

Evaluation of the safety and general toxicology of GA inhalation

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Background

Airway peak pressure (P_{peak}) was assessed as an indicator of bronchoconstriction; and white blood cell counts, inflammatory parameters and bronchi histopathology for detecting systemic or local inflammation.

Eight pigs are randomly divided into three groups. Pigs in each group are sedated differently:

- Using Mannitol
- Using GA s.c.
- Using GA inhaled

For each group one or more measurements of Peak inspiratory pressure were collected.

Also, immune cell measurements before and after were used to compare the effect differences between the three groups.

Objective

Given a measurement error on P_{peak} of 2 cmH₂O, to show that the effect of method GA Inhaled is equivalent to the effect of both GA s.c. and Mannitol.

Summary

Method

Ppeak was modelled using a regression with random intercepts for each pig and each day, with independent residuals, and with a variance component for each day. Model control showed an acceptable model fit. Margins and marginal effects were estimated.

A “Two Onesided Test” (TOST), Schuirmann (1987), was used to test equivalence in effects between GA inhaled and both GA s.c. and Mannitol with respect to an measurement error on Ppeak of 2 cmH2O.

Comparison of blood cell counts was done based on similar regression to Ppeak. The effects (POST - PRE) were compared as differences with confidence intervals between Mannitol and GA inhaled and between GA s.c. and GA inhaled in Forest plot.

Results

The effect if GA inhaled was tested equivalent (max P-value = 0.00) to the effects of both GA s.c. and Mannitol with respect to an measurement error on Ppeak of 2 cmH2O. The TOST tests are visually confirmed by Figure 2.

Regarding different types of blood cell counts, a few comparisons between Mannitol and GA inhaled and between GA s.c. and GA inhaled were significant. Sometimes in favour of GA inhaled, and sometimes not.

Ppeak, safety

Data

Table 1: Metadata for the variables in the dataset

Name	Index	Label	Value Label	Format	Value Label	Values	n	unique	missing
group	1	Group	group	%12.0g1	"Mannitol"	2 "GA s.c." 3 "GA inhaled"	503	3	0
pig	2	Pig	pig	%10.0g1	"Pig 1"	2 "Pig 2" 3 "Pig 3" 4 "Pig 4" 5 "Pig 5" 6 "Pig 6" 7 "Pig 7" 8 "Pig 8"	503	8	0
day	3	Day	day	%10.0g1	"Day 1"	2 "Day 2" 3 "Day 3"	503	3	0
ppeak	4	Ppeak		%10.0g			500	10	3
time	5	Time	time	%10.0g1	"Pre"	2 "Post"	503	2	0

Analysis

Estimation by a random intercept regression model (-mixed-)

The regression model for Ppeak is a random intercept regression with random intercepts for each pig and each day. The residuals are modelled as independent with a variance component for each day. Model control in previous document shows an acceptable model fit.

```
mixed ppeak i.group##i.time ||pig: ||day:, noheader nogroup ///
      covariance(unstructured) residuals(independent, by(day))
estimates store ppeak
```

Presenting the estimates of the means

Estimates of the means with 95% estimates over group and time are estimated. See Table 2 and Figure 1.

```
margins group#time
marginsplot, title("") name(m1, replace) legend(size(small) on position(6) ring(0)) ///
      ytitle(Estimated means and 95% CI, size(vsmall)) xtitle("") ///
      xlabel(1(1)3, labsize(vsmall)) xmtick(0.5(1)3.5, nolabels noticks) ///
      ylabel(5(5)25, labsize(vsmall)) angle(horizontal) format(%2.0f) ///
      plotopts(lpattern(blank))
```

Also, the effect (POST - PRE) with 95% confidence interval for each group. See Table 2.

```
estimates restore ppeak
margins r.time, within(group)
```

Table 2: Estimated means (PRE, POST) and effect (POST - PRE) with 95% CI.

