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Artículo de investigación

Thyroid toxicity of oral overdose of potassium iodide in female new zealand rabbits (*oryctolagus cuniculus*)

Signos clínicos y efectos tóxicos de la sobredosis oral del yoduro de potasio en conejos (Oryctolagus cuniculus) hembras Nueva Zelanda

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ABSTRACT

This work contains the initial outcomes from studies conducted to analyze thyroid toxicity associated with potassium iodide overdose. This project is based in the histopathological finding of Hürthle cells in a thyroid gland obtained from a rabbit housed in an animal facility. The goal of this study is to report thyroid toxicity associated with oral administration of iodide through clinical signs, histopathology and ultrasound scan. The iodide was used as potassium iodide (KI) iodide in female New Zealand rabbits (Oryctolagus Cuniculus) at a concentration of 200 ppm (200mg/L) in drinking water administered over a 4-week period. Rabbits treated with KI showed distress, slight lymphopenia and neutropenia. Ultrasound valuation of the thyroid gland revealed multifocal cystic space. Histopathology evaluation showed follicular cells with vacuolar degeneration and parafollicular depletion. Preliminary results of the present study indicate that potassium iodide may disrupt thyroid function by direct cytotoxicity to follicular epithelial cells. Although we have to increase animal numbers, include male rabbits and optimize potassium iodide dose, the toxicological model presented in this work represents an opportunity to study toxicological disorders affecting the thyroid gland.

Key words*:* Potassium iodide, cytotoxicity, thyroid, rabbits.

RESUMEN

Este trabajo contiene los primeros resultados iniciales sobre la toxicidad asociada con la sobredosis de yoduro de potasio. Este proyecto se basó en el hallazgo histopatológico de células de Hürthle en una glándula tiroides obtenida de un conejo proveniente de bioterio. El objetivo del estudio es reportar la toxicidad tiroidea asociada con la administración oral de yoduro a través de signos clínicos, histopatología y ultrasonido. El yoduro fue utilizado como Yoduro de Potasio (KI) en conejos *(Oryctolagus cuniculus)* hembras Nueva Zelanda a una concentración de 200 ppm en agua potable administrada durante un período de 4 semanas. Los conejos tratados con KI mostraron estrés, ligera linfopenia y neutropenia. La valoración ecográfica de la glándula tiroides reveló espacios quísticos multifocales. La evaluación histopatológica mostró células foliculares con degeneración vacuolar y depleción de células parafoliculares. Los resultados preliminares indican que el conejo Nueva Zelanda es un modelo experimental factible para estudiar los efectos toxicológicos de altas dosis de KI en el agua potable. Aunque es necesario implementar algunos cambios en estudios futuros, tales como aumentar el número de animales utilizados, optimizar la administración de KI y proporcionar un perfil hormonal, el modelo de conejo presentado en este trabajo representa una atractiva oportunidad para evaluar los trastornos toxicológicos que afectan a la glándula tiroides.

Palabras clave: Yoduro de Potasio, citotoxicidad, tiroides, conejos.

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INTRODUCTION

Microscopic observations of thyroid tissues from a normal adult New Zealand rabbit, housed in the Universidad Centroccidental "Lisandro Alvarado" (Cabudare-Venezuela) Animal Facility, revealed Hürthle cells (oncocytic). These cells are also observed in diverse thyroid pathological conditions in humans [1], including autoimmune thyroiditis and multinodular goiter and in the thyroids of patients receiving radiotherapy for head and neck, as well as systemic chemotherapy [2]. One of the most important environmental factors that triggers thyroid dysfunction in both humans and animal models is excessive iodide intake [3]. The present study examines the toxicological effects of iodide, when used as potassium iodide (KI), on the thyroid of rabbits as an animal model. The goal of the study is to report clinical signs and toxic effects of oral KI in New Zealand rabbits. The Hürthle cells (HC) are follicular-derived epithelial cells. These large polygonal cells are characterized by the presence of abundant cytoplasmic acidophilic granules (mitochondria), and a large hyperchromatic round to oval nucleus with a prominent nucleolus [1]. KI is an inorganic compound commonly used as an additive used for "iodizing" table salt as a nutritional supplement for populations that are at risk for iodine deficiency [4]. Toxicological studies in rabbits indicate they can tolerate levels up to 500 mg/kg. However oral administration during late gestation at levels of 250 mg/kg can cause higher offspring mortality [5].

