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An innovative investigative approach to characterize the effects observed in a combined fertility study in male and female rats

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ABSTRACT

This paper describes the characterization of male- and female-mediated effects in a standard ICH rat fertility and early embryonic development study with a discontinued clinical small molecule. In the standard study, the test item had no effect on the number of treated females becoming pregnant, but litter sizes were reduced at the high dose level. In the treated male rats, increased incidences of abnormal sperm, decreases in average sperm path and straight line velocities, and minimal retention of mature sperm in the seminiferous tubules were observed at all dose-levels tested. These findings were unexpected in view of a lack of histopathological changes in the reproductive organs of either gender in 4-week repeat dose studies in rats and monkeys. A follow-up fertility study was conducted using an innovative flexible study design and a single high-dose level. In the first instance, treated male rats were mated with untreated females, followed by necropsy of a subset of males. The intention was then to re-mate the males after an 8-week wash-out period with either treated or untreated females depending on the outcome of the first mating. On this occasion, litter sizes were not affected, but the testicular effects were reproduced. A second mating with treated females reproduced the reduced litter sizes due to increased pre- and post-implantation loss, demonstrating that the effect on fecundity was female-mediated. The testicular changes in males were shown to be reversible after a 12-week recovery period.

1. Introduction

A fertility and early embryonic development study (herein referred to as “fertility study”) is a routine component of the nonclinical reproductive toxicity testing package for novel pharmaceuticals. This study is designed to test for toxic effects on male and female gamete maturation, copulatory performance, fertilization, zygote development and implantation (Barrow, 2009; Parker, 2006). The fertility study is generally conducted in rats according to the study design suggested in the ICH S5 (R2) guideline, “Detection of toxicity to reproduction for medicinal products and toxicity to male fertility” (ICH, 2005). According to this guideline, a combined fertility study incorporating treatment of both male and female animals can be performed for medicinal compounds that are not expected to induce marked reproductive toxicity (Lerman et al., 2009) based on the known pharmacological activity and general toxicity profile of the drug. As shown in Fig. 1A, in a combined fertility study, each treatment group typically consists of 20 male and 20 female rats. Males are dosed once daily for at least 14 days before and during pairing and until approximately 14 days after completion of the mating period. The males are then necropsied. Females are dosed for 14 days before and during pairing and until gestation day (GD) 6, inclusive. Effects of the drug on the following aspects of fertility and development are assessed: mating behavior in males and females, estrous cycle, ovulation, oviductal transport, early embryonic development up to implantation, and implantation of the conceptus on the uterine wall. In males, effects on sperm parameters (motility, counts and morphology) can also be evaluated when not already assessed in repeat dose toxicity studies.

No effects on testes and ovaries were noted in repeat dose toxicity studies of up to 4 weeks duration in rats and monkeys conducted with a discontinued antiviral small molecule. In dose-range-finding (DRF) and pivotal embryo-fetal toxicity (EFD) studies in rats and rabbits (both starting dosing on GD6) no evidence of teratogenicity was detected in rats up to 450 mg/kg/day or in rabbits up to 50 mg/kg/day (data not shown). In a combined male and female rat fertility study (Fig. 1A) conducted recently with this test item an increased incidence of pre-

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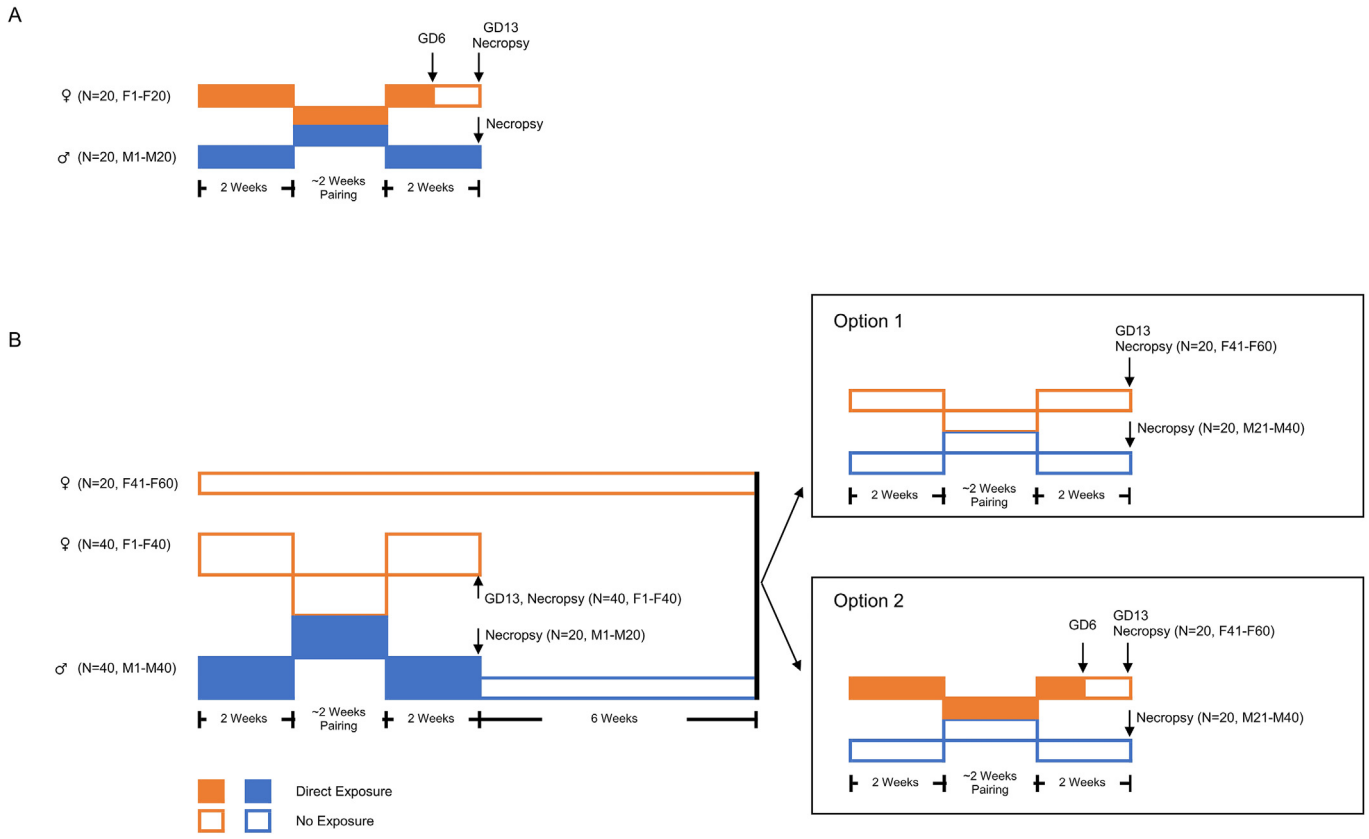

