF

Continuous electroencephalographic monitoring and selective shunting reduces neurologic morbidity rates in carotid endarterectomy.

J Vasc Surg. 1997 Apr;25(4):620-8.

Prokop A, Meyer GP, Walter M, Erasmi H

Validity of SEP monitoring in carotid surgery. Review and own results.

J Cardiovasc Surg (Torino). 1996 Aug;37(4):337-42

Quinn MJ, Fitzgerald DJ

Ticlopidine and Clopidogrel

Circulation 1999;100:1667-72

Rao GH, Johnson GJ, Reddy RK, and White JG

Rapid return of cyclo-oxygenase active platelets in dogs after a single oral dose of aspirin.

Prostaglandins 1981;22(5):761-772

Reininger CB, Reininger AJ, Steckmeier B, Greinacher A, Lasser R, Schweiberer L

Platelet response to vascular surgery--a preliminary study on the effect of aspirin and heparin.

Thromb Res 1994;76(1):79-87

Reiter R, Rescu U, Sinzinger H

Do human platelets express COX-2?

Prostaglandins, Leukotrienes and Essential Fatty Acids 2001; 64(6):299-305

Rerkasem K, Bond R, Rothwell PM

Local versus General Anaesthesia for Carotid Endarterectomy

Cochrane Database Systematic Review 2004

Robless PA, Tegos TJ, Okonko D, Mansfield AO, Nicolaides AN, Mikhailidis DP, Stansby G

Platelet activation during carotid endarterectomy and the antiplatelet effect of Dextran 40.
Platelets 13(4):231-239, 2002

Rocca B, Secchiero P, Ciabattini G, Ranelletti FO, Catani L, Guidotti L, Melloni E, Maggiano N, Zauli G, Patrono C

Cyclo-oxygenase-2 expression is induced during human megakaryopoiesis and characterises newly formed platelets
Proc Natl Acad Sci USA 2002; 99(11):7634-9

Roth GJ, Stanford N, and Majerus PW

Acetylation of prostaglandin synthase by aspirin.
Proc Natl Acad Sci U S A 1975; 72(8):3073-3076

Rothwell PM, Eliasziw M, Gutnikov SA, Fox AJ, Taylor DW, Mayberg MR, Warlow CP, Barnett HJM

for the carotid endarterectomy Trialists' Collaboration
Analysis of pooled data from the randomised controlled trials of endarterectomy for symptomatic carotid stenosis
Lancet 2003; 361:107-16

Rothwell PM, Gutnikov SA, Warlow CP

for the European Carotid Surgery Trialists' Collaboration
Reanalysis of the final results of the European Carotid surgery trial
Stroke 2003 Feb;34(2):514-23

Ruggeri ZM

Mechanisms initiating platelet thrombus formation
Thromb Haemost 1997; 78:611-6

Sacco RL, Ellenberg JH, Tatemichi TK, Hier DB, Price TR, Wolf PA

Infarcts of undetermined cause: the NINCDS Stroke Data Bank.
Ann Neurol. 1989 Apr;25(4):382-90

Sacco RL, Boden-Albala B, Gan R, Chen X, Kargman DE, Shea S, Paik MC, Hauser WA

Stroke incidence among white, black and Hispanic residents of an urban community: the Northern Manhattan Stroke Study

Am J Epidemiol 1998 Feb 1;147(3):259-68

Sacco RL, Smith DB, Alberts M, Mustone-Alexander L, Rader D, Ross JL, Raps E, Ozer MN, Brass LM, Malone ME, Goldberg S, Booss J, Hanley DF, Toole JF, Greengold NL, Rhew DC

Prevention of a first stroke: a review of guidelines and a multidisciplinary consensus statement from the National Stroke Association.