MATERIAL AND METHODS

KI- induced toxicity: KI toxicity was induced following the protocol of Soliman et al (2001) [6] with some modifications. Briefly, Potassium Iodide (KI) (Sigma-Aldrich catalog Nº 221945) was administrated in the drinking water at a concentration of 200 ppm (200mg/L) over a period of 4 weeks. KI-supplemented water was tasted by the principal investigator (PI) to evaluate palatability and potential rejection responses. Daily food and water consumption, as well as body weight, were also monitored.

Experimental design, animal housing arrangements, and management

This project was conducted following the principles of the 3Rs: Replacement, Reduction and Refinement, which are the guiding principles for the ethical use of animals in toxicological studies. They were established by the Animal Welfare Act (AWA), United States (2000) [7]. This policy was implemented in an effort to reduce the number of animals used for toxicological studies in vivo. Five adult New Zealand rabbits (5 female), age 3 months, identified as R1 to R5, weighing 2.167.5 ± 442.73 g, were enrolled in a pilot study to develop KI toxicity. Five rabbits consisted of 4 females that received KI in their drinking water at a concentration of 200 ppm over a period of 4 weeks. The untreated control animal (1 female) was used as a prognosis value of clinical and histopathological evaluation parameters.

Animals were obtained from the Universidad de Carabobo Animal Resources Facility. All procedures were performed in accordance with the published guidelines of the Institutional Animal Care and Use Committee (IACUC) regarding the use of rabbits for research. Furthermore, procedures and protocols were reviewed and approved by the Medicine School Bioethics Committee of the Universidad Centroccidental "Lisandro Alvarado" under control code CBDCS-01-2015. Rabbits were housed in an animal facility at the Veterinary School, in a controlled environment maintained at a temperature of 25-26 °C, with 50-55 % relative humidity. Rabbits were exposed to a 12:12 light/dark cycle. Animals were housed in previously sterilized stainless steel cages. General health was monitored over the course of 24 days, with body weight and animal behavior observed and recorded on a daily basis. To evaluate the general health condition of the rabbits, samples of peripheral blood and feces were taken. To perform a manual differential white cell count, blood samples were collected by puncturing the saphenous vein using a 23-gauge needle. The thin blood smears were air-dried and stained with Hemacolor® rapid staining kit (Merck Millipore catalog number 111661). This procedure was done twice; first at day zero (0) before treatment began, and then on the last day of treatment (day 32). Both processes were conducted the same day, respectively, as the pre-treatment and post-treatment thyroid ultrasound study. Animals were properly sedated (see above for procedures).

Animal's sensory stimulation, monitoring animal physiological condition, and staff training in rabbit handling

Before starting the experiments, the staff responsible for routine management of the animals was trained by the PI in handling, restraining, monitoring and sampling rabbits. Animals were housed using the principle of environmental enrichment; the animals were removed from their cages so that they could receive gentle touching/petting on the ears, dorsal and abdominal area. After that, rabbits were rewarded with small pieces of clean, fresh vegetables (carrots, cauliflower, kale, and broccoli) in order to establish a positive interaction with humans that facilitated cooperative behavior during sampling and handling. When cleaning and changing cages, the animals were taken to a fenced playground space (2x2 m^2) where they

expressed their natural behavior of jumping, running, scratching and grooming. Potable tap water was administrated ab libitum and it was changed daily. Diet consisted of 100 g of balanced commercial food containing 0.02 PPM of iodide per animal, hay (also working as a bed), and fresh vegetables (carrots, celery, chard). In each cage, 1/3 of the floor consisted of a solid resting area made of wood.

Clinical experimental endpoint

The clinical end point was defined as the time of prolonged inappetence, marked loss of body condition (≥20% body weight lost), evidence of severe infection, rough hair coat, and postural changes, together with lethargy or reluctance to move.

Thyroid gland ultrasound

A thyroid gland ultrasound study was performed in order to detect changes in volume, measurements of thyroid gland dimensions in two axes, and morphological grayscale imaging (shape, echogenicity, borderline, cystic spaces, nodularity and septations). This study was carried out in the Imaging Service of the Veterinary Hospital "Humberto Ramirez Daza" Universidad Centroccidental Lisandro Alvarado, at the Veterinary School. Ultrasounds were performed using a MINDRAY team vet DC-3 model 2011 with multifrequency transducer microconcex C2 model 6 (5-6 MHz). For thyroid ultrasound scanning, rabbits were sedated using 25 mg/kg of ketamine hydrochloride (Ketaset® Fort Dodge) IM. The neck area just below the gland was shaved using clippers (Wahl®), then 70% isopropyl alcohol was applied to the area using a gauze strip. A coupling gel for ultrasound was applied.

