Reproductive Toxicology 45 (2014) 39–44

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Reduced post-natal versus pre-natal incidence of bent long bones and scapulae in a preliminary investigation using the Han Wistar rat

Terri Mitchard*, Jane Stewart

AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, United Kingdom

ARTICLE INFO

Article history:
Received 6 August 2013
Received in revised form
20 December 2013
Accepted 2 January 2014
Available online 15 January 2014

Keywords:
Chondrodystrophy
Han Wistar rat
Fetus
Embryofetal development
Post-natal development
Skeletal malformations

ABSTRACT

There is a "chondrodystrophy" syndrome in the Han Wistar rat fetus that manifests as characteristic skeletal abnormalities such as bent and/or short long bones, and is classified as permanent detrimental abnormalities (major malformations). This pilot study investigated whether these defects resolve after birth.

Han Wistar rats were dosed during organogenesis either with vehicle or test article. Examination of gestation day 20 fetuses showed a slightly increased incidence (11%; 11/101) of skeletal abnormalities in the high dose fetuses compared with 6% (4/67) in control fetuses, whereas no skeletal abnormalities were present in the 205 pups examined on post-natal day 21. The probability of having zero litters containing pups with skeletal abnormalities was $p < 0.0000001$. This very low probability suggests that these defects recover by weaning and supports the hypothesis that these fetal findings in the Han Wistar are probably not permanent abnormalities and therefore are potentially reclassifiable as minor malformations.

1. Introduction

A "chondrodystrophy" syndrome in the Han Wistar rat fetus that manifests as characteristic multiple skeletal abnormalities, such as bent and/or short bones, has been previously reported [1]. The long term monitoring of background skeletal abnormality incidences in control fetuses showed variable incidences over time of bent long bones and scapula and other anomalies such as kinked (wavy) ribs, brachygnathia, cleft palate and kyphosis/lordosis. These abnormalities were reported for the Han Wistar rat supplied by Harlan UK Ltd. Similar skeletal abnormalities, except for kyphosis/lordosis, were also reported for control Charles River Sprague Dawley fetuses. There is a spectrum of effects seen in the Han Wistar rat fetus ranging from the most severe with multiple effects such as those reported by Wilby et al. [1] to less severe manifestations comprising of single bent limbs or scapulae.

Of all the findings that can form a part of this "chondrodystrophy" syndrome, previous published literature has predominately concentrated on the occurrence, pathogenesis and reversibility of wavy ribs in rat fetuses [2,3]. However, wavy ribs are often seen in combination with bent and short scapula, humerus, radius and ulna [2]. A range of pharmaceuticals and chemicals has been reported to increase the incidence of wavy ribs and bent/short long bones/scapula in various rats strains including the Wistar. These include pharmaceuticals such as doxaminol [4], cyclophosphamide [5], indacrinone, azosemide and furosemide [6], dimethadione [7], lithium carbonate [8] and tretinoin [9], herbicides such as 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid [10] and pesticides such as linuron, malathion and methoxychlor [11].

Currently within this laboratory, bent/short bones (scapula, humerus, radius, ulna, femur and tibia) in rat fetuses are classified as major malformations i.e. permanent detrimental abnormalities. However, it is possible that these abnormalities seen prenatally may remodel after birth, as is seen with wavy ribs, and are transient abnormalities. Therefore, it is important for risk assessment to investigate whether these bent/short bones resolve after birth and therefore could be re-classified as minor abnormalities.

In a preliminary investigative embryofetal development study where a test article was administered, some of these skeletal abnormalities were evident in fetuses from both control and test article-dosed animals. This study also included an additional cohort of animals that were dosed to the end of organogenesis and were then allowed to litter and the in utero exposed pups were assessed on post-natal day (PND) 21. This design provided the opportunity to compare the incidence and appearance of these particular limb observations in gestation day (GD) 20 fetuses versus PND21 pups and from that make better informed judgement as to whether these types of limb observations in the fetal are permanent, detrimental changes.

* Corresponding author at: AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. Tel.: +44 1625519902.
E-mail address: terri.mitchard@astrazeneca.com (T. Mitchard).

0890-6238/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.reprotox.2014.01.001

Table 1
Design of Investigative study.