JAMA 1999; 281(12):1112-1120

Santos MT, Valles J, Marcus AJ, Safier LB, Broekman MJ, Islam N, Ullman HL, Aznar J

Downregulation of human platelet reactivity by neutrophils. Participation of lipoxygenase derivatives and adhesive proteins

J Clin Invest 1993;92(3):1257-65

Sarti C, Rastenyte D, Cepaitis Z, Tuomilehto J

International Trends in Mortality and Stroke, 1968 to 1994

Stroke 2000;31; 7:1588-1601

Savi P, Herbert JM

Pharmacology of Ticlopidine and clopidogrel

Haematologica 2000;85:73-77

Schneider JR, Droste JS, Schindler N, Golan JF, Bernstein LP, Rosenberg RS

Carotid endarterectomy with routine electroencephalography and selective shunting: Influence of contralateral internal carotid artery occlusion and utility in prevention of peri-operative strokes.

J Vasc Surg. 2002 Jun;35(6):1114-22.

Shimizu Y, Kato H, Lin CH, Kodama K, Peterson AV, Prentice RL

Relationship between longitudinal changes in blood pressure and stroke incidence

Stroke 1984 Sep-Oct; 15(5):839-46

Shinton R, Beevers G

Meta-analysis of relation between cigarette smoking and stroke.

BMJ 1989; 298:789-794

Spencer MP

Transcranial Doppler monitoring and causes of stroke from carotid endarterectomy.

Stroke 1997;28(4):685-91

Stansby G, Mikhailidis DP

Systematic review of antiplatelet therapy for the prevention of myocardial infarction, stroke or vascular death in patients with peripheral vascular disease.

Br J Surg 2001;88(6):787-800

Stendel R, Hupp T, al Hassan AA, Brock M

The use of direct intra-operative Doppler ultrasonography in carotid thromboendarterectomy. A prospective study.

Acta Neurochir (Wien). 1998;140(9):925-31; discussion 930-1.

Sutherland M, Shankaranarayanan P, Schewe T, Nigam S.

Evidence for the presence of phospholipid hydroperoxide glutathione peroxidase in human platelets: implications for its involvement in the regulatory network of the 12-lipoxygenase pathway of arachidonic acid metabolism.

Biochem J 353(1):91-100, 2001

Treiman RL, Cossman DV, Foran RF, Levon PM, Cohen JL, Wagner WH

The influence of neutralizing heparin after carotid endarterectomy on post-operative stroke and wound haematoma

J Vasc Surg 1990; 12:445-6

Valenti D, Gaggiano A, Berardi G, Ferri M, Mazzei R, Roda G, Palombo D

Intra-operative assessment of technical defects after carotid endarterectomy: a comparison between angiography and colour duplex scan.

Cardiovasc Surg. 2003 Feb;11(1):26-9

van Pampus EC, Huijgens PC, Zevenbergen A, Twaalfhoven H, van Kamp GJ, Langenhuijsen MM

Influence of aspirin on human megakaryocyte prostaglandin synthesis

Eur J Haematol 1993 May;50(5):264-8

Vaughn CJ

Prevention of stroke and dementia with statins. Effects beyond lipid lowering.

Am J Cardiol 2003 Feb 20;91(4A):23B-29B

Ware JH, Muller JE, Braunwald E

The futility index. An approach to the cost-effective termination of randomized clinical trials.

Am J Med 1985 Apr 78(4):635-43

Weber AA, Zimmermann KC, Meyer-Kirchrath J, Schror K

Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance

Lancet 1999 353(9156):900

Weber AA, Przytulski B, Schumacher M, Zimmermann N, Gams E, Hohlfeld T, Schror K

Flow cytometry analysis of platelet cyclooxygenase-2 expression: induction of platelet cyclooxygenase-2 in patients undergoing coronary artery bypass grafting.

Br J Haematol 2002;117(2):424-6

Weber AA, Przytulski B, Schanz A, Hohlfeld KS

Towards a definition of aspirin resistance: a typological approach

Platelets 2002 13:37-40

Whisnant JP, Brown RD, Petty GW, O'Fallon WM, Sicks JD, Frye RL

Effect of time since onset of risk factors on the occurrence of ischaemic stroke.