Histopathological evaluation

Necropsy was performed in the Veterinary School Microscopic Anatomy Laboratory at Universidad Centroccidental “Lisandro Alvarado”. Prior to euthanasia, rabbits were pre-medicated with Fentanyl at 0.03 mg/kg (Innovar-Vet®); sedation status was evaluated after 5 minutes by observing the animal reaction to a gentle ear punch. Once the animal had reached proper sedation, euthanasia was performed via an overdose of pentobarbital 100 mg/kg administered by IV into the forearm cephalic vein. Once death was verified by auscultation, terminal body weight was measured, and 1 ml of heparin was injected into the heart, followed by a blood perfusion performed manually using a 50 ml syringe and a 15 gauge 1 inch blunt end needle filled with a neutral pH phosphate buffer saline solution and then 10% neutral buffered formalin. Gross anatomy characteristics and incidence of lesions in organs were recorded using photography (Sony HDR-11). The tissue samples were fixed by immersion in 10% neutral buffered formalin for 72 hours. To facilitate histopathological studies, routine staining was accomplished using hematoxylin and eosin (H&E), 1 cm^2 samples were collected and processed by using the paraffin technique at the Universidad Centroccidental “Lisandro Alvarado” Veterinary School Histopathology Laboratory. Using a rotary microtome, tissue transverse sections were cut at a thickness of 4µm awere then stained with H&E and observed microscopically. The histopathological parameters evaluated include inflammatory cell infiltration, epithelial cell degeneration, atrophy, hyperplasia, necrosis, and fibrosis. Two semi-quantitative score systems were designed to grade clinical signs and histopathological tissue injuries. For clinical signs scoring, a semi-quantitative clinical score of severity was established, where: 0 = none (0%), 1 = minimal (5%), 2 = moderate (25%) and 3 = strong (≥50 %). In this system, percentage (%) is applied to the histopathological variables as follows: For histopathological injuries, grading in parenchymal target organ tissue lesions were identified, recorded in a contingency table, and scored using a histopathology grading system from 0 to 4.

RESULTS

The original plan was to develop a sub-chronic condition by administrating KI in a 90-day study. However, rabbits showed severe clinical signs of distress at week 4; the administration of KI was suspended, and clinical interventions were implemented as described in the next paragraphs. After KI treatment was interrupted, the animals were observed over a 5-day period to monitor the rabbit’s recovery.

General health condition and daily body weight during pre-treatment and treatment periods

Oral test of KI treated water palatability by the PI indicates this formulation has a normal taste and should not be rejected by rabbits.

Assessment of clinical and behavioral signs.

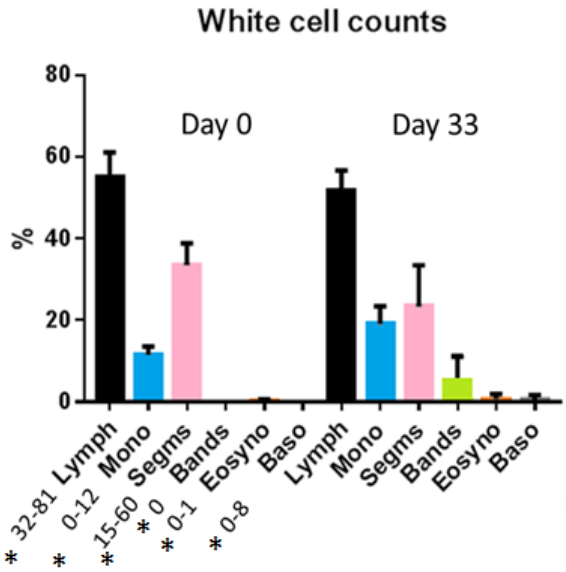
The condition of animals was recorded daily using the following criteria: Physical appearance, body weight, posture, water and food consumption, response to stimuli and position of ears. Differential cell counts in blood smears were determined by counting 100 nucleated cells on Hemacolor® stained blood smear. No numeric or morphological abnormalities were observed in blood samples at day zero (0) before treatment. However, at the clinical end point (day 28) after first treatment, rabbits receiving KI at a concentration of 200 ppm experienced an increase in the percentage of monocytes and a left shift, characterized by the presence of immature stages of neutrophils (bands) (Figure 1). A slight reduction in circulating lymphocytes and a mild neutropenia were also noticed. The cumulative score was used to determine clinical experimental endpoint, and the severity of clinical

symptoms as an indicator of the degree of distress. Female rabbits 1 to 4 identified as R1-R4, which received KI at a concentration of 200 ppm in drinking water over a 4-week period reached higher scores (9-14), while the control, untreated female rabbit identified as R5 presented lower scores. During the 4th week (day 26) treated animals displayed a hunched posture and down-turned ears.

