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Spontaneous lesions in endocrine glands of experimental Wistar rats and beagle dogs

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ABSTRACT

A retrospective analysis was undertaken at Zydus Research Centre to understand the incidences of spontaneous lesions in endocrine glands of Wistar rats and beagle dogs. The data from a total of 841 Wistar rats (418 males and 423 females) and 144 beagle dogs (72 males and 72 females) was used from placebo/vehicle treated control group of different non-clinical toxicity studies. The lesions in various endocrine glands were classified according to the species and age of the animals at termination of study. Among the endocrine glands, the highest numbers (types) of spontaneous lesions were observed in adrenal glands followed in descending order by pituitary, thyroid, endocrine pancreas and parathyroid glands in Wistar rats. In beagle dogs, highest numbers (types) of spontaneous lesions were seen in adrenals followed by thyroid, endocrine pancreas, pituitary and parathyroid gland. In adrenal glands of Wistar rats, the incidences of cortical cell vacuolation, hemorrhages and hemangiectasis/peliosis were increased with age. Incidence of peliosis at ~110 weeks of age was higher in female rats. Among the proliferative lesions in rats, higher incidences of cortical cell hyperplasia was observed followed by medullary hyperplasia, complex pheochromocytoma, cortical cell adenoma and cortical adenocarcinoma. In beagle dogs, the incidences of hemangiectasis and cortical cell vacuolation in adrenal glands were higher in 18–21 months aged dogs in both the sexes as compared to 10–12 months of age. In pituitary gland, the incidences of cystic changes were higher in older rats and dogs and the incidences were more in beagles as compared to rats. In thyroid glands, C-cell (parafollicular cells) hyperplasia/complex was observed more frequently in both the species. Few incidences of cystic changes were observed in parathyroid of 18–21 months aged beagle dogs. In endocrine pancreas, few incidences of islet-cell vacuolation, atrophy and hyperplasia were observed in both the species. The Islet cell hyperplasia was found to be more frequent in male rats at ~110 weeks of age.

1. Introduction

The endocrine system helps to regulate and maintain several systemic functions by synthesizing and releasing hormones directly into the bloodstream for chemical signaling of target cells. The major areas of control and integration include responses to stress and injury, growth and development, absorption of nutrients, energy metabolism, water and electrolyte balance, reproduction, and lactation. Thus, the variation in these physiological functions in response to direct or indirect stimulation including advancement of age largely reflects in the form of morphological or structural changes.

Knowledge of spontaneous histomorphological variations in endocrine organs of laboratory animals used in routine toxicity studies will assist in accurate and meaningful interpretation of findings in animal safety/toxicity studies. Keeping in view of the regulatory requirements for safety assessment of new drugs and various new chemical entities which are likely to affect the endocrine system, it is essential to compile the background histopathological data of key endocrine glands such as adrenals, pituitary, endocrine pancreas, thyroid and parathyroid glands, which are routinely evaluated in toxicity studies. The review of literature suggests that chemically induced lesions of the endocrine organs are most commonly encountered in the adrenal glands, followed in descending order by the thyroid, pancreas, pituitary, and parathyroid glands (Frith et al., 2000). Historical control data are especially useful in assessment of increased incidence of uncommon neoplasms, when increase in frequency is

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marginal compared to concurrent controls, and in evaluation of genetic drift within a particular strain (Haseman et al., 1989, 1984). As noted previously by Walsh and Poteracki (1994) spontaneous lesions have been extensively described and quantitated in commonly used Fischer 344 (F344) rats and in Sprague-Dawley rats but most reports in Wistar rats are restricted to specific organ systems.

The main objective of this article is to provide a data base of spontaneous lesions of endocrine glands in routinely employed pre-clinical laboratory animal species (Wistar rats & beagle dogs) at one place for easy and quick reference to toxicologists and toxicologic-pathologists. This will aid in the interpretation of animal toxicology studies and to determine their significance further in risk assessment. An attempt was made to compile the data in accordance to duration of toxicity studies and also to arrive at a meaningful conclusion about the frequency and pattern of occurrence of lesions as related to the age of the animals.

2. Materials and methods

This retrospective analysis was conducted using histopathological data from control (vehicle/placebo treated) animals of toxicity studies conducted between year 2005 and 2014 at Zydus Research Centre, Cadila Healthcare Ltd., Ahmedabad, Gujarat, India.