	PRE	LB	UB	POST	LB	UB	POST - PRE	LB	UB
Mannitol	19.414	18.177	20.652	19.938	18.777	21.099	0.523	0.050	0.997
GA s.c.	20.395	19.140	21.650	21.178	20.016	22.340	0.783	0.263	1.303
GA inhaled	19.322	18.468	20.175	20.534	19.714	21.355	1.213	0.942	1.483

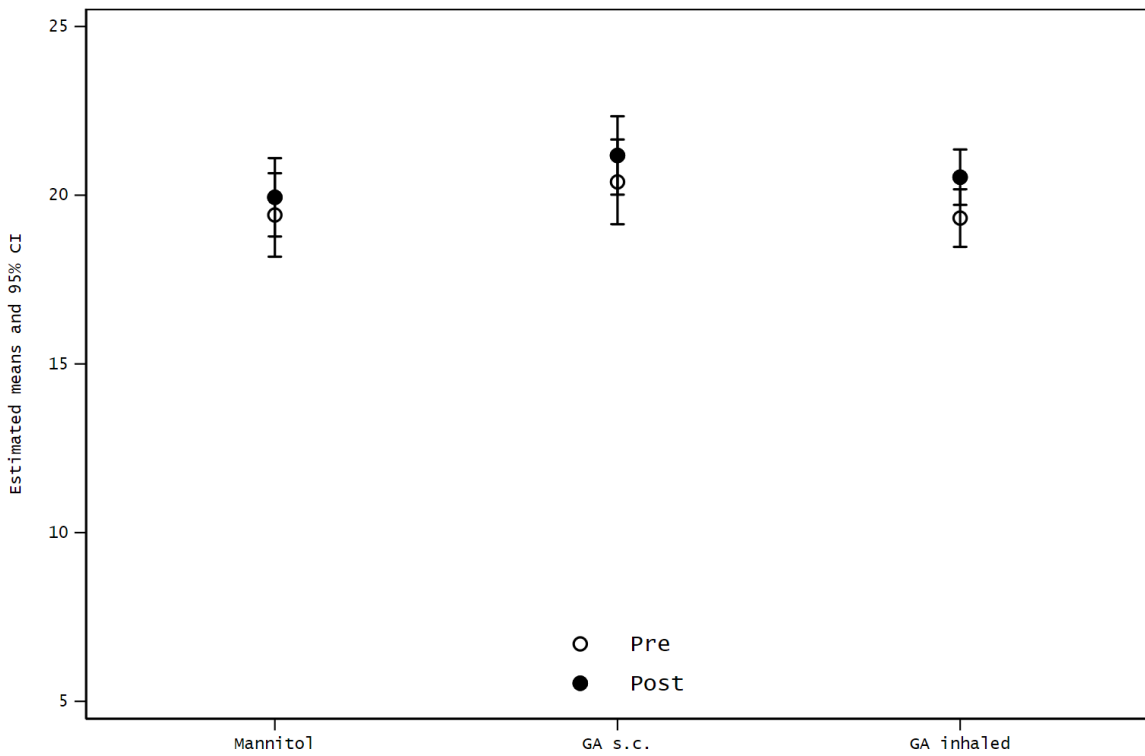


Figure 1: Marginsplot over group and time. The means of Post are greater than Pre for each group.

Equivalence test (TOST)

The uncertainty in measurement on Ppeak is 2 cmH2O. Only absolute differences above 2 cmH2O are clinically relevant.

TOST (two-one-sided t-tests) are described in Schuirmann (1987). The idea is to test the differences in effects (Δ) outside the interval -2 and 2 by two one-sided tests:

- $H_0 : \Delta \leq -2$ vs $H_1 : \Delta > -2$, and
- $H_0 : \Delta \geq 2$ vs $H_1 : \Delta < 2$.

By rejecting both hypotheses the difference in effect must lie inside the interval of -2 and 2, ie be equivalent with respect to clinical relevance. Here, the normal distribution is used when evaluating the tests. The P-values from the TOST comparison are in Table 4. The two tests from the TOST on the effect of Mannitol vs the effect of GA inhaled are both rejected, hence acceptance of the hypothesis that the two effects are equivalent. Likewise for the effect of GA s.c. vs GA inhaled. This is visualised in Figure 2.

The estimation is done by `-margins-` and the estimated difference in effects are summarized in Table 3.

```
estimates restore ppeak
margins r.time, over(rb3.group)
matrix tbl = r(table) // Saves data for Figure 2
```

Table 3: Comparing effect (Post - Pre) with 95% CI.

