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Sensitivity of male reproductive endpoints in nonhuman primate toxicity studies: A statistical power analysis

G.D. Cappon[a,*], D. Potter[a], M.E. Hurtt[a], G.F. Weinbauer[b], C.M. Luetjens[b], C.J. Bowman[a]

[a] Worldwide Research & Development, Pfizer Inc, Groton, CT, USA
[b] Covance Laboratories GmbH, Münster, Germany

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ABSTRACT

To determine the sensitivity of male reproductive toxicity endpoints in NHPs we performed a power analysis of routine and triggered endpoints using control data from sexually mature Asian and Mauritian NHPs. The power to detect a 50% change from control was 13–30% for male reproductive organ weights, ~30% for testicular volume, 6–66% for seminal analyses and 10–78% for male hormones. Overall, male reproductive endpoints have poor power (less than 80%) to detect a 50% change from control with a group size of 3 monkeys. Confidently identifying adverse male reproductive effects with these endpoints would likely require specialized study designs with larger group sizes. Triggering of non-routine endpoints in cases where there is special concern for male reproductive toxicity is unlikely to increase sensitivity to detect adverse effects.

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1. Introduction

The potential for pharmaceuticals to cause developmental and reproductive toxicity has traditionally been evaluated in a battery of studies; embryo-fetal development (EFD) studies in rats and rabbits, fertility and early embryonic development study in rats, and pre- and postnatal development (PPND) study in rats. However, for many biopharmaceuticals nonhuman primates (NHPs) represent the only pharmacologically-relevant animal model. While the routine testing paradigm used for small animals can be performed with NHPs [1], there are pragmatic and animal use considerations to taking the 3 study approach when NHP is the only option as described by ICH S6(R1) guidance on “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals” [2]. For developmental toxicity assessment, instead of running separate EFD and PPND studies an enhanced PPND (ePPND) study was developed [3,4]. For identifying potential effects on male reproduction, given that mating studies are generally not practical in NHPs and functional fertility endpoints lack sensitivity owing to comparatively low mating success, histopathological and organ weight assessments from repeat dose studies in sexually mature animals represent the standard approach. This is consistent with the approach to assess male reproductive toxicity for small molecules where the male fertility study is not required until Clinical Phase III [5]. For small molecules toxicity to male reproduction can be detected by standard histopathological examination in a rodent repeated-dose toxicity study of at least 2-week duration and the additional endpoints (e.g., mating assessment) included in an ICH guideline male fertility study [6] do not provide additional sensitivity for detection of toxicity to male reproduction (for review see [7]).

However, if the pharmacological activity or previous findings for a biological compound raises specific cause for concern regarding potential impact to male reproduction the ICH S6(R1) guideline [2] calls for the addition of specialized assessments such as sperm count, sperm morphology/motility, and male reproductive hormone levels to the chronic repeat dose toxicity study if NHPs are the only relevant species. To understand the value of including these additional endpoints we performed a power analysis with data collected from control animals in toxicity studies conducted in sexually mature cynomolgus monkeys.

2. Methods

2.1. Animals

This investigation comprises data from 194 purpose-bred male cynomolgus monkeys (long-tailed macaque, *Macaca fascicularis*). These animals were control animals in nonclinical safety assessment studies and ranged in age from 3.1 to 9.2

* Corresponding author at: Pfizer Worldwide Research & Development, Eastern Point Road, MS 8274-1260, Groton, CT 06340, USA. Tel.: +1 860 715 1691.
E-mail address: Gregg.d.cappon@pfizer.com (G.D. Cappon).

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years with a mean age of 5.2 years, and necropsy weights of 2.1–9.8 kg. Sexual maturity was initially tested by the presence of sperm in a semen sample (for review Luetjens and Weinbauer [9]). The animals were either of mainland Asian origin or Mauritian origin. Data were collected between January 1997 and May 2010. Animals were single- or group-housed at Covance facilities (Münster, Germany) under a 12:12 h light:dark cycle and in a controlled environment with >8 air changes/hour and temperature and relative humidity ranges of 19–25 °C and 40–70%, respectively. Twice daily the animals were offered a commercial pellet diet for primates (Ssniff P10, Ssniff Spezialdiäten GmbH, Soest, Germany) supplemented with fresh fruit and bread added as food supplement. Tap water was provided ad libitum. Housing and handling of animals was in accordance with the German Animal Welfare Act and an institutional animal care and use committee (IACUC) reviewed each of the studies to which theses control animals were assigned.

Male reproductive organ weights and testicular volume were combined for paired organs. Semen parameters (ejaculate weight, sperm number, motility, and morphology) were determined as described previously [8]. The ultrasound method of measuring testicular volume is presented, as the power analysis of testicular volume was essentially the same whether measured with caliper or ultrasound (data not shown). Reproductive hormones (testosterone, luteinizing hormone [LH], follicle stimulating hormone [FSH] and inhibin B) were determined using validated immuno- and bioassays as reported earlier [8].

