An Assessment of Continuous Intravenous and Subcutaneous Infusion Dosing in Embryo-fetal Development Studies – Maternal and Embryo-fetal Parameters

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Introduction

Intravenous (IV) infusion or subcutaneous (SC) infusion, via indwelling catheters, are dosing routes sometimes used in nonclinical reproductive toxicity studies to mimic the proposed clinical dose route and also the treatment regimen. The feasibility of using the IV infusion route has been established for many years in our laboratory for ICH reproductive toxicology studies; more recently SC infusion methods have been developed and utilized for SC infusion dosing of ICH 3 embryo-fetal development studies in Sprague Dawley CD® IGS (Crl:CD[SD]) rats and New Zealand White rabbits. Consideration of the control data sets from these studies allows for the examination of potential effects of dose route on maternal, ovarian/uterine and fetal parameters, including evaluation of embryo-fetal mortality, growth and dysmorphology.

Experimental Procedures

Comparisons were made to data from studies conducted with standard routes (oral gavage, IV bolus and subcutaneous injection). Catheters were implanted into the vena cava vs the femoral vein (IV infusion) or subcutaneously in the lumbar region (SC infusion) of groups of 20-25 female rats or rabbits while under general anesthesia. The catheter was linked to an infusion pump by a jacket/tether attached to a swivel outside the cage. SC catheter implantation occurred prior to mating or insemination due to the longer healing times (10 days) prior to dose initiation required following the surgery. Animals were housed in standard caging; for rats, solid bottomed cages with corn cob bedding was used. Rats were dosed from Gestation Day (GD) 6 to 17/18, and rabbits were dosed from GD 7 to 19/20. A variety of vehicles were successfully administered by both IV and SC infusion routes, including physiological saline, lactated Ringers solution, proprietary placebos and cyclodextrins. Daily continuous IV infusion volumes of 1x the blood volume/day for physiological solutions and ½ the blood volume/day for non-physiological solutions were well tolerated for both rats and rabbits. For SC infusion, due to slow absorption, dose volumes and infusion rates must be much lower, approximately 10% of volumes and rates used for IV infusion dosing. In-life assessments included mortality, clinical observations, body weight and food consumption were conducted. A cesarean section was performed on GD 21 for rats and GD 29 for rabbits and the fetuses were weighed and examined externally. All rabbit fetuses and ½ of the rat fetuses were given a fresh visceral dissection, and the rat heads were examined using Wilson's technique. The skeletons of all rabbits and the remaining ½ of the rats were stained with Alizarin Red S and examined.

Table 1 – Maternal Performance and Uterine Findings – NZW Rabbits

Parameter	Intravenous Infusion	Subcutaneous Infusion	Control Range - Standard Routes
Mortality/Study	0 - 1	0 - 0	0 - 2
No. Does with Abortion	0 - 2	0 - 0	0 - 1
Pregnancy Rate (%)	81.8 – 100	90.9 – 100	81.8 – 100
No. Corpora Lutea/Doe*	10.3 (9.7 – 11.4)	10.2 (10.0 – 10.5)	9.5 (8.4 – 11.0)
No. Implants/Litter*	8.8 (8.4 – 9.7)	8.7 (8.0 – 9.6)	8.6 (7.7 – 10.1)
Pre-Implantation Loss (%)*	13.2 (6.8 – 16.7)	14.1 (8.4 – 18.2)	9.2 (3.1 – 17.1)
No. Live Fetuses/Litter*	8.3 (7.6 – 8.9)	8.4 (7.6 – 9.5)	8.3 (6.9 – 9.7)
No. Dead Fetuses/Litter*	0.01 (0.0 -0.1)	0	0.01 (0.0 – 0.3)
No. Early Resorptions/Litter*	0.4 (0.2 – 0.7)	0.1 (0.1 – 0.3)	0.3 (0.0 – 0.7)
Total No. of Resorptions/Litter*	0.5 (0.3 – 0.8)	0.2 (0.1 – 0.3)	0.4 (0.0 – 0.8)
Post Implantation Loss (%)*	5.7 (3.0 – 9.9)	2.9 (0.8 – 4.3)	4.1 (0.6 – 11.5)

*Data are presented as the average of the study means (range of study means)

Table 3 – Maternal Performance and Uterine Findings – Sprague Dawley Rats

Parameter	Intravenous Infusion	Subcutaneous Infusion[&]	Control Range - Standard Routes
Mortality/Study	0	0	0 - 1
No. Dam with Total Resorption	0 - 1	0	0 - 1
Pregnancy Rate (%)	92.0 - 100	95.5	84.0 - 100
No. Corpora Lutea/Dam*	15.5 (14.9 – 16.2)	17.5	15.7 (13.9 – 20.1)
No. Implants/Litter*	13.7 (13.2 – 14.5)	15.5	13.8 (11.6-17.6)
Pre-Implantation Loss (%)*	11.3 (6.8 – 15.0)	8.7	11.5 (3.3 – 19.2)
No. Live Fetuses/Litter*	13.1 (12.5 – 14.0)	14.5	13.1 (11.1 -16.6)
No. Dead Fetuses/Litter*	0.01 (0.0 – 0.1)	0	0 (0.0 – 0.0)
No. Early Resorptions/Litter*	0.7 (0.0 – 1.0)	1	0.7 (0.2 – 1.4)
Total No. of Resorptions/Litter*	0.7 (0.4 – 1.0)	1	0.7 (0.2 – 1.5)
Post Implantation Loss (%)*	5 (3.4 – 8.3)	6.5	5.6 (1.6 – 10.4)

*Data are presented as the average of the study means (range of study means)

[&] Based on one study (21 pregnant rats examined).

