The postnatal development and growth of the cardio-respiratory system in Sprague-Dawley rats

Ancuta Apreutese¹, Cedric Gordon¹, Roy Forster³, Andrew Graham¹, Robert Tavcar¹, Bernard Palate³, Julius Haruna¹ and Marie-Odile Benoit-Biancamano²

TOXICOLOGY SERVICES
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- Infusion
- Dermal
- Reproductive toxicology including minipigs and NHPs
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- Ecotoxicology: wide range of test species

SAFETY PHARMACOLOGY
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- Respiratory: plethysmography / JET telemetry
- CNS / EEG
- Early safety pharmacology

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- Bioanalysis: LC-MS/MS, GC-MS/MS, LC-ICP/MS, ELISA, RIA
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- Immunology: 10-color flow cytometer, Lumines, Mesoscale

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CiToxLAB in France
+33 (0) 32 29 26 26
contact.france@citoxlab.com
B.P. 563 - 27005 Evreux Cedex, France

CiToxLAB in North America
+1 888 353 2240
contact.northamerica@citoxlab.com
445, Armand-Frappier Blvd, Laval, Quebec, H7V 4B3, Canada

CiToxLAB in Denmark
+45 56 86 15 00
contact.scanlab@citoxlab.com
Hestehavevej 36A, Elby, DK-4623 Lille Skensved, Denmark

CiToxLAB in Hungary
+36 88 543 500
contact.hungary@citoxlab.com
Vezprény, Szabadidápogúszta, H-3200, Hungary

Media Services Ltd
+81 3 3666 9915
citoxlab@mediaservices-jp.com
Fujif 16 Bldg 7F
1-11-2 Nihombashi Kabayacho,
Chuo-ku, Tokyo 103-0021, Japan

Croen Research Inc.
+82 31 888 9390
customerservice@croen.co.kr
Advanced Institutes of Convergence Technology - B-6th Fl, 864-1, lui-dong,
Yeongtong-gu, Suwon-si - Gyeonggi-do,
443-270, Korea

Stemina
+1 608 204 0104
info@stemina.com
www.stemina.com
504 South Rosa Road, Suite 150 Madison,
Wisconsin 53719, USA
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Ancuta Apreutese1,2, Cedric Gordon1, Roy Forster3, Andrew Graham1, Robert Tavcar1, Bernard Palate1, Julius Haruna1 and Marie-Odile Benoit-Biancamano2
1 CitoxLAB in North America, Laval, QC, Canada; 2 Faculté de Médecine Vétérinaire, Université de Montréal, STH-Hypotite, QC, Canada; 3 CitoxLAB in France, BP 563, 27005 Evreux cedex, France

INTRODUCTION
In recent years, juvenile toxicity studies have been an area of significant concern in preclinical development of drugs and have been target of academic interest as well. Added to other issues of testing strategies (e.g. route of administration, target organs, age of pups), monitoring of landmarks of postnatal histomorphological development in rodents is a critical component in study design and a fundamental element of such studies. In the work presented here, we have investigated the histomorphological changes of cardio-respiratory system in rat pups over the first month of life.

MATERIALS AND METHODS

The 51 pups in this study were the progeny of 6 time-mated Sprague-Dawley rats dams Crl:CD (SD) purchased from Charles River Laboratories Canada Inc. (ST-Constant, QC) at Day 0 of pregnancy. The tissues were obtained from pups at post-natal day (PnD) 1, PnD2, PnD4, PnD6, PnD8, PnD10, PnD14, PnD17, PnD21, PnD24, PnD26, PnD28 and PnD30. When possible, equal number of females and males were used for each occasion. The body weight and heart weight were recorded for each time point and the organ weight to body weight ratio was calculated; the results were statistically evaluated using a dispersion diagram and a polynomial regression. The thoracic aorta, heart, trachea and lungs were collected and placed in 10% buffered formalin. After dehydration, the tissues were treated and stored in 70% ethanol until histological processing. The fixed samples were trimmed, processed and paraffin-embedded. Sections were cut at 4μm thickness and stained with hematoxylin and eosin (H&E). The immunohistochemical demonstration of vimentin within lung samples was performed using the kit Dako Envision HRP(AEC) (a rabbit monoclonal antibody diluted at 1:4000) and used according to manufacturer instructions. In order to clarify the distinction between collagen and elastin fibers within the aortic wall, Masson’s trichrome stain was employed.

RESULTS AND DISCUSSION

During the first month of life, both body and heart weight increased with age, and most significantly after PnD21. The heart weight relative to body weight was higher at birth and progressively decreased until PnD30, suggesting that by the end of first month of life the heart weight increased at a less steep rate than body weight (Figure 1). As a broad generalization, at birth, most organs showed increased cellularity, the interstitium was edematous and contained loosely arranged mesenchymal cells. Qualitative changes in the aortic wall from PnD1 to PnD30 were indicative of the adaptive growth response of the thoracic aorta to the rapidly increasing size of the whole body. When compared with the perinatal period, the thoracic aorta showed an approximately 1.5 fold increase in luminal diameter after the first week of life and around 3-fold increase at the end of the first month. The wall appears hypercellular and elastic fibers are less prominent (Figure 2); subsequently the proportion between components changed in favor of elastic fibers addition (Figure 3). The rat heart is morphologically immature at birth and grows by cell hyperplasia over the perinatal period followed by cell hypertrophy around the third postnatal week. The neonatal cardiomyocytes are elliptical to elongate, scant and short, with a single, central nucleus. The intercalated discs are poorly developed, the cross striations are less prominent (Figure 4). An increase in the myocardial contractility function appears to be associated with enlarged cellular size by addition of new, orderly sarcomeres leading to the appearance of cross-striations, visible around PnD10. Through the first month of life, the tracheal lumen increased approximately 4-5 folds. At birth, the trachea was lined by immature columnar goblet-ciliated epithelium, the first subepithelial glands became visible around one week after birth. The newborn rat has no alveoli and breathes with diffuse pattern; while the lung, trachea and aorta were more discreetly remodeled by apoptosis and/or mitosis.

CONCLUSION
As in human, the cardio-respiratory system of Sprague-Dawley rats is immature at birth. The lungs and airways reached maturity 3 weeks after birth while cardiac myocyte hypertrophy was noticed until PnD30. Tissue remodeling by apoptosis and/or mitosis was minimal in the lung, trachea and aorta and marked within the heart, especially at PnD4. These results will serve as database of background age-related changes of neonatal and juvenile rats in preclinical toxicologic studies.