Group	Animals	Formulation	Dose levels	Dosing period	Fetal/pup assessment
Caesarean groups					
1	10 females	Control	Control	GD6-17	GD20
2	10 females	Test article	Low dose		
3	10 females	Test article	Mid dose		
4	10 females	Test article	High dose		
Littering groups					
5	10 females	Control	Control	GD6-17	PND21
6	10 females	Test article	Mid dose		
7	10 females	Test article	High dose		

This paper describes the outcome of this study and includes probability calculations to indicate whether the findings seen in this study support the hypothesis that these skeletal abnormalities remodel after birth and hence are not permanent detrimental abnormalities and are potentially reclassifiable as minor rather than major malformations.

2. Materials and methods

2.1. Study design

Four groups of 10 mated female Wistar Hannover rats (caesarean groups) were dosed with a low, medium or high dose level of test article or with control formulation from GD6 to 17. This administration period covered the period from implantation to closure of the hard palate and the completion of major embryonic organogenesis. Scheduled maternal necropsy was performed on GD20 (Table 1).

A second cohort of animals (littering groups), consisted of three groups of 10 mated female Wistar Hannover rats that were dosed with the medium or high dose level of test article or with control formulation from GD6 to 17 (Table 1). These animals were allowed to litter and the pups were necropsied on PND21.

At scheduled necropsies for both cohorts, the fetuses or pups were weighed, sexed and examined for external anomalies. The pectoral girdle, hind and forelimbs of both fetuses and pups were assessed for skeletal abnormalities after staining with alizarin red S.

There are background fetal morphology data available for this substrain of rat in this laboratory.

In a previous embryofetal development study, 9.5% of control litters (2/21 litters) had at least one affected fetus with bent scapulae and/or thickened humerus. Based on that recent background information, calculations revealed a 63% probability of having one or more affected control litters if group sizes of 10 groups were used in this study. In addition, a group size of 10 animals would allow an approximately three-fold increase in bent scapulae and/or thickened humerus to be detected as statistically significantly different when compared against the probable control incidence.

The study was conducted in accordance with the relevant UK Animal Welfare Laws.

2.2. Animals

Female time-mated Wistar Hannover rats (HsdHan:WIST), aged approximately 11 weeks old, were delivered from Harlan UK on GD1 or 2 (GD0 = day that plugs were found in the mating cage). All animals were healthy when delivered and before they started dose administration.

Maternal animals were randomly allocated to consecutive study cages as they were taken from the delivery boxes. Body weights were checked to ensure that group mean weights for the test article dosed groups were within 5% of the mean weight for the relevant control group at the time of allocation. The caesarean groups were dosed 6 weeks before the littering groups to ensure that skeletal defects occurred at the dose levels tested.

2.3. Housing

The animals were acclimatised to the test facility for at least 4 days prior to the start of dosing on GD6. Maternal animals were housed individually or with their litter in tinted solid bottomed plastic cages (Model Type 2000P, Tecniplast, Italy). Females from each group were placed together on the same racks. Control animals were placed in a separate rack. Clean cages were provided at least every two weeks.

The animals were provided with Tapvei aspen wood chips, soft nesting material (sizzlenest) and polycarbonate tunnels (supplied by Datesand, UK). The littering rats had their tunnels removed and sizzlenest exchanged for paper shavings (Beta-shred F.D.A. supplied by Datesand, UK) prior to expected parturition.

Water from the site drinking water supply was provided in water bottles and pelleted diet (Harlan Teklad 2918C diet) was freely available. Tapvei chew sticks (supplied by Datesand, UK) were also given.

The animal room was maintained at values for temperature and relative humidity of 19–23 °C and 40–70%, respectively. The animal room was illuminated by artificial light from fluorescent tubes on an approximately 12 h light/dark cycle.

2.4. Necropsy procedures

Adult females were euthanased by halothane inhalation prior to scheduled necropsy. Scheduled necropsies for caesarean section were performed on GD20. Females and pups in the littering groups were necropsied on PND21.

The uterus and ovaries were examined and the number of corpora lutea and number and type of implantations were recorded for animals in the caesarean section groups. Individual fetal weights were recorded. Each fetus had a detailed examination for external malformations and variants, which included examination of the oral cavity, and was sexed externally. Fetuses were then killed by subcutaneous injection of sodium pentobarbital and eviscerated, processed and stained with alizarin red S [12].

For littered females euthanased on PND21, the uterus was examined and the number of implantation scars was recorded. Each pup had a detailed examination for external malformations and variants, which included examination of the oral cavity, and was sexed externally. Pups were then killed by administration of halothane. The pectoral girdle, hind and forelimbs were detached, processed and stained with alizarin red S. When pups were found dead, if not cannabalised, they were examined for external anomalies, processed and stained with alizarin red S [12].