Table 2 – Fetal Evaluations – NZW Rabbits

Parameter	Intravenous Infusion	Subcutaneous Infusion	Control Range - Standard Routes
Fetal Weight (g) - Males	44.2 (41.8 – 45.7)	44.7 (43.6 – 46.7)	43.1 (40.4 – 47.9)
Fetal Weight (g) - Females	42.9 (40.3 – 44.2)	43.2 (41.8 – 44.9)	42.2 (38.4 – 46.4)
Abnormalities – % of Litters (Fetuses) Affected			
Cleft palate	0.0 – 5.3 (0.0 – 0.7)	0	0.0 – 4.8 (0.0 – 0.6)
Hydrocephaly	0.0 – 5.3 (0.0 – 1.9)	0	0.0 – 4.8 (0.0 – 0.6)
Omphalocele	0	0.0 – 5.0 (0.0 – 0.5)	0.0 – 5.3 (0.0 – 0.6)
Interventricular septum defect	0.0 – 10.5 (0.0 – 1.2)	0	0.0 – 11.0 (0.0 – 1.4)
Dilatation of the ascending aorta	0.0 – 10.5 (0.0 – 1.2)	0.0 – 4.8 (0.0 – 0.6)	0.0 – 5.0 (0.0 – 0.6)
Stenosis of the pulmonary trunk	0.0 – 10.5 (0.0 – 1.2)	0.0 – 4.8 (0.0 – 0.6)	0.0 – 5.0 (0.0 – 0.6)

Table 4 – Fetal Evaluations – Sprague Dawley Rats

Parameter	Intravenous Infusion	Subcutaneous Infusion[&]	Control Range - Standard Routes
Fetal Weight (g) - Males	6.06 (5.63 – 6.25)	6.08	5.89 (5.53 – 6.27)
Fetal Weight (g) - Females	5.73 (5.33 – 5.89)	5.76	5.68 (5.24 – 5.93)
Abnormalities – % of Litters (Fetuses) Affected			
Anophthalmia	0.0 -4.6 (0.0 – 0.35)	0	0.0 – 4.2 (0.0 – 0.32)
Omphalocele	0.0 – 4.6 (0.0 – 0.38)	0	0.0 – 9.1 (0.0 – 6.8)
Microcaudia	0.0 – 4.6 (0.0 – 0.38)	0	0.0 – 4.0 (0.0 – 0.31)
Digits of the forepaws absent	0.0 – 4.8 (0.0 – 0.72)	0	0
Hydrocephaly	0.0 – 4.6 (0.0 – 0.70)	0	0
Interventricular septum defect	0.0 – 4.6 (0.0 – 0.65)	0	0.0 – 5.0 (0.0 - 0.74)
Lung lobes fused	0.0 – 4.6 (0.0 – 0.74)	0	0.0 – 5.0 (0.0 – 0.74)
Subclavian artery, retroesophageal	0	4.8 0.7	0.0 – 5.3 (0.0 – 0.85)

[&] Based on one study (21 litters examined).

Results

Mortality rates, body weight gain and food consumption were comparable across treatment routes (data not shown). There were no clear differences in pregnancy rate, number of animals with total resorption or that aborted, or the ovarian, uterine or fetal parameters in rats and rabbits treated by IV or SC infusion compared to more standard routes (Tables 1 and 3). The slightly higher pre-implantation loss noted for one of the SC infusion rabbit studies was likely related to administration of a high volume of a non-physiological vehicle (cyclodextrin); such vehicles have been noted previously to have an effect on embryo-fetal viability.

For fetal weights, overall mean of study means (with range in parentheses) were comparable (Tables 2 and 4).

Consideration of the fetal abnormalities showed no differences attributable to either IV or SC infusion (Tables 2 and 4) for the incidence of malformations observed. Similarly the external, visceral and skeletal variations (data not shown) were unaffected.

Conclusion

It was concluded that IV and SC dose administration by infusion did not adversely affect embryo-fetal development in Sprague Dawley rats and New Zealand White rabbits. Ovarian and uterine parameters were comparable; the slightly higher pre-implantation loss noted for SC infused rabbits was possibly due to the high concentration of non-physiological components in the vehicle. Fetal weights and incidences of fetal findings were also comparable regardless of the dose administration method.

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