Age at termination was in the range of 10 weeks to 2.2 years for Wistar rats and 10 months to 1.9 years for beagle dogs. Percent incidence of lesions in rat and dog at different age was compiled and tabulated. The data from a total of 841 Wistar rats (418 males and 423 females) and 144 beagle dogs (72 each male and female) was used for analysis. The data was analyzed and interpreted both biologically and statistically. Chi-square test for trend followed by Fisher's exact test was applied by using Graph Pad Prism ver.6.0 software to analyze and compare the age relation in the incidence of spontaneous lesions.

Animals were kept under the species specific identical housing and husbandry conditions and were maintained and supplied by Animal Research Facility of Zydus Research Centre. Wistar rats were procured from Harlan laboratory, USA. After receipt of the animals, they were quarantined and housed in individually ventilated cages (IVC) made of polysulfonate with autoclaved corn cob as bedding material. Beagle dogs were procured from Beijing Marshall Biotechnology Co., Ltd., and housed individually in kennels. Individual kennel were provided with animal enrichment items and co-mingling was permitted daily for some hours. Both rats and dogs were kept in environmentally controlled rooms with 22 ± 3 °C temperature and 30–70% relative humidity with 12/12 h light/dark cycle. Purified water and standard laboratory animal diet was provided. Proximate analysis of feed nutrient content and microbial contaminant was checked batch wise or periodically. Quality of water was checked periodically for ensuring acceptable limits of total dissolved solute and microbial contamination as per Standard Operating Procedures of Zydus Research Centre.

Necropsy of all the animals was performed and was subjected for gross examination. The tissues of interest were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm thickness, stained with hematoxylin and eosin and subjected for microscopic evaluation. All the studies were conducted in AAALAC (Association for Accreditation and Assessment of Laboratory Animal Care) accredited facility in compliance with national and international guidelines for animal welfare and all the procedures used in these studies were reviewed and approved by the Institutional Animal Ethics Committee.

Table 1
Incidences of spontaneous lesions in Wistar rats (%).

Organ/finding	Male			Female		
	10–12 weeks (n = 191)	32–34 weeks (n = 119)	~110 weeks (n = 108)	10–12 weeks (n = 197)	32–34 weeks (n = 118)	~110 weeks (n = 108)
Adrenal Glands						
Cortical cell vacuolation	1	2.5[a]	17.6[b,c]	0.5	3.4	13.9[b,c]
Capsular thickening	0	0	4.6[b,c]	0	0	1.9
Cystic changes	0	0	0.9	0	0	0
Accessory cortical tissue	2.1	2.5	3.7	2	2.5	4.6
Hemangiectasis/ Peliosis	0	0	2.8	0	3.4[a]	11.1[b,c]
Congestion	1	3.4	1.9	1	0.8	12.0[b,c]
Hemorrhage	0	2.5	0.93	0	1.7	5.6[b]
Thrombus- venous/ arterial	0	0	0	0	0	1.9
Hemosiderosis	0	0	0.9	0	0	0
Extramedullary hematopoiesis	0	0	0.9	0	0	0.9
Cortical cell hypertrophy, *zona fasciculata*	0	0	3.7[b,c]	0	0	3.7[b,c]
Cortical cell hyperplasia	0	0	5.6[b,c]	0	0	5.6[b,c]
Medullary hyperplasia	0	0	3.7[b,c]	0	0	2.8[b]
Pheochromocytoma	0	0	1.9	0	0	0
Complex pheochromocytoma	0	0	0.9	0	0	1.9
Cortical adenoma	0	0	0.9	0	0	0.9
Cortical adenocarcinoma	0	0	0	0	0	0.9
Parathyroid						
Parathyroid hyperplasia	0	0	3.7[b,c]	0	0	5.6[b,c]
Chief cell hypertrophy	0	0	0	0	0	0.9
Endocrine Pancreas						
Islet cell hyperplasia	0	1.7	13.0[b,c]	0	1.7	0.9
Atrophy of Islets	0	0	3.7[b,c]	0	0	1.9
Islet cell carcinoma	0	0	0	0	0	0.9
Islet cell vacuolation	0	1.7	0	0	0.8	0

[a] Significant at $p < 0.05$ between 10–12 weeks and 32–34 weeks.
[b] Significant at $p < 0.05$ between 10–12 weeks and ~110 weeks.
[c] Significant at $p < 0.05$ between 32–34 weeks and ~110 weeks.