Note on the statistical evaluation of a randomized, cross-over bioavailability study.

Henrik E. Poulsen & Per Juul. Danish National Board of Health, September 1990.

In Denmark the large number of bioavailability studies submitted for registration of generic drugs has necessitated a local consensus on the requirements for such a study from a statistical point of view based on the Nordic and EC guidelines. This note does not represent a thorough statistical consideration, but rather is intended to be a "statistical first aid" for non-statisticians.
Therefore an example of the results of a study has been included.
It is assumed that the assessor is capable of calculating the mean and standard deviation of a sample, and that the concepts of significance testing, type I and type II errors are known.

PLANNING OF A TRIAL

Five concepts are invariably linked together in a randomized cross-over bioavailability trial:

N	the number of individuals in the trial
α	the significance level (risk of type I error = false positive)
β	the operational characteristic (risk of type II error = false negative)
SD	the standard deviation
SED	the standard error of the mean difference ($SD/\sqrt{n}$)
Delta	the minimum acceptable difference between the two treatments considered relevant.

By tradition values are usually chosen as $2\alpha=0.05$, $\beta=0.20$ and Delta=0.20. In the planning of the trial you must have an estimate (or guess) for SD of the differences between the treatments. Then the number of individuals can be calculated as

$$N = \left(\frac{t_{2\alpha} + t_{\beta}}{K} \right)^2 \quad ; \quad \text{where} \quad K = \frac{\text{Delta}}{SD}$$

and the values for $t_{2\alpha}$ and t_{β}, is taken from Table 1

Table 1

2α or β	$t_{2\alpha,df=11}$	$t_{2\alpha,df=\infty}$	$t_{\beta=\infty}$
0.01	3.11	2-58	2.33
0.05	220	1.96	1.65
0.10	1.80	1.65	1.28
0.20	1.36	1.28	0.84

df= degrees of freedom

EVALUATING THE RESULT'S OF A TRIAL

After conduction of the trial the results should be presented as the mean difference, e.g. in AUC, between the two formulations. The difference should be 0 if the two formulations are identical. This is tested by a conventional paired t-test by calculating the t-distributed test value as

test value = mean difference/SED

If the test value is greater than the $t_{2\alpha}$ value in Table 1, it is concluded that the difference is significantly different from 0.

In addition to the result of the significance test, the actual difference observed and its 95% confidence limits should be given. The confidence limits are calculated as:

mean difference $\pm$ $t_{2\alpha}$ x SED

$t_{2\alpha}$ is taken from the table above (or from a t-table using the correct degrees of freedom), and SED is the standard error of the differences between the formulations.

AN EXAMPLE

Among the usual pharmacokinetic parameters involved in the evaluation of bioavailability data, most emphasis is normally given to the AUC Consequently only AUC data are given in the following random, cross-over, single dose, relative bio availability study.

	Prep A	Prep B	A-B	B/A
	234	382	-148	1.63
	438	242	196	0.55
	465	338	127	0.73
	193	258	–65	1.34
	312	308	4	0.99
	314	186	128	0.59
	230	196	34	0.85
	249	179	70	0.72
	201	212	-11	1.05
	439	317	122	0.72
	281	192	89	0.68
	211	308	- 97	1.46
mean	29725	259.83	37.42	0.94
SD	98.73	68.90	103.86	0.36
SED ($SD/\sqrt{12}$)			29.98	0.10

Testing difference between the two formulations:

test value = 37.42/29.98 = 1.25

The t-value from Table 1 is 2.20 (df=11). Therefore the observed difference is not different from 0. The 95% confidence limits for the observed difference are

37.42 ± 2.20 x 29.98 = -28.54 to 103.38

Since the observed confidence limits exceed the acceptable delta (20% of the mean value of all AUC observations =56), the study does not rule out a real difference of that magnitude.

Testing difference based upon ratio B/A

The 95% confidence limits for the observed ratio B/A is

0.94 ± 2.20 x 0.10 = 0.72 to 1.16

The observed ratio is not different from 1.0. However, since the confidence limits exceed the acceptable delta (a 20% reduction of the ratio to 0.8), the study does not rule out a real difference of that magnitude.

Based an the information in the present study how many persons would be required to fulfil the demands of $2\alpha=0.05$; $\beta=0.20$; Delta=0.20;

If we assume no difference between the two formulations, the mean of all AUC's are 278.54. The Delta is 20% of that value. The SD of the differences is 103.86. The required number of persons therefore is

$N= ((1.96 + 0.84)/K)^2 = 27.25$
(K= 0.2 x 278.54/103.86)

The board would therefore require 28 persons in such a trial. lt should be noted that the Danish experience is, that SD during the planning is often assumed smaller than eventually observed.

Table 2 Relationship between sample size, SD of the difference between two formulations and Delta, for $2\alpha=0.05$. SD is considered known and is given as % of the mean (based on df = ∞).

SD	Delta	N(β=0.2)	N(β=0.1)
20%	20%	8	11
25%	20%	13	17
30%	20%	18	24
40%	20%	32	43
50%	20%	50	66
80%	20%	126	169

Toxicology table

Type of drug	Phase I		Phase 11		Phase III	
	Clinical trial	**Toxicology**	**Clinical trial**	**Toxicology**	**Clinical trial**	**Toxicology**
Drugs for oral or parenteral use	Small single dose to few people	Acute toxicology minimum 2 species. Subacute2species 3 doses, 14 days	Single dose	As Phase I	Single dose	As Phase I
			1-2 weeks	Subacute 2 species 4 weeks	1-2 weeks	Chronic 2 species 1-3 months
		Special tests	1~ 3 months	Chronic 2 species 3 months	1-3 months	Chronic 2 species minimum 3 months
			6 months or more	Chronic 2 species 6 months-2 years Special tests	6 months or more	Chronic 2 species 6 months-2 years Special tests
Inhalation anaesthetics	One anaesthesia	Acute 4 species Subacute 3 hour exposure 5 subsequent days	One anaesthesia	As Phase I Special test	One anaesthesia	As Phase I Special test
Drugs for dermal application	Single dose	Acute, oral 2 species Dermal exposure 24 hours Observation 2 weeks	Single dose Short-time (2 weeks)	As Phase I Subacute dermal 3 weeks Observation 2 weeks Sensitivity testing guinea pig	Brief period Long-term (unlimited)	As Phase II 3-6 months dermal Special tests
Drugs for local opthalmic application	Single dose	Acute oral and local 2 species Rabbit irritation test	Brief period	Subacute 2 species 3 weeks	Brief period Long-term	As Phase II Chronic 3-6 months
Drugs for vaginal or rectal application	Single dose	Acute oral and local 1 ~ 2 species	Brief period	Chronic local 2 species 3weeks-3months	Brief period Long-term	As Phase II Chronic local 3 months or more
Combinations, full assay for each single component	Brief period	Acute	Brief period	Rat and dog 1-3 months	Brief period Long-term	As Phase II As Phase II
Hormone contraceptives, oestrogens and progestagens	Brief period (1 month)	Acute, Subacute rat, dog, and primate 90 days	3 months	Chronic rat, dog, primate, 1 year	3-12 months	Chronic rat, dog, primate 2 years
Contact lenses and lens fluid	Brief period	Acute and subacute tests, locally. Oral acute for fluids	Long-term	Chronic. rabbit 3 weeks or more	Long-term	As Phase II