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Full Length Article

Investigation into the effect of microsampling on mouse fetuses and pregnant mice in the embryofetal development study design

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ABSTRACT

The effects on fetal weights and maternal health of taking 32 μL blood microsamples at the end of organogenesis in a mouse embryofetal development (EFD) study design was investigated with the aim of reducing satellite animal usage. The effects of warming, handling and sampling either 3 or 6 times on gestation day 16 was evaluated.

Maternal body weight gain was transiently reduced when animals underwent warming and handling with or without microsampling. Fetal weights on gestation day 18 were reduced after 6 occasion warming and handling alone or taking samples, but not when sampling was limited to 3 timepoints.

Taking 3 microsamples of 32 μL had no permanent adverse effects on maternal health or toxicologically significant effects on fetal development (measured by fetal weights). This regimen could be used to generate composite toxicokinetic profiles using only 6 main test animals in mouse EFD studies provided sampling procedures were matched across groups.

1. Introduction

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has published two harmonised tripartite guidelines relevant to embryofetal development studies. The Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials (ICHM3(R2)) [1] requires the assessment of effects on embryofetal development by pharmaceuticals is performed before the inclusion of women of childbearing potential in long duration and larger scale clinical trials. The technical requirements for these studies, usually performed in a rodent and non-rodent species, are detailed in ICHS5 (R2) [2].

Toxicokinetic evaluation of compound exposure is performed in these studies to prove exposure, determine any differences between pregnant and non-pregnant animals and to provide information on margins to the eventual maximum recommended human dose for labelling purposes [2–4].

When the mouse is selected as the rodent species, there are limitations in blood sample volume due to the small size of the mouse. In our facility no more than 15% of total blood volume can be taken in any 28-day period under our Home Office Licence, therefore a maximum of 0.3 mL can be taken from a 25 g mouse. This means that even for a non-pregnant mouse, to obtain a six-time point toxicokinetic profile from an individual animal requires a sensitive analytical assay and a "microsampling" approach.

However, in addition to general physiological constraints regarding blood volume loss, pregnant mice are considered sensitive to various stressors and as a species have a tendency to clusters of malformations of unclear origin [2]. It has been noted that commonplace methods of handling mice (such as catching and lifting by the tail) can induce high anxiety [5] which is undesirable in pregnant mice. Even assuming that blood sampling at the end of organogenesis (typically gestation day 16) is after the period where procedural stresses could cause fetal malformations, the extremely truncated period of post-organogenesis fetal maturation in the mouse (when fetal weight is increasing very rapidly) means there is little recovery time between end of organogenesis blood sampling and the caesarian section necropsy examination, which are typically scheduled for gestation day 18. For all these reasons, the handling, warming and repeated needle puncture sampling of pregnant mice is rarely done in those animals that are also being used for assessment of fetal growth and morphology. This has traditionally resulted in additional, satellite, animals being included for toxicokinetic blood sampling in murine embryofetal development studies.

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Table 1
Phase 1 Groups and Treatments.

Group	Animals	Treatment	Procedures
1	17 Females	Control	No warming, handling or blood sampling
2	17 Females	Handling Control	Warming and handling for TK but no blood sampling
3	16 Females	TK sampling	Warming, handling and TK blood sampling

Microsampling in mice has been successfully introduced into the AstraZeneca laboratories for general toxicology studies to reduce the blood sample volume requirement. Therefore, in order to avoid the use of satellites and reduce animal numbers, this study was designed to investigate whether toxicokinetic samples could be taken from main group animals in the embryofetal development study design without causing detrimental effects on fetal development or maternal health. Fetal weights were selected as the indicator of development (since major organogenesis is complete by gestation day 16) and maternal body weight gain, clinical signs and necropsy observations as the indicators of maternal health. This study was powered to pick up a 10% change in fetal weights, which was considered to be biologically significant. Therefore, this study used fewer animals than a standard embryofetal development study, which is designed to detect changes in malformation rates.

To simulate the conditions of a mouse embryofetal development study, the animals were dosed during organogenesis, Gestational Day (GD) 5–16, with a vehicle, 0.5% w/v hydroxypropyl methyl cellulose and 0.1% w/v polysorbate 80, which is commonly used as a control and vehicle in toxicology studies.