Water consumption and estimation of the mean daily oral intake of Iodide in drinking wáter

The average water consumption and Iodide intake is summarized in Table I.

Figure 1. Differential white cell count on hemacolor® stained blood smears rabbits enrolled on a pilot study of oral toxicity of potassium iodide at 200 ppm in rabbits.*



* Data is expressed as mean±SD. During day zero leucocytes mean from all the six rabbits enrolled in this study were as follows: lymphocytes (lymph) 55±6.01; monocytes (mono) 11.5±2.07; segment neutrophil (segms) 33.5±5.32; bands 0; eosinophils (eosyno) 0.16±0.41 and basophils (baso) 0. During the clinical endpoint (day 28) the values were: lymp 51.67±5; mono 19±4.43; segms 23.33±10; bands 5.17±5.85; eosyno 0.66±1.21; baso 0.5±1.23. When these values were compared with reference values (**), an increase in monocytes and bands was noticed, together with a reduction on mature neutrophils. **References values: *Labor fürKlinischeDiagnostik*GMBH & CO. KG [11]

ID	24 days Pretreatment (ml/rabbit/day) minus KI	28 day Treatment (ml/rabbit/day) plus KI*	KI intake during 28 days treatment mg of drinking water
R1	191.10±38.48	201.50±43.22	8.06 mg/day
R2	195.70±39.72	267.40±74.29	10.70 mg/day
R3	251.30±44.15	249.50±64.12	9.98 mg/day
R4	263.20±36.04	218.00±41.77	8.72 mg/day
R5	302.90±92.97	290.50±45.18	0.00 mg/day

*Values for water intake means ± SD for individual rabbit over a period of 24 days pretreatment and 28 days of treatment respectively. Absolute Iodide intake as Potassium Iodide (KI) is indicated as mg/ml

Table I. Water consumption and Iodide intake of rabbits during both 24 days pre-treatment and 28 days treatment periods.*

Morphological and ultrasound study of thyroid gland

Macroscopically thyroid gland is composed of two lobes joined by a thin isthmus of connective tissue containing glandular tissue. Microscopically, the thyroid gland is surrounded by a loose connective tissue capsule, which emits thin septa or trabeculae dividing each of the two lobes into smaller parenchymal segments of lobules. Each lobule is composed of two populations of parenchymal secretory cells, epithelial follicular cells (FC) forming a single cubic epithelium assembling rounded structures or follicles filled with storage material or colloid (Figure 2A), and the parafollicular cells (PC), which are ovoid cells usually forming a cluster of cells localized within the interstitial connective tissues surrounding each follicle (Figure 2A). In female rabbits treated during 4 weeks with KI at 200 ppm in drinking water, thyroid samples were scored according to the injury severity between 1 and 2, FC showed a diverse degree of vacuolar degeneration (hydropic changes). FC with hydropic changes presented a pale cytoplasm, nuclei turned ovoid or flattened and were localized close to the basal membrane; some of them showed karyorrhesis and karyolisis (Figure 2 panels C and D). No inflammatory infiltration was detected in thyroid from KI treated rabbits. Ultrasound evaluation was performed before treatment (day zero) and during the last day of treatment (day 28, week 4). Representative ultrasound images are shown in Figures 3 and 4. During the designed day zero (0) before treatment, all the rabbit thyroid glands presented an echotexture homogenous hypoechoic with fine echotexture and smooth surface. At day 28 of treatment, ultrasound study revealed enlargement of the thyroid gland lobules, together with a higher echogenicity Cystic spaces were detected in treated female rabbits but in the untreated rabbit (Figure 4).

Figure 2:Photomicrographs of sections from thyroid rabbits no exposed (panels A and B) and exposed to ki at a concentration 200 ppm in drinking water for 4 weeks (panels C and D). Thyroid follicle lumens are indicated by asterisks. a) Black arrow indicates folicullar cells (FC), parafolicullar cells (PC) are indicated using a blue arrow. b) Hürthle-like cells, notice that some of these cells present a heterochromatic nuclei (straight black head arrow), while other possess euchromatic ones (white head arrow). c) Tissue sample from treated female rabbit, notice the vacuolar aspect of FC (black arrows), dashed arrow shows a fc presenting a pyknotic nucleus, red arrows indicates vacuolated epithelial cell within follicle cavity, blue head arrows indicates a sample preparation artifact. d) at higher magnification (100x) can be detailed the vacuolar aspect of FC, as well as the nuclei abnormalities., h&e paraffin sections, panels A, B, c ocular magnification 40x, bar scale=50µm

