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Reproductive and Developmental Assessments in an Alternate Rodent Species: The Guinea Pig (*Cavia porcellus*)

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Abstract

Regulatory guidelines for developmental and reproductive toxicology (DART) studies require selection of "relevant" animal models as determined by kinetic, pharmacological, and/or toxicological data. As regulatory demands evolve and laboratory techniques are enhanced, the use of alternate species that more closely mimic the metabolic profile of a drug in humans or demonstrate activity similar to man only in that species are being explored. The domestic guinea pig, *Cavia porcellus*, is one such alternate animal model that is again gaining attention in the field of reproductive and developmental toxicology. Guinea pigs are accepted as an animal model in a wide variety of biological research, including immunology assessments, infectious disease, diabetes, dermatology, and phototoxicology. Rocca and Wehner[1] described the advantages of utilizing the guinea pig for DART studies in instances when the drug candidate is pharmacologically inactive in traditional animal models such as rats, mice, and rabbits. Although the guinea pig is a viable animal model in biomedical research, real or perceived limitations that suggest that the guinea pig may be a questionable model for DART assessments include: limited historical control data, variability in pregnancy rates, a long estrous cycle, small and variable litter size, long gestation, relative maturity at birth, and breeding[1]. Other inherent disadvantages that have been identified include: limited dosing techniques, particularly repeat oral dosing, and the nature of the sperm to stack or form rouleaux of cells while developing motility that limits the evaluation of this endpoint. We have developed new innovations to firmly establish the guinea pig as an accepted model for DART assessments and meet the growing regulatory expectations for nonclinical safety testing. Our experience with novel techniques in oral dosing procedures, blood collection and male reproductive assessments has allayed the concerns in using this test system as a DART model. Description of these techniques along with a presentation of historical control data from our work with the guinea pig demonstrates consistent success in evaluating critical endpoints when traditional test systems are not appropriate.

Objectives

The objectives of this presentation are to summarize:

1.Oral dose route feasibility in guinea pigs (short-term vs. long-term dosing using standard vehicles).

2.Historical comparison of viable routes of administration in guinea pigs.

3.Biological sample collection routes and volumes.

4.Sperm assessments (motility and concentration) in male guinea pigs.

5.Ovarian, uterine, and litter data generated in Dunkin-Hartley and Hartley guinea pigs.

Materials and Methods

Oral (Capsule) Dosing

1.Prefilled lock ring capsules (two-piece, hard shell gelatin capsules) and a stainless steel pill syringe.

2.The capsule and pill syringe were inserted into the oral cavity, the capsule was ejected, the pill syringe was withdrawn, and the animal was observed to ensure the capsule had been swallowed.

Oral (Gavage) Dosing

1.Jacket restraints, a bite guard/gag, suitable lubricant (KY Jelly), Nelaton rubber catheter (French size 8), a 20 gauge 1½ inch stainless steel gavage needle and an appropriately sized syringe.

2.Vehicle: R.O. deionized water or aqueous 0.5% (w/v) methylcellulose (400 cps)

3.The rubber catheter was passed through the bite guard/gag directly into the esophagus.

Dosing Techniques

Figures 1-3 illustrate the methods of repeat oral dose administration that have been used in guinea pigs. Capsule administration as well as oral gavage (using flexible tubing) are both feasible techniques for repeat dose administration in this species. Each technique takes into account the unique anatomy of the guinea pigs' oral cavity.

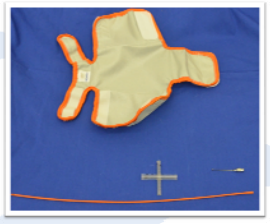

Figure 1. Oral Dosing using French Catheters and Stainless Steel Gavage Needle

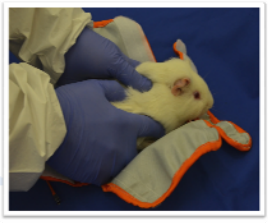

Figure 2. Jacket Restraint Technique for Guinea Pigs

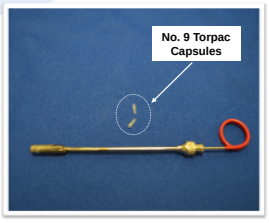

Figure 3. Oral Dosing using a Stainless Steel Pill Dosing Apparatus and No. 9 Torpac Capsules