Neurology 2002 Mar 12;58(5):787-94

White JS, Sirinek KR, Root HD, Rogers W

Morbidity and Mortality of Carotid endarterectomy: rates of occurrence in asymptomatic and symptomatic patients

Arch Surg 1981 Apr;116(4):409-12

Wilkinson JM, Rochester JR, Sivaguru A, Cameron IC, Fisher R, Beard JD

Middle cerebral artery blood velocity, embolisation and neurological outcome during carotid endarterectomy : a prospective comparison of the Javid and Pruitt-Inahara shunts

European Journal of Vascular and Endovascular Surgery 1997;14:399-402

Wober C, Zeithofer J, Asenbaum S, Claeys L, Czerny M, Wofl G, Grubhofer G, Polterauer P, Deecke L

Monitoring of median nerve somatosensory evoked potentials in carotid surgery.

J Clin Neurophysiol 1998 Sep;15(5):429-38.

Xiao Z, Theroux P

Platelet activation with unfractionated heparin at therapeutic concentrations and comparisons with a low-molecular-weight heparin and with a direct thrombin inhibitor.

Circulation 1998;97(3):251-256,

Young B, Moore WS, Robertson JT, Toole JF, Ernst CB, Cohen SN, BroderickJP, Dempsey RJ. Hosking JD

An analysis of Perioperative Surgicla Mortality and Morbidity in the Asymptomatic Carotid Atherosclerosis study

Stroke 1996 Dec;27(12):2216-24

Weber AA, Przytulski B, Schanz A, Hohlfeld J, Schror K

Towards a definition of aspirin resistance: a typological approach

Platelets 2002 Feb; 13(1):37-40

Wells J, Nicosia S, Wale C, Smith LJ, Buchanan MR

Thrombin generation in patients undergoing carotid endarterectomy: implications in acute vessel wall closure and antithrombotic therapy.

Thromb Res 1994;75(4):419-426,

WHO MONICA Project Principal Investigators.

The World Health Organisation MONICA project (monitoring trends and determinants in cardiovascular disease): A major international collaboration.

J Clin Epidemiol 1988; 41: 105-114

Zampella E, Morawetz RB, McDowell HA, Zeiger HE, Varner PD, McKay RD, Halsey JH Jr.

The importance of cerebral ischemia during carotid endarterectomy.

Neurosurgery. 1991 Nov;29(5):727-30; discussion 730-1

Zed PJ

Low-molecular-weight heparin should replace unfractionated heparin in the management of acute coronary syndromes.

J Thromb Thrombolysis 1999; 8(2):79-87

References from books, reports and websites

Carotid Artery Surgery, A problem-based approach. Edited by A Ross Naylor and William C Mackey

Department of Health, Burdens of Disease 1996

Department of Health Publications (www.dh.gov.uk/PublicationsAndStatistics)
CHD, Chapter 2. Preventing CHD in high risk patients www.dh.gov.uk

Measurements of platelet function. Harker LA, Zimmerman TS (Eds)
Edinburgh/London/Melbourne/New York: Churchill Livingstone; 1983

The Doctors Lounge Internet Resource
www.thedoctorslounge.net/medlounge/articles/stroke_risk/strokerisk7.htm

National Service Framework (NSF) guidelines

NICE guidelines TA090 May 2005

ONS 1998, 2001

Platelets in thrombotic and non-thrombotic disorders: pathophysiology, pharmacology and therapeutics. Edited by Paolo Gesele, Clive Page, Valentin Fuster and Jos Vermynen
Cambridge University Press, 2002

Report by the comptroller at auditor general / HC452 Session 2005-6 16/11/05

Report from National Audit Office, Dept of Health, Reducing Brain Damage: Faster Access to better stroke care

World Health Report 2004

Appendix 1

Ethics Committee Approval Letters

Melanie Sursham
Direct Dial 0116 258 8610
9 November 2001

Gwendolen Road
Leicester
LE5 4QF

Mr Paul Hayes
University Lecturer in Surgery
Department of Surgery
Clinical Sciences
The Leicester Royal Infirmary

Tel: 0116 2731173
Fax: 0116 2588577
DX 709470 Leicester 12

Dear Mr Hayes

Variation in aspirin sensitivity during surgical procedures – our ref. no. 6478

I have received your letter dated 2 November responding to the Research Ethics Committee's concerns in connection with the above study together with the amended patient information sheets.