	Effect	LB	UB
Mannitol - GA Inhaled	-0.689	-1.234	-0.144
GA s.c. - GA Inhaled	-0.429	-1.015	0.157

Table 4: P-values for the two tests in TOST for each difference of effects. Both the effects of Mannitol and GA s.c. are equivalent with the effect of GA inhaled.

	Mannitol - GA Inhaled	GA s.c. - GA Inhaled
H0: $\Delta \leq -2$	1.00	0.00
H0: $\Delta \geq 2$	1.00	0.00

Figure 2 is generated by the command below.

```
matrix colnames tbl = "Mannitol - GA Inhaled" "GA s.c. - GA Inhaled"
coefplot matrix(tbl), ci((5 6)) ///
mlabel(string(@b, "%5.2f") + " (" + string(@l1, "%5.2f") + "; " + string(@ul, "%5.2f") + ")") ///
mlabposition(12) mlabsize(vsmall) name(m2, replace) ///
xline(0, lcolor(red) lwidth(thin)) xline(-2 2, lcolor(red) lwidth(medium)) ///
xscale(range(-2.5 2.5)) xlabel(-2(1)2, labsize(vsmall)) ///
ylabel(, labsize(vsmall)) xtitle("Differences in effects and 95% CI", size(vsmall)) ///
caption("GA inhaled larger", size(vsmall) position(7) orientation(horizontal) ring(0)) ///
note("GA inhaled smaller", size(vsmall) position(5) ring(0))
```