Fig. 1. Schematic diagrams of (A) the standard combined fertility study in male and female rats according to ICH S5, and (B) the investigative follow up study. (A) Each group consists of 20 male and 20 female rats. Males are dosed once daily for 14 days before and during pairing and until approximately 14 days after completion of the mating period. The males are then necropsied. Females are dosed for 14 days before and during pairing and until GD6, inclusive. (B) Each group consists of 40 male and 60 female rats. All males are dosed once daily for at least 14 days before and during the first pairing with 40 un-dosed females and up to approximately 14 days after completion of the mating period. After the completion of the dosing period, 20 males are necropsied while the remaining 20 are retained un-dosed for approximately eight weeks for recovery assessment. The 40 paired females are necropsied on GD 13 to assess pregnancy parameters. Following assessment of uterine and implantation data, if a male-mediated effect is determined, the remaining un-dosed 20 females are paired with the recovery males (option 1); if a male-mediated effect is not determined, the remaining 20 females are dosed for 14 days before and during a second pairing with the previously treated males (off-dose for 8 weeks) with continued dosing of females up to GD 6 (option 2). For both options, the females are necropsied on GD 13 to assess pregnancy parameters. The recovery males are necropsied approximately 14 days after completion of the mating period (i.e. after approximately 12 weeks of recovery).

implantation loss associated with a lower mean number of live embryos was observed. In male rats, sperm evaluation indicated an increased incidence of abnormal sperm and decreases in average sperm path and straight line velocities. Histopathological examination of the testes revealed minimal retention of mature sperm in the seminiferous tubules. A further study (Fig. 1B) was designed to determine the male- or female-mediated origin of the pre-implantation loss and to investigate the reversibility of the effect(s). In this follow-up study, males were initially paired with un-treated females. The mated females were submitted to necropsy on presumed GD13 and half of the males were necropsied. The remaining males were retained un-dosed for a recovery assessment and a second mating with treated or untreated females depending on the outcome of the first mating. Since no effects on fertility were detected following the first mating, the recovery males were paired with treated females. The treated females were submitted to necropsy on GD13 and the remaining males were necropsied after a total of 12 treatment-free weeks.

This novel study design allowed both characterization of the observed effect on fertility (i.e. male- or female-mediated) and a determination of the reversibility of the testicular lesions using the minimal number of animals.

2. Materials and methods

2.1. Test substance formulation

The test item, which is an antiviral small molecule, was provided by F. Hoffmann-La Roche Ltd (Basel, Switzerland). It was formulated for dosing as a suspension in a vehicle comprised of 2% hydroxypropylcellulose, 0.1% polysorbate 80 (Tween 80), 0.09% methylparaben, and 0.01% propylparaben in purified water. The prepared dose suspensions were stored refrigerated (2 °C–8 °C) until administration within the 14-day confirmed stability period of the test item in the vehicle. The control and the test item dose preparations were removed from the refrigerator and allowed to warm for at least 30 min before administration and were stirred until completion of dosing.

2.2. Animals and husbandry

The studies were conducted at Sequani Limited (Ledbury, UK). Male and female rats of the Crl:WI(Han) strain were supplied by Charles River (Margate, UK). For the initial combined fertility study, the animals were 6–7 weeks of age on arrival and were acclimated to the laboratory housing for at least 11 days. On the first day of dosing the