I have noted the standardised dose of aspirin.

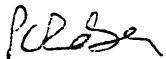
If the Surgeon in charge of the case is agreeable to patients having aspirin prior to thyroid surgery and if it is not thought to put the patients at extra risk of bleeding, then we would not object.

Thank you for clarifying the volume of blood, we had understood it to be seven samples of 70ml each but I note it is seven samples of 10ml each.

On behalf of the Leicestershire Research Ethics Committee and by Chairman's action, the revised patient information sheet (thyroid) version 2B, Nov.01 and patient information sheet (CEA) version 2, Nov.01) are approved and final approval is, therefore, given to allow the study to proceed.

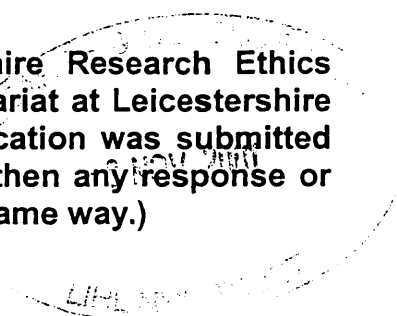
Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when ethics committee approval is given.

Yours sincerely



P G Rabey
Chairman
Leicestershire Research Ethics Committee

(NB All communications relating to Leicestershire Research Ethics Committee must be sent to the Committee Secretariat at Leicestershire Health Authority. If, however, your original application was submitted through a Trust Research & Development Office, then any response or further correspondence must be submitted in the same way.)



Leicestershire, Northamptonshire
and Rutland



Ethics Administration
Direct Dial: 0116 258 8565

Strategic Health Authority

Our ref: pgr/ela/cd021101

8 November 2002

Miss Sally Webster
Research Fellow in Vascular Surgery
Dept of Surgery
Leicester Royal Infirmary

Gwendolen Road
Leicester
LE5 4QF

Tel: 0116 273 1173
Fax: 0116 258 8577
Mini Com: 0116 258 8640
DX 709470 Leicester 12

Dear Miss Webster

Re: Randomised trial comparing the effects of standard Unfractionated Heparin and Low Molecular Weight Heparin (FragminTM, dalteparin) on platelet aggregation during Carotid Endarterectomy (ethics ref: 6863)

Your application to undertake the above mentioned research was considered by the Leicestershire Research Ethics Committee at its meeting held on 1st November 2002. Your application was approved.

The committee asks that you make the following amendments to the patient information sheet:

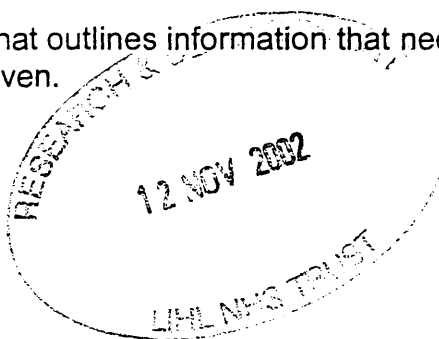
In paragraph 1 the 's' should be removed at the end of the word 'involve', and under question 1 – the purpose of the study, last sentence, this should be amended to say 'This study has been designed to look at the effects of two different types of heparin in platelets *during surgery for Carotid Endarterectomy*'.

Please send us a copy of the amended patient information sheet for our records.

Your attention is drawn to the attached paper that outlines information that needs to be observed when ethics committee approval is given.

Yours sincerely

P G Rabey
Chairman
Leicestershire Research Ethics Committee



(N.B. All communications related to Leicestershire Research Ethics Committee must be sent to the Committee Secrerariat at Leicestershire, Northamptonshire and Rutland Health Authority. If, however, your original application was submitted through a Trust Research & Development Office, then any response or further correspondence must be submitted in the same way).

