Reproductive Toxicology 64 (2016) 105–115

Contents lists available at ScienceDirect

Reproductive Toxicology

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

"All pigs are equal" Does the background data from juvenile Göttingen minipigs support this?

Bianca Feyen[a,*], Laure Penard[b], Marjolein van Heerden[a], Pierluigi Fant[b], Edward Marsden[b], Sandra De Jonghe[a], Maria Desmidt[a], Sien Maki Mousa[a], Graham Paul Bailey[a]

[a] Preclinical development and safety, Janssen Research & Development, A Division of Janssen Pharmaceutica NV, Turnhoutseweg 30, B-2340 Beerse, Belgium
[b] Wil Research, a Charles River Company, 329 impasse du Domaine Rozier, 69210 Saint-Germain-Nuelles, France

ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 20 April 2016
Accepted 21 April 2016
Available online 22 April 2016

Keywords:
Minipig
Juvenile
Background data
Growth parameters
Clinical pathology
Histopathology

ABSTRACT

For pediatric indications requiring juvenile toxicity testing, the rat is the preferred species. However, for some drugs it might not be an appropriate model or regulatory agencies may also request a non-rodent species. Due to the relatively recent use of Göttingen minipigs, little background data are available. This shortage of historical data can raise concerns with respect to interpretation, thus potentially discouraging investigators. This article presents background data from 82 piglets collected at different ages. The data described show the normal variations and changes which are important in the interpretations of these studies. Age-related changes were observed for several cardiac and clinical pathology parameters and in the haematopoietic tissues. Therefore, all pigs were not considered equal. It can be concluded that these data can be used as guidance, to support the concurrent study control data but cannot completely replace them.

1. Introduction

The Göttingen minipig has become more popular as a non-rodent species in routine repeat dosing safety testing during the last few years. It is also a promising model for juvenile toxicity studies now that a pediatric assessment is required for all new drug applications in the USA [1] and Europe [2]. For pediatric indications requiring juvenile toxicity testing, the rat is still the preferred species because it is the most convenient and widely studied species [3]. However, for some drugs it might not be an appropriate model or regulatory agencies may request both rodent and non-rodent juvenile studies, for example, when a drug is being developed for a pediatric-only indication. The most common non-rodent species for juvenile studies is the dog, but this is likely to change as the advantages of using minipigs become more widely known and more background data become available. Juvenile studies in non-human primates are often also possible but such studies are only performed as a last resort due to numerous ethical and practical challenges.

The minipig represents good similarity to man in terms of many organ systems [4–7], including postnatal development [8–10]. Overall, it is closer to the stage of development at birth of a human neonate than that of a rodent. It also has many practical advantages over the dog. Pregnant females in the numbers required to perform such studies may be obtained relatively easily with adequate planning. The gestation period is significantly shorter and litter size is larger than in non-human primates and to a lesser degree the dog. Piglets can be readily cross fostered and temporary removal from the mother is well tolerated. Piglets are weaned earlier than puppies and they reach sexual maturity earlier than both dogs and non-human primates.

Due to the relatively recent use of minipigs in this field, only a few studies have been conducted within each age range and therefore very little background data from juvenile minipigs are currently available. This shortage of historical control data can clearly raise concerns with respect to interpretation of results, thus potentially discouraging investigators from using this animal model which, as described above, presents many advantages over the dog or monkey.

Abbreviations: ECG, electrocardiography; EDTA, ethylenediaminetetraacetic acid; EMH, extramedullary haematopoiesis; H.E., hematoxylin-eosin; HP-β-CD, hydroxy propyl-β-cylodextrin; MCV, mean cell volume; MCH, mean cell haemoglobin; PND, Post Natal Day.

* Corresponding author.
E-mail address: bfeyen@its.jnj.com (B. Feyen).

http://dx.doi.org/10.1016/j.reprotox.2016.04.019
0890-6238/

Table 1
Overview of study designs, number of piglets included and different parameters recorded at various timepoints.

Treatment	Study	Dose volume (ml/kg/day)	Treatment period (PND)	Number of animals		Data collection period (PND)	Cardiac evaluation[2]	Clinical Pathology[3]	Post mortem[4]
				Male	Female				
Untreated	A	NA	NA	3	5	5	/	Week 1	/
	A+C	NA	NA	11	11	1–36	Week 5	Week 5	Week 5
Water control	A	10	1–28	1	1	1–29	Week 4	Week 4	Week 5
	A	10	1–58	0	1	1–58	Week 4	Week 4+9	Week 9
	C	2.5	1–35	5	7	1–36	Week 5	Week 5	Week 5
	C	2.5	1–35	6	0	1–64	Week 8	Week 9	Week 9
Vehicle (HP-β-CD)	B	5/2.5[1]	1–21	0	1	1–22	/	Week 3	Week 3
	B	2.5	1–28	2	2	1–29	/	Week 4	Week 4
	B	3.75	1–28	0	2	1–29	/	Week 4	Week 4
	C	2.5	1–14	4	2	1–15	Week 2	Week 2	Week 2
	C	2.5	1–35	5	7	1–36	Week 5	Week 5	Week 5
	C	2.5	1–35	5	1	1–64	Week 8	Week 9	Week 9