The study was conducted in two phases. In the first phase, three treatments were compared: No warming, handling or blood sampling; warming and handling but no blood sampling; or warming, handling and blood sampling. The animals allocated to blood sampling had 6 blood samples of 32 μL taken and the animals allocated to warming and handling only had procedures performed 6 times to match this group. After the results from this phase were analysed, a second phase was conducted to investigate whether effects on fetal weights seen in the first phase could be ameliorated. Two treatments were compared: No warming, handling or blood sampling; or warming, handling and blood sampling. The animals allocated to blood sampling had 3 blood samples of 32 μL taken and the animals allocated to warming and handling only had procedures performed 3 times to match this group. All blood samples in both phases were taken after the last vehicle dose on GD16.

2. Materials and methods

2.1. Study design

In both phases, mated female CD1 mice were dosed by oral gavage with 0.5% w/v hydroxypropyl methyl cellulose and 0.1% w/v polysorbate 80 at a dose volume of 5 mL/kg from Gestational Day (GD) 5–16. The day of mating was regarded as GD 0. This administration period covered the period from implantation to closure of the hard palate and the completion of major embryonic organogenesis. Animals were allocated to treatment groups on GD15 based on body weight gain between GD3 and 15 to ensure an even distribution of pregnant animals. Scheduled maternal necropsy was performed on GD18. The uterus and ovaries were excised and the uterus opened and examined for implantations. The placentae were examined for gross abnormalities. The fetuses were removed, weighed, sexed and examined externally for gross defects.

Animals were picked up mainly by cupping or occasionally by the base of the tail depending on the handler, animal and the stage of pregnancy. Mice in later stages of pregnancy with a heavy gravid uterus were always cupped supporting their abdomen. Therefore, by the time that the mice underwent blood sampling, the mice were always cupped.

Table 2
Phase 2 Groups and Treatments.

Group	Animals	Treatment	Procedures
4	13 Females	Control	No warming, handling or blood sampling
5	12 Females	TK sampling	Warming, handling and TK blood sampling

All protocols and experiments were performed under the authority of a valid Home Office Project Licence and conformed to UK Governmental regulations regarding laboratory animal use and care [6].

2.1.1. Phase 1

In Phase 1, three groups of 16 or 17 mated female CD1 mice were allocated to treatment groups (Table 1). On Day 16, the three groups were treated as follows: No warming, handling or blood sampling (Group 1: 17 females), warming and handling but no blood sampling (Group 2: 17 females) or warming, handling and blood sampling (Group 3: 16 females). The animals allocated to blood sampling had 6 blood samples of 32 μL taken at 0.5, 1, 2, 4, 6 and 24 h post-dose. The animals allocated to Group 2 were warmed and handled in the same way as animals in group 3 at 0.5, 1, 2, 4, 6 and 24 h post-dose.

The group sizes were based on the number of pregnant animals, using data from a recent study, required to detect a 10% change in fetal weights (combined sexes, male and female weights) using a two-sided *t*-test at the 5% significance level with 80% power, which is 13 animals per group. Charles River advised that time mated CD1 mice have a typical pregnancy rate of 75%. Taking this into account, 19 mated females were estimated to be needed to provide 13 pregnant animals with an 80% certainty. However, since the pregnancy rates in the first subset of 10 animals per group were better than expected, 16-17 animals per group were used to ensure that at least 13 pregnant animals per group were achieved.

2.1.2. Phase 2

For Phase 2, the fetal weights in the control group from Phase 1 were used to refine the power analysis used to determine group size. The group sizes were based on the number of pregnant animals required to detect a 10% change in fetal weights (combined sexes, male and female weights) using a two-sided *t*-test at the 5% significance level with 80% power, which is 9 animals per group. Assuming an ongoing pregnancy rate of 75%, 13 mated females were required to provide 9 pregnant animals with an 80% certainty. One animal had to be euthanised prior to allocation due to intercurrent disease and therefore only 12 females were allocated to Group 5.

In Phase 2, two groups of 12 or 13 mated female CD1 mice were allocated to treatment groups (Table 2). On GD16, the two groups were treated as follows: No warming, handling or blood sampling (Group 4: 13 females) or warming, handling and blood sampling (Group 5: 12 females). The animals allocated to blood sampling each had 3 blood samples of 32 μL taken, in a composite design, either being bled at 0.5, 2, and 6 h or at 1, 4 and 24 h post-dose.