Food and Chemical Toxicology 121 (2018) 467–471

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Soy-deficient diet induces renal lesions in juvenile rats

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ARTICLE INFO

Keywords:
Juvenile
Isoflavone
Preclinical Toxicity
Diet
Mineralization
Basophilic tubules

ABSTRACT

Certified LabDiet® 5K96 Advanced Protocol™ Verified Casein Diet 10 IF (5K96) is a commercial diet low in soy isoflavones developed for use in developmental and reproductive toxicity (DART) studies, especially those designed to detect endocrine disruptors. The objective of this study was to determine the incidences and severities of 5K96-associated renal lesions in control F_0 and F_1 cohorts of rats fed the 5K96 diet. Kidneys from control animals of four DART studies involving Sprague-Dawley rats fed the 5K96 diet, were evaluated microscopically. Mineralization and basophilic tubules were present in high incidence/severity in males and females compared to historical controls fed conventional diets. F_1 cohorts were affected to a far greater degree than F_0 cohorts, and females were affected more than males. Consideration of target tissue and mode of action should be given before automatically incorporating the 5K96 diet into DART study designs, and caution should be exercised when identifying and interpreting renal toxicity in the F_1 cohorts of such studies.

1. Introduction

Diet has been known to both exacerbate and mitigate spontaneous kidney disease of adult rats. Increased incidence and severity of chronic progressive nephropathy (CPN), a background tubulo-interstitial lesion primarily in adult male rats, has been attributed to high protein and/or caloric content of certain diets (Hard et al., 2013; Keenan et al., 2000). On the other hand, decreased severity of CPN has been associated with diets containing soy as its protein source, since soy contains isoflavones with estrogenic-like activity (Rao, 2002). Since males are primarily affected with CPN and soy mitigates CPN, it is apparent female hormones protect against certain tubulo-interstitial diseases of the kidney. In order to minimize the occurrence of CPN in adult rats on preclinical safety studies, most conventional rodent diets (e.g., LabDiet® 5001) are soy-based with controlled protein and caloric content.

Mineralization is another background lesion in rats that can be exacerbated or mitigated by diet. Diets with a calcium: phosphorus (Ca:P) molar ratio < 1.00 have been associated with mineralization, especially in adult female rats (Reeves et al., 1993). Unlike the situation with CPN where female hormones mitigate disease, female hormones *promote* mineralization (Rao, 2002; Reeves et al., 1993). In order to minimize the occurrence of mineralization in adult female rats on preclinical safety studies, commercial rodent diets with a Ca:P molar ratio of at least 1.3 are commonly used (Reeves, 1997; Reeves et al., 1993).

Even though most commercial diets have been successfully adjusted to control protein and caloric intake (to help minimize CPN), and to optimize Ca:P ratio (to help minimize mineralization) in the adult rat, these adjustments may not sufficiently protect the developing juvenile kidney from renal lesions. In a series of extended one-generation reproductive toxicity (EOGRT) studies at Charles River, we recognized that a soy-free commercial diet was associated with exceptionally high incidences and severities of basophilic tubules and mineralization in the control F_1 cohorts, which were fed 5K96 from the time of weaning, as compared to the F_0 adult cohorts. Regardless of cohort, females were affected to a greater degree than males for both types of lesions. These observations prompted a more complete retrospective analysis of rat kidneys from four DART studies, each of which employed the same soy-free diet. The purposes of this analysis was to: i) determine the incidence and severity of renal lesions in various cohorts of rats on DART studies employing soy-free diets; ii) provide historical control data for future studies utilizing this diet; and iii) highlight how 5K96-induced renal lesions may complicate interpretation of test article-related renal toxicity.

Abbreviations: BUN, Blood Urea Nitrogen; CPN, Chronic Progressive Nephropathy; Cr, Creatinine; DART, Developmental And Reproductive Toxicity; EOGRT, Extended One-Generation Reproductive Toxicity; H&E, Hematoxylin and Eosin; GLP, Good Laboratory Practices; OECD, Office of Economic Cooperation and Development; PND, postnatal day; U.S. FDA, United States Food and Drug Administration

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https://doi.org/10.1016/j.fct.2018.09.044
Received 16 August 2018; Received in revised form 18 September 2018; Accepted 20 September 2018
Available online 21 September 2018
0278-6915/