A: historical control study; B: pilot study; C: main study; /: no evaluation performed.
[1] 5 ml/kg on PND1 and 2 and then 2.5 ml/kg from PND3 due to vehicle-related liquid feces.
[2] Week 4 data were not reported.
[3] Not all parameters measured were measured at each timepoint, week 3 data were not reported.
[4] Histology was not performed at each timepoint, week 4 organ weight data were not reported, week 3 macroscopic data were not reported.

The purpose of this article is to present data for routine parameters (body weight, physical development, clinical pathology, cardio-vascular function and post mortem observations including histopathology) evaluated in juvenile toxicity studies from newborn minipigs up to the age of 9 weeks to support interpretation of findings in neonatal and juvenile toxicology studies and to discuss the feasibility of performing these type of studies from Post Natal Day (PND) 1 onwards. In addition, the use of hydroxy propyl-β-cylodextrin (HP-β-CD) as a vehicle in juvenile minipigs was also evaluated.

2. Materials and methods

2.1. Test system

Multiparous pregnant female Göttingen minipigs were supplied by Ellegaard, Dalmose, Denmark. They arrived at the test facility approximately 3 weeks before littering. The litter size varied between 5 and 12 piglets. At the start of treatment, on PND 1, selected piglets weighed between 309 and 737 g. The background data presented in this article were generated from 82 piglets from 17 different litters. The piglets were either untreated or dosed via oral gavage with water or vehicle (aqueous solution containing 30% w/v HP-β-CD [Roquette], HCl to pH = 2 ± 0.1).

The studies were conducted in an AAALAC-approved laboratory and all animals were treated humanely and cared for in accordance with the European [11] and French [12,13] regulations.

2.2. Animal husbandry

Following arrival at the test facility, the pregnant females were observed daily during a 3-week acclimatization period. The animals were housed in an air-conditioned building (a minimum of 10 air changes per hour) under the following environmental conditions: room temperature 22 + 3 °C and relative humidity 35–70%. The rooms had a lighting cycle of 12 h light (artificial) and 12 h dark (except when forced lighting was required to complete technical procedures). The mothers (together with their litters) were housed singly in double pens of approximately 4.0 m^2 with sawdust as bedding. A heating lamp was provided for the piglets when necessary.

A pelleted complete commercial diet (SMP (E) SQC, Special Diet Services) was provided to the mothers. From PND 10, yogurt and diet were provided mixed or separated to the piglets up to and after weaning (PND 28), i.e. up to 7 weeks of age. The animals had ad libitum access to drinking water.

2.3. Pre-treatment procedures

Piglets were examined daily for physical condition from birth onwards, including the umbilical cord for the first 5 days after birth. All piglets received an intramuscular injection (1 ml, corresponding to 100 mg) of iron (Ferdelta B12, Coophavet or Fer dextran B12, Franvet), 24–48 h after birth.

In the main study, piglets were pre-selected for the study shortly after birth, based on weight (a minimum weight of approximately 300–350 g) and defined physical/functional development parameters (open eyes, stance and gait, and suckling behaviour). The objective was to standardize litter size to not more than 8 piglets (ideally 4 males and 4 females). In cases of surplus, cross fostering was performed if possible or piglets were retained at the disposal of the test facility for generating additional background data, including post mortem examination in the fetal morphology laboratory for the presence of any congenital malformations.

2.4. Study designs (Table 1)

The juvenile package contained both pilot and definitive studies with treatment commencing on Post Natal Day 1 (PND 1).

In the pilot study (study B), a single vehicle group was included and piglets were treated orally via gavage from PND 1 to PND 21 (1 female) or PND 28 (2 males and 4 females). In this study, one litter contained all dose groups, including the test article dosed piglets.

In the main study (study C), untreated, water and vehicle control groups were included and piglets were treated from PND 1 to PND 14 or PND 35. Some piglets (11 males and 1 female) were retained for a treatment-free period of 4 weeks after completion of the 5-week dosing period. All piglets in any given litter were allocated to the same treatment group with the exception of one containing piglets receiving water or the vehicle.

A historic control study (study A) was also performed to help develop and improve certain technical procedures for the juvenile minipigs (1 male and 2 females were treated orally from PND 1 to PND 28 or 58 with water, remaining piglets were not treated) and to provide some additional background data using spare piglets not