Table 1. Summary of Mortality Based on Route of Administration

Period	2004-2006	2009-2011	2003-2004	2012-2013	2010
Route of Administration	IM	SQ	Oral (Capsule)	Oral (Gavage)	IV (Saphenous)
No. of Studies	3[a]	5	4	2	1
No. of Guinea Pigs	51	87	59	38	9
No. of Dose Attempts	51	1213	849	690	9
No. of Mortalities	1[b]	2[c,d]	1	1[e]	0
No. of Dose Holidays	NA	NA	NA	2[f]	NA
Maximum Duration of Dose Holiday	NA	NA	NA	8[f]	NA
No. of Successful Dose Attempts	51	1213	848	680	9
% Success Rate	100	100	99.9	98.5	100
Dose Volume	2 mL/kg (1 mL/site)	1.54 to 2 mL/kg	Up to 4/day[g]	10 mL/kg	1.7 to 3.9 mL/kg

IM = intramuscular; SQ = subcutaneous; IV = intravenous; NA = not applicable
[a] Conducted in neonatal Crl:IAF(HA)-hr guinea pigs on PND 2.
[b] A single female was found dead 3 days after a single administration.
[c] One mortality occurred in a catheterized animal; death was attributed to embolism.
[d] One mortality occurred in an animal with kidney lesions; cause of death could not be determined.
[e] One animal was euthanized due to limb injury.
[f] One animal was given a dose holiday on 2 occasions (2 days and 8 days in duration); successful administration was achieved for an additional week following the dose holiday.
[g] Based on a 0.2 mL fill volume in the capsules used.

Observations

1.Repeat oral dosing techniques (capsule and oral gavage) were successfully demonstrated in the guinea pigs.
2.Oral dosing by capsules is limited by the size of the capsule and the number of capsules that could be administered per day to achieve a specific dose.
3.During method development, oral gavage dosing was successfully performed on 494 out of 504 possible occasions (98% success rate).
4.The survival rate for repeat oral dosing techniques (capsules and oral gavage with flexible tubing) is comparable.

Blood Collection Procedures

Table 2 outlines acceptable routes of blood collection in adults, fetuses, and/or pups. Pooling of fetal samples by litter is sometimes required in order to meet volume specifications.

Table 2. Summary of Blood Collection Techniques and Acceptable Volumes

Biological Sample Collections	Adult	Amniotic Fluid	Fetuses[a]	Fetuses[a]	Pup[b]
	N=13	N=1	N=1	N=1	N=3
Route of Survival Collection	Jugular	NA	NA	NA	NA
Maximum No. of Collections in 24 Hours	3[b]	NA	NA	NA	NA
Maximum Volume Obtained	3.0 mL	NA	NA	NA	NA
Range of Volumes Obtained at Collection	0.3 to 1.5 mL	NA	NA	NA	NA
Route of Terminal Collection	Vena Cava	NA	Umbilical	Carotid	Vena Cava
Maximum Volume Obtained	20 mL	1.3 mL	0.315 mL	0.220 mL	2 mL
Range of Volumes Obtained at Collection	2.0 to 20 mL	1.0 to 1.3 mL	0.005 to 0.315 mL	0.010 to 0.220 mL	2 mL

[a] Fetal samples were collected on DG 30.
[b] Terminal samples obtained via the vena cava from neonatal Crl:IAF(HA)-hr guinea pigs on PND 4.
[c] Assumes that a terminal collection can be performed as a fourth time point.
NA = not applicable

Reproductive Endpoints

Tables 3 and 4 summarize the historical data for male and female reproductive endpoints.