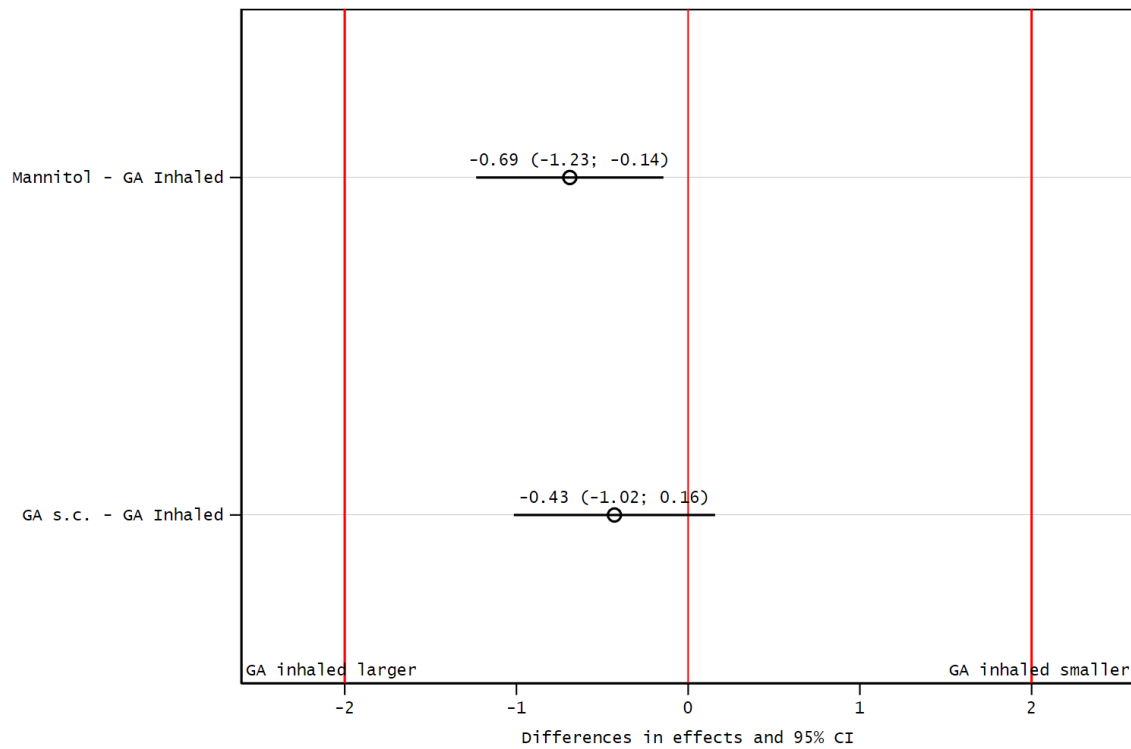


Figure 2: Comparing the effect of GA Inhaled with both GA s.c. and Mannitol given a measurement error of 2 cmH₂O (red vertical lines at -2 and 2). Even though there is a significant larger effect for GA Inhaled with respect to the effect of Mannitol (confidence interval above red line at 0), both confidence intervals lay within the bounds of -2 and 2, ie they are clinically equivalent with respect to those bounds.

Blood cell counts, toxicology

Data

Table 5: Metadata for analyse data

Name	Index	Label	Value Label	Name	Format	Value	Label	Values	n	unique	missing
pig	1	pig	pig	pig	%10.0g	1	"Pig 1"	2 "Pig 2" 3 "Pig 3" 4 "Pig 4" 5 "Pig 5" 6 "Pig 6" 7 "Pig 7" 8 "Pig 8"	48	8	0
group	2	Group	group	group	%10.0g	1	"Mannitol"	2 "GA s.c." 3 "GA inhaled"	48	3	0
day	3	Day	day	day	%10.0g	1	"Day 1"	2 "Day 2" 3 "Day 3"	48	3	0
time	4	Time	time	time	%9.0g	1	"PRE"	2 "POST"	48	2	0
cd16plus	5	CD16+			%10.0g				48	48	0
cd172aplus	6	CD172a+			%10.0g				48	48	0
cd18plus	7	CD18+			%10.0g				48	48	0
cd3plus	8	CD3+			%10.0g				48	47	0
cd4aplus	9	CD4a+			%10.0g				48	46	0
cd79aplus	10	CD79a+			%10.0g				48	47	0
cd8aplus	11	CD8a+			%10.0g				48	48	0
granulocytes	12	Granulocytes			%10.0g				48	48	0
lymphocytes	13	Lymphocytes			%10.0g				48	48	0
monocytes	14	Monocytes			%10.0g				48	47	0
nk_cells	15	NK cells			%10.0g				48	48	0

Analysis

The regression model for the counts is a random intercept regression with random intercepts for each pig and each day. The residuals are modelled as independent with a variance component for each day. The same as in previous analyses. No TOST is performed, since no limits are provided.

Table 6: Effect (POST - PRE) in blood cell counts pairwise compared for Mannitol, GA s.c., and GA inhaled

		POST - PRE (1000)	95% CI Lower bound	95% CI Upper bound	P-value
Mannitol - GA inhaled	Granulocytes	-371.559	-1675.011	931.892	0.58
	Lymphocytes	-600.228	-1766.418	565.963	0.31
	Monocytes	-185.553	-368.392	-2.714	0.05
	NK cells	940.991	481.487	1400.495	0.00
	CD16+	1089.271	334.413	1844.129	0.00
	CD172a+	506.379	-609.545	1622.304	0.37
	CD18+	-128.595	-2093.569	1836.380	0.90
	CD3+	-2524.216	-4637.911	-410.522	0.02
	CD4a+	-335.007	-635.561	-34.453	0.03
	CD79a+	225.541	-664.021	1115.103	0.62
	CD8a+	556.788	-18.506	1132.082	0.06
GA s.c. - GA inhaled	Granulocytes	342.263	-961.189	1645.714	0.61
	Lymphocytes	555.062	-611.128	1721.253	0.35
	Monocytes	-37.991	-220.829	144.848	0.68
	NK cells	172.974	-286.530	632.478	0.46
	CD16+	1310.976	556.119	2065.834	0.00
	CD172a+	1054.084	-61.840	2170.009	0.06
	CD18+	-1711.756	-3676.731	253.218	0.09
	CD3+	-1192.422	-3306.116	921.273	0.27
	CD4a+	-42.357	-342.911	258.197	0.78
	CD79a+	-287.705	-1177.267	601.857	0.53
	CD8a+	500.086	-75.209	1075.380	0.09
Mannitol - GA s.c.	Granulocytes	-713.822	-2218.919	791.275	0.35
	Lymphocytes	-1155.290	-2501.890	191.311	0.09
	Monocytes	-147.562	-358.686	63.562	0.17
	NK cells	768.017	237.427	1298.607	0.00
	CD16+	-221.705	-1093.340	649.929	0.62
	CD172a+	-547.705	-1836.264	740.854	0.40
	CD18+	1583.162	-685.795	3852.119	0.17
	CD3+	-1331.794	-3772.478	1108.890	0.28
	CD4a+	-292.650	-639.700	54.400	0.10
	CD79a+	513.246	-513.931	1540.424	0.33
	CD8a+	56.702	-607.590	720.995	0.87