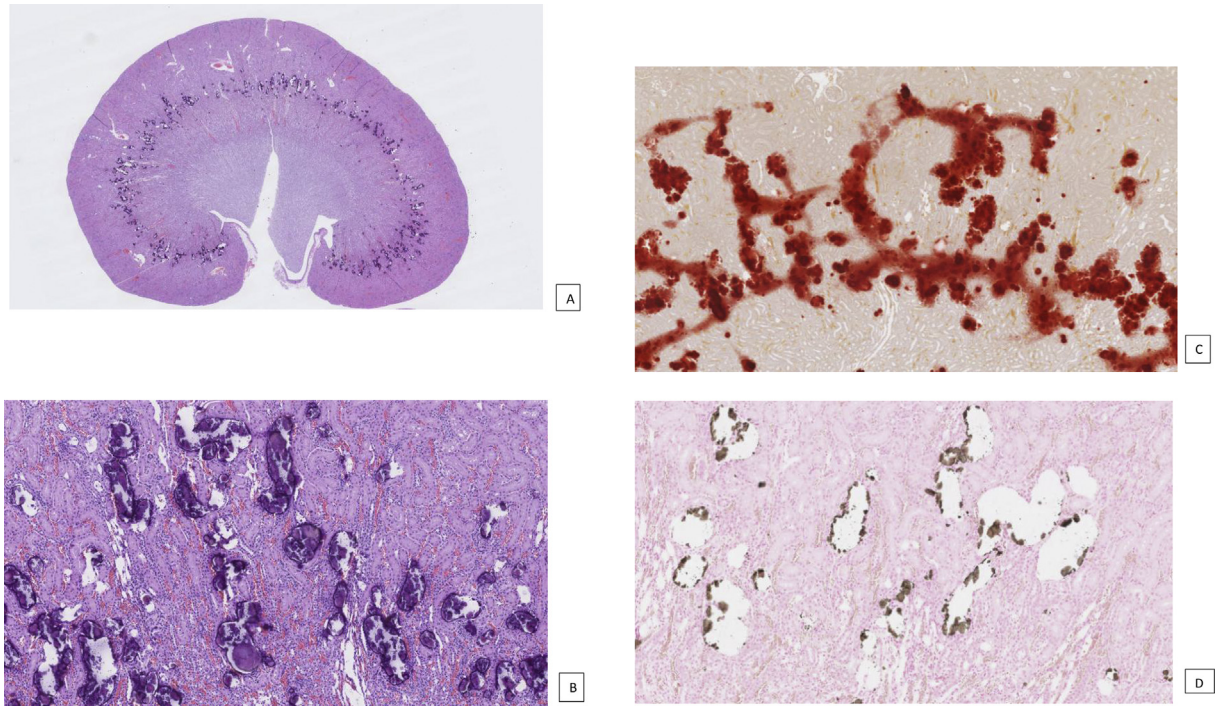

Fig. 1. 5K96-Induced Renal Mineralization. This series of images depict renal mineralization (moderate) at the cortico-medullary junction in an F_1 control female fed 5K96 diet. (A) There was a dark blue band of mineral between the cortex and the outer stripe of the medulla. 1.0 x, H&E. (B) There was no disruption of the adjacent parenchyma. 8.0 x, H&E. (C) This mineral was strongly positive for calcium. 6.0 x, Alizarin Red. (D) The mineral was moderately positive for phosphate. 9.5 x, von Kossa. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

Four DART studies, each involving Sprague-Dawley rats [(Cr1:CD (SD) rats from Charles River Laboratories)] fed the soy free diet Certified Rodent LabDiet® 5K96 Advanced Protocol™ Verified Casein Diet 10 1F (5K96; PMI Nutrition, St. Louis, MO) were reviewed. Each study was designed in compliance with Office of Economic Cooperation and Development (OECD) Guidelines 443: Extended One-Generation Reproductive Toxicity (EOGRT) studies (Office of Economic Cooperation and Development (OECD), 1998). F_0 cohorts (adult males and females) were fed the 5K96 diet *ad libitum* during acclimation, mating, pregnancy, and lactation (for a minimum of 10 weeks), and F_1 cohorts (i.e., rat pups) were fed this same 5K96 diet for approximately 10 weeks, from weaning (PND 21–22) until scheduled necropsy (approximately PND 90 or 12 weeks of age).

Each control and test article-treated group on these studies had 25 animals of each sex. The route of administration of test article varied between studies. Test article was administered by whole body inhalation (Study 1), by oral dietary administration (Study 2), or by oral gavage (Studies 3 and 4). For each of the test articles in Studies 1 through 4, the kidneys or any portion of the urinary system were not considered a potential target tissue. In the interest of confidentiality, test article identity cannot be revealed in this report, but since this report is limited to the evaluation of control animals, test article identity is not relevant.

Complete hematology, serum chemistry and urinalysis parameters were obtained prior to necropsy following overnight fasting, and kidney weights (along with body weights and multiple organ weights) were obtained at necropsy.

Five μm sections of both kidneys from control and test article-treated F_0 and F_1 males and females were fixed in 10% formalin, processed routinely, and stained with hematoxylin and eosin (H&E). Selected tissues were stained with von Kossa and/or alizarin red to help characterize the nature of the mineral deposits. The slides were evaluated microscopically by board-certified veterinary pathologists, and the severities of findings were based on a 5 - point grading scale (1: minimal, 2: mild, 3: moderate, 4: marked, 5: severe). The average (mean) incidence and average severity of each finding in each study was determined.

Data from the control groups from each of the four DART studies were compared to Charles River-Ashland historical control data set for Crl:CD(SD) rats (updated August–September 2015). This historical control data set is based on studies conducted at Charles River-Ashland in Sprague Dawley rats fed commercially available conventional rodent diet, most commonly but not exclusively, Certified Rodent LabDiet® 5001 (Purina Mills International Nutrition, St. Louis, MO).

The studies were conducted at Charles River - Ashland in compliance with OECD Principles of Good Laboratory Practice (GLP) (Office of Economic Cooperation and Development (OECD), 1998), and U.S. Code of Federal Regulations (21 CFR Part 58; 50 CFR Parts 160 and 792). The use of animals was carried out in accordance with *Guide for the Care and Use of Laboratory Animals* (National Research Council (NRC), 2011).

3. Results

In the control F_0 and F_1 females and in F_1 males, 5K96 diet was associated with increased incidence and/or severity of renal mineralization and basophilic tubules, when compared to age- and sex-matched historical controls (Figs. 1 and 2; Table 1). Females were affected to a greater extent than males for both lesions, and F_1 cohorts were affected to a greater extent than F_0 cohorts.

Mineralization was characterized by deposition of mineral at the corticomedullary junction, along the outer most portion of the outer stripe of the medulla (Fig. 1). Small deposits of mineralization were occasionally present in the inner stripe of the medulla. At all severities present (minimal to marked), the mineral did not cause degenerative changes in adjacent parenchyma. Mineral stained strongly positive with alizarin red (which stains calcium) (Puchtler et al., 1969) and moderately strongly positive with von Kossa (which is nonspecific for calcium