Appendix 2

The Dextran Wash-out Study

Background and Objectives

Post-operative carotid thrombosis is the principle cause of early stroke following CEA. 50 – 60% of patients with high grade embolisation will go on to suffer a thrombotic stroke. Early post-operative monitoring of blood flow in the middle cerebral artery using Transcranial Doppler (TCD) can identify patients with sustained, high rates of embolisation (Lennard et al, 1997; Gaunt et al, 1998; Spencer, 1997; Levi et al, 1997) and this can be abolished by commencing an incremental intravenous infusion of Dextran solution (Lennard et al, 1998). However, identification of patients with high rates of emboli requires continuous TCD monitoring for three hours following surgery for all patients. This is both labour-intensive and expensive, requiring trained and experienced technicians for the monitoring, meaning that for most small units it is too impractical to implement. Aside from the practical issues with TCD monitoring, the use of intravenous Dextran therapy is associated with a small risk of serious complications (bleeding, multi-organ failure).

It was postulated that Dextran applied directly to the endothelium intra-operatively, could be as effective an anti-thrombotic agent as it is when used intravenously. This would thereby reduce the need for intensive post-operative monitoring and also the potential risk of adverse effects.

Methods

Ethical Approval and Study Size

Ethical approval was obtained from the Leicestershire Regional Ethics Committee prior to commencement of the study. A power calculation estimated that 140 patients would be required for a study with an 80% chance of detecting a 30% reduction in the overall number of emboli (based on a mean embolus count of 20, with a standard deviation of 23). This study was not powered to detect a difference in post-operative complications. Informed consent was obtained from patients due to undergo CEA. All patients with a suitable TCD window were included, subject to informed consent.

Patient recruitment and randomisation

From December 2001 to December 2002, 72 patients with an accessible cranial window for TCD were randomised with a computer generated random number programme, and allocated with a closed envelope technique to receive either 10% Dextran 40 in normal saline or Heparinised Saline (10 000 units in 1000 millilitres) as the intra-operative irrigation solution.

Operative details

The surgeon, anaesthetist, vascular monitoring technician and data analyst were blinded to the type of irrigation solution. A minimum volume of 60 millilitres was used in each patient. There was no maximum limit to the amount used.

CEA was performed by one of four consultants or supervised higher surgical trainee, under normocarbic, normotensive general anaesthetic, with administration of 5000 units unfractionated heparin prior to shunting, routine shunting, completion angiography, Dacron patching and intra-operative TCD monitoring. Each patient was monitored for three hours post-operatively with TCD, and emboli quantified with off-line analysis.

Data and Statistical Analyses

The two groups were analysed for patient demographics and the magnitude of emboli in the first three post-operative hours. The TCD data was recorded onto digital audiotape for off-line analysis by an independent observer. As the groups were not normally distributed, non-parametric statistical analyses were employed. The Mann-Whitney test was used, with data displayed as median values. A p-value of <0.05 was considered statistically significant. A planned interim analysis using the principle of Ware's futility index (Ware et al, 1985) was carried out after the first 70 patients. The futility index is a published method using mathematical formulae to justify the early termination of a trial, if it is unlikely to show any benefit for the tested treatment. This analysis showed no difference in the embolus counts between the two groups, and suggested that even if the study were continued to its planned conclusion (or further) no significant difference would be detected. The study was therefore terminated at 72 patients.

Results

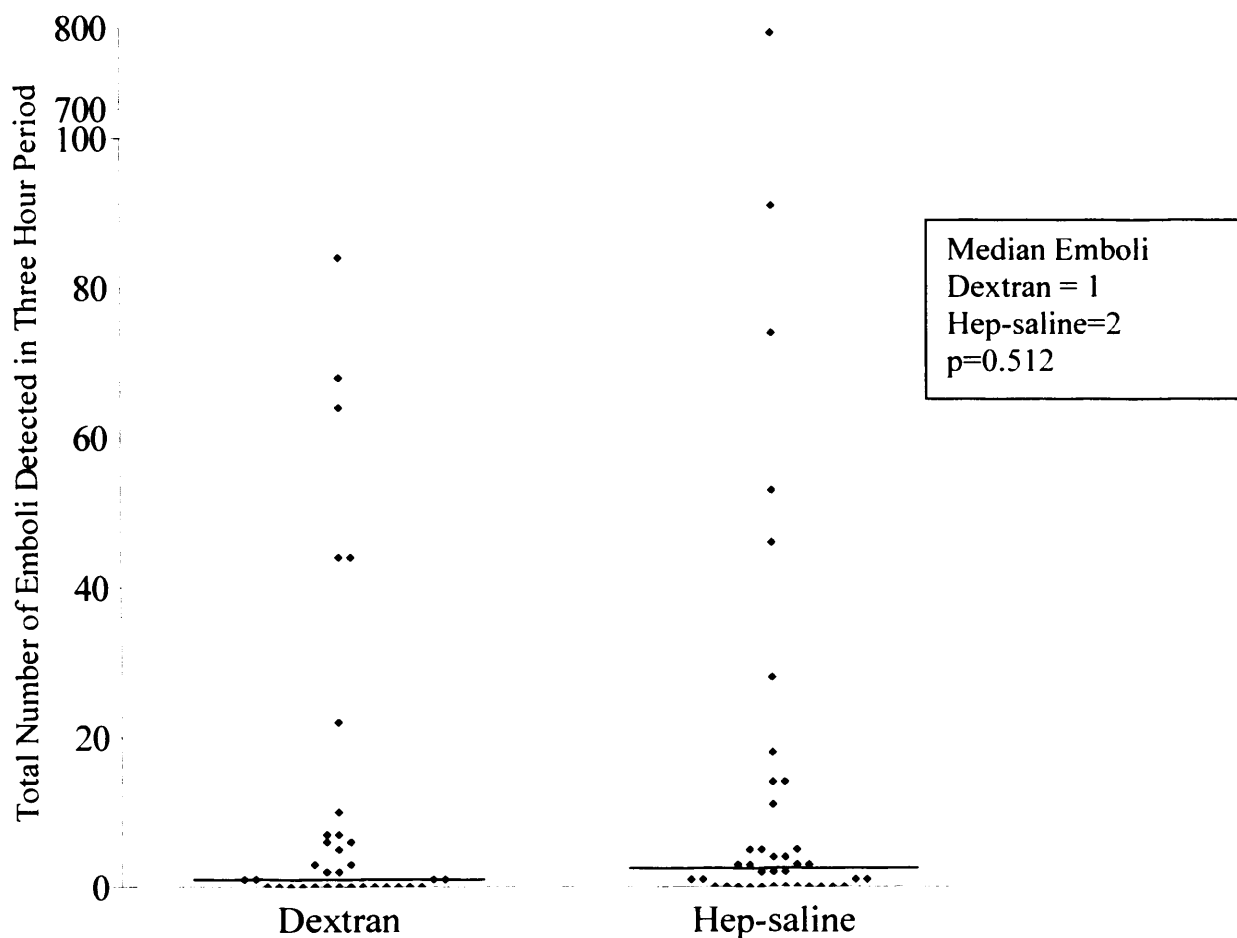
The study was terminated after an interim analysis using Ware's Futility Index. Seventy-two patients with an accessible cranial window for TCD underwent CEA during this twelve-month period. 34 were randomised to the Dextran group and 38 to the Heparinised-saline. The groups were well matched in terms of patient demographics (table A1).

Table A1 Patient Demographics

	Dextran (34)	Heparinised-saline (38)	P-value
Median Age	71 (50-84)	71 (52-80)	0.663
Sex (Male)	23 (67.6%)	30 (78.9%)	0.463
Median Weight	78kg (61-114)	80kg (58-103)	0.595
Hypertension	26 (76.5%)	33 (86.8%)	0.323
Previous MI	8 (23.5%)	7 (18.4%)	0.563
Current smoker	9 (26.5%)	3 (7.9%)	0.169
Aspirin	28 (82.4%)	32 (84.2%)	0.186
Aspirin + Dipyridamole	9 (26.5%)	7 (18.4%)	0.396
Op side left	14 (41.2%)	15 (39.5%)	0.882
Symptomatic : Asymptomatic	25:9	34:4	0.179

In the first three post-operative hours, the median number of emboli detected in the Dextran group was one (range 0-84) and two (range 0-794) in the Heparinised saline group, ($p=0.5116$, Mann-Whitney test). Figure A1 details the overall distribution of emboli detected in the two groups.

Figure A1 Post-operative Emboli Counts



The figure shows the numbers of emboli detected in the middle cerebral artery by TCD monitoring in the first three post-operative hours. Median emboli count was 1 in the group receiving Dextran and 2 in the Heparinised-Saline group (Hep-Saline). $p=0.512$

The majority of patients had fewer than 30 emboli detected during the first three hours of monitoring (29/34 = 85.3% of Dextran group; 33/38 = 86.8% of hep-saline group). (Table A2) There was no difference in the requirement for IV Dextran infusion (one in the normal saline group, none in the Dextran group).

Table A2 Post-operative Emboli Counts

	Dextran	Hep-sal	p-value (Mann-Whitney)
Less than 30 emboli	29/34 (85.3%)	33/38 (86.8%)	0.85
More than 30 emboli	5/34 (14.7%)	5/38 (13.2%)	
Median	1	2	0.35

Discussion

Post-operative thrombotic stroke is a devastating complication of carotid endarterectomy, occurring in 2-3% of cases. Through a series of research projects and audits, we have been able to identify those patients at high risk of post-operative stroke and have shown that thrombosis is preceded by sustained high-rate embolisation into the cerebral circulation (Lennard et al, 1999). Using the TCD to monitor flow in the middle cerebral artery, emboli can be identified, and treatment regimes aiming to reduce or prevent embolisation can be evaluated. Although intravenous Dextran is very effective at reducing the rates of embolisation, with therapeutic effects beginning within 20 minutes of commencement of the infusion (Hayes et al, 2001) this treatment can be associated with systemic complications (cardiac failure, bleeding and multi-organ failure). If Dextran were efficacious when applied locally to the endarterectomy zone, many of these adverse events would be overcome, but the hypothesis of this study has not been supported by the results.

The mechanism of action of Dextran has, until recently, been unclear. It was first used in the 1950's as a plasma-volume expander, but was soon noted to confer beneficial anti-thrombotic properties. Subsequent studies have shown that Dextran reduces platelet adhesiveness, and increases lysability of ex vivo-formed thrombi. These effects were thought to be due to the effect of Dextran on Factor VIII. (Bergqvist, 1982). It has also been suggested by Aberg et al 1979, that the effects of Dextran are due to impairment of the factor VIII bound to platelet surface, which enables platelets to function normally (by supporting platelet adhesion to the exposed sub-endothelium and for initiating and stabilising of ristocetin induced platelet aggregates).

Noorman et al in 1997 showed that Dextran is also an antagonist for the mannose receptor in the liver, thereby inhibiting tissue-type Plasminogen activator (tPA) binding and clearance. This leads to higher levels of free, active tPA in the circulation. As tPA is a

potent fibrinolytic agent working by converting Plasminogen into plasmin, (which then cleaves fibrin into soluble degradation products) leading to increased fibrinolysis.

Given the likely mechanism of action of Dextran when given systemically in conjunction with the results of this latest study, it is logical to conclude that exposure of the sub-endothelium to small volumes of Dextran does not reduce the number of emboli seen following CEA, and cannot be used as an alternative to intravenous Dextran.

Conclusions

This study has shown no evidence to support anecdotal reports suggesting that irrigation of the endarterectomy zone reduces post-operative embolisation after CEA. It would appear that the exposure of sub-endothelial collagen to small volumes of irrigation solution was either insufficient to exert a clinically significant effect or that intravenous Dextran exerts its beneficial effect via a different mechanism, when given systemically.