Table 3. Summary of Sperm Motility, Count and Concentration

VAS DEFERENS	Number Motile	Mean	291.4	Range	161.2 – 421.6
	Motile Percent	Mean	92.1	Range	91.6 – 92.5
	Static Count (Nonmotile)	Mean	23.1	Range	12.9 – 33.3
	Total Count[a]	Mean	314.5	Range	174.0 – 454.9
CAUDA EPIDYDMIS	Sperm Count[b]	Mean	262.4	Range	199.1 – 325.6
	Sperm Density	Mean	730.87	Range	643.38 – 818.35

Animal Source: Elm Hill Breeding Laboratories; Strain: Dunkin Hartley; No. of Studies/Period: 2 (N=32) / 2011 – 2012
a.5 fields evaluated
b.20 fields evaluated

Table 4. Summary of Ovarian, Uterine and Litter Parameters in Dunkin-Hartley and Hartley Guinea Pigs

Animal Source	Elm Hill Breeding Laboratories		Charles River Laboratories, Inc.		Covance Research Products, Inc.	
Strain	Dunkin-Hartley		Crl:(HA)BR-Hartley		Hra:(DH)fBR (Dunkin Hartley)	
Period	2011 – 2012		2003 – 2004		2003 – 2004	
No. of Studies	2		6		6	
No. of Female Guinea Pigs Tested	53		52		52	
No. Pregnant	51		41		44	
No. Found Dead	0		1		2	
No. Aborted	0		1		1	
No. With a Single Conceptus Litter	0		1		0	
No. Pregnant at Examination	51		39		42	
Day of Examination	DG 30		DG 60		DG 60	
Parameters	Mean or %	Range/Study Mean or %	Mean or %	Range/Study Mean or %	Mean or %	Range/Study Mean or %
% Pregnant	96.4	91.7 – 100	80.1	50.0 – 100	89.3	25.0 – 100
Avg No. Corpora Lutea	4.9	4.8 – 5.0	5.7	3.0 – 6.2	5.0	3.0 – 5.5
Avg No. Implants	4.4	-	4.9	3.0 – 6.0	4.5	3.0 – 5.5
Avg % Preimplantation Loss	10.4	8.3 – 12.0	NC	NC	NC	NC
Avg No. Live Fetuses	4.0	3.9 – 4.0	4.7	2.0 – 5.1	4.3	3.0 – 5.5
Avg No. Dead Fetuses	0.0	-	0.0	-	0.0	-
Avg No. Resorptions	0.4	-	0.2	0 – 3.0	0.2	0 – 0.5
Avg No. Early Resorptions	0.4	-	0.2	0 – 3.0	0.1	0 – 0.2
Avg No. Late Resorptions	0.0	-	0.0	0 – 0.1	0.1	0 – 0.5
Avg % Postimplantation Loss	10.4	10.2 – 10.6	NC	NC	NC	NC
Avg % With Any Resorptions	25.5	24.1 – 27.3	15.4	0 – 100.0	14.3	0 – 50.0
Avg % With Total Litter Loss	1.9	0 – 3.4	0.0	-	2.4	0 – 4.5
Avg % With One Or More Live Fetuses	98.1	96.6 – 100	100.0	-	97.6	95.4 – 100
Avg % Resorbed Conceptuses/Litter	NA	NA	3.7	0 – 50.0	3.8	0 – 12.5
Avg Fetal Body Weight (grams)	NA	NA	64.49	56.64 – 67.76	55.64	34.56 –62.90

NC = not calculated; NA = not applicable

Conclusions

•Dose administration in guinea pigs can be successfully performed via intramuscular, subcutaneous, or intravenous (saphenous) injection or via oral (capsule or gavage) administration.
•Acute and repeat dosing techniques have been successfully demonstrated.
•Biological samples can be obtained from adults, fetuses, and pups at variable volumes. The sample volume for the fetuses and pups depends on the age at collection.
•Values for sperm motility and sperm concentration are comparable to the historical ranges for other rodents commonly used at the Testing Facility.
•Despite limitations that are noted in the literature[1], guinea pigs can be used to evaluate the potential effects of reproductive or developmental toxicants.

References

1.Rocca MS, Wehner NG. The guinea pig as an animal model for developmental and reproductive toxicology studies. Birth Defects Research B Development Reproduction Toxicology 2009;86(2): 92-7.

Acknowledgements

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