2.2. Power calculations

For all endpoints, power calculations were performed assuming that a two-sided two-sample *t*-test ($\alpha = 0.05$) would be used for the study-specific analysis. Because some endpoints included multiple measurements over time for a given animal, a variance components analysis was used to calculate between-animal and within-animal estimates of variability. These components were subsequently used to perform the power calculations, assuming that a single measurement per animal would be taken. All calculations were performed in R (Version 2.15.1).

2.3. Subgroup analyses

To evaluate the suitability of combining subgroups for the purposes of estimating variability for power calculations, two analyses were performed for each endpoint. First, Levene's test was used to evaluate differences in biological variability across source and housing sub-groups. Similarly, analysis of variance (ANOVA) models were used to assess the impact of source and housing on the average magnitude of each endpoint. Analyses were conducted in R (Version 2.15.1).

3. Results

3.1. Assessment of the dataset

The group sizes, means and coefficient of variations (CV) for each measure for all animals combined, and for each of the 4 major subgroups of animals based on source and housing condition (i.e., Mauritian or Asian sourced, and individual or group housed) are presented in Table 1 and visualized with box and whisker plots (Fig. 1A–M). A qualitative comparison of the CVs (Table 1) and 25th/75th percentiles of values (Fig. 1A–M) shows some variation among groups, but in general there is overall consistency between the subgroups and there are no obvious patterns in the variation to suggest any biologically meaningful differences across endpoints between subgroups (for example, Mauritian single housed have the smallest CV for ejaculate weight but the second highest CV for sperm count). This cursory evaluation suggests that combining data from all animals will provide the best overall representation of the biological variability for the purpose of power calculations.

In order to more rigorously assess the validity of pooling data across subgroups for the purposes of estimating endpoint variability, two statistical methods were employed. First, Levene's test was used to assess the equality of variances across the different subgroups. As indicated in Table 2, for 9 of the 13 endpoints Levene's test had a *P*-value ≤ 0.05, indicating that there are differences in within-group variances across the 4 subgroups presented in Table 1. Second, an Analysis of Variance (ANOVA) model was used for each endpoint to assess whether housing and/or source contributed to the overall variation (Table 2).

There were no significant effects of source on reproductive hormone levels; all animals for which this data was collected were group housed so housing was not assessed. There were no

Table 1
Comparison of means and coefficient of variations for each endpoint when combined or separated by NHP source and housing conditions.

	All males combined			Asian sourced group housed			Mauritian sourced group housed			Asian sourced single housed			Mauritian sourced single housed		
Endpoint	*N*	Mean	CV	*N*	Mean	CV	*N*	Mean	CV	*N*	Mean	CV	*N*	Mean	CV
Testes weight (g)	188	36.67	0.33	11	45.68	0.23	87	37.42	0.28	51	38.50	0.38	39	30.08	0.30
Epididymides weight (g)	180	5.75	0.30	11	7.31	0.25	87	5.70	0.24	48	6.37	0.33	34	4.49	0.26
Seminal vesicles weight (g)	152	9.60	0.59	11	12.95	0.41	77	8.17	0.52	34	14.55	0.42	30	6.46	0.66
Prostate weight (g)	185	2.08	0.46	11	3.53	0.40	87	2.10	0.46	48	2.15	0.31	39	1.57	0.42
Testicular volume (mL)	240	34.65	0.30	49	36.5	0.34	184	34.16	0.29	7	34.44	0.29	NA	NA	NA
Ejaculate weight (g)	340	0.50	1.32	29	0.66	0.88	133	0.60	1.42	149	0.42	1.20	29	0.28	0.89
Sperm count ($10^6\times$)	347	138.05	1.29	28	182.32	1.17	143	106.30	0.98	147	166.52	1.65	29	107.53	1.55
Sperm motility (%)	343	68.69	0.19	27	73.57	0.16	144	65.44	0.18	143	70.96	0.19	29	69.07	0.25
Sperm morphology (% normal)	418	68.01	0.27	28	80.66	0.15	100	79.79	0.17	262	62.67	0.29	28	63.30	0.30
Testosterone (nmol/L)	472	24.60	0.74	NA	NA	NA	158	23.89	0.65	240	26.49	0.80	74	20.01	0.59
LH (U/L)	106	19.00	0.99	NA	NA	NA	NA	NA	NA	52	20.57	1.01	54	17.49	1.01
FSH (ng/mL)	98	11.24	0.17	NA	NA	NA	NA	NA	NA	52	10.74	0.16	46	11.82	0.16
Inhibin B (pg/mL)	82	746.17	0.33	NA	NA	NA	NA	NA	NA	44	789.64	0.30	38	695.84	0.35

NA, not available; N, number of observations; CV, coefficient of variation; FSH, follicle stimulating hormone; LH, luteinizing hormone.

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