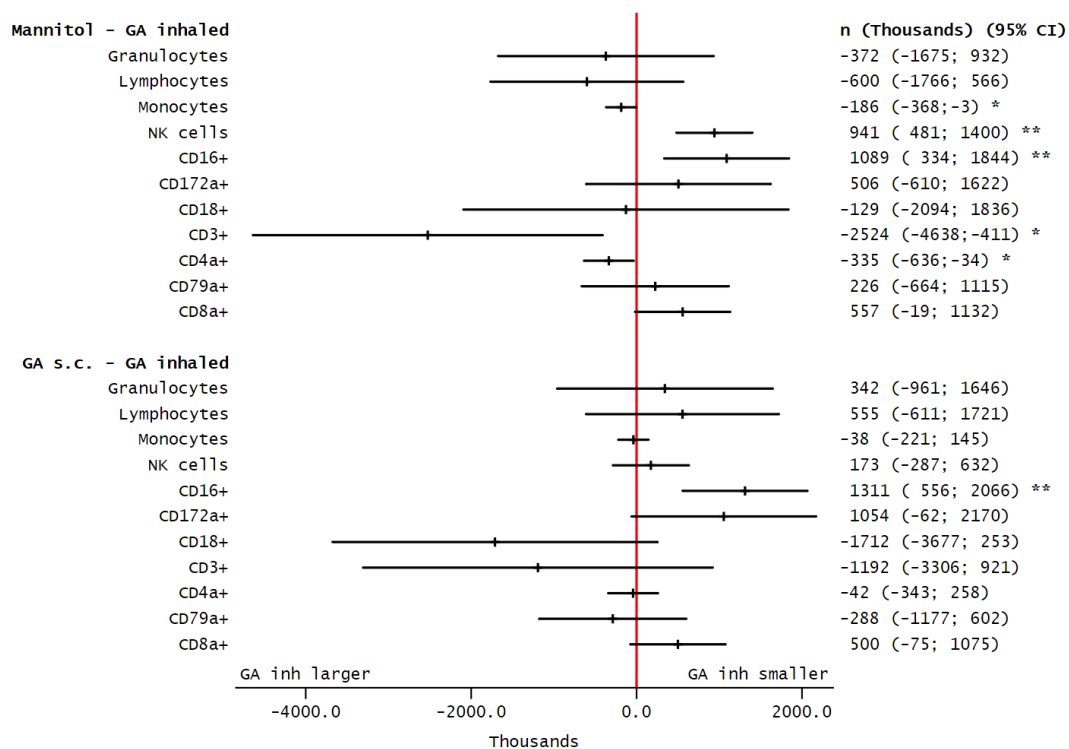


Figure 3: Change in effect (POST - PRE) in blood cell counts compared for Mannitol and GA s.c. vs GA inhaled. Effect (POST - PRE) is the increase in blood cell count for Mannitol, GA s.c., and GA inhaled. The effects are compared as differences between Mannitol and GA inh as well as differences between GA s.c. and GA inhaled. The red line at zero should be within the shown confidence intervals if there are no difference Mannitol and GA inhaled or GA s.c. and GA inhaled. One * means significant at level 5%, ** at level 1%.

References

Schuirman, Donald J. 1987. "A Comparison of the Two One-Sided Tests Procedure and the Power Approach for Assessing the Equivalence of Average Bioavailability." *Journal of Pharmacokinetics and Biopharmaceutics* 15 (6):657–80. <https://doi.org/10.1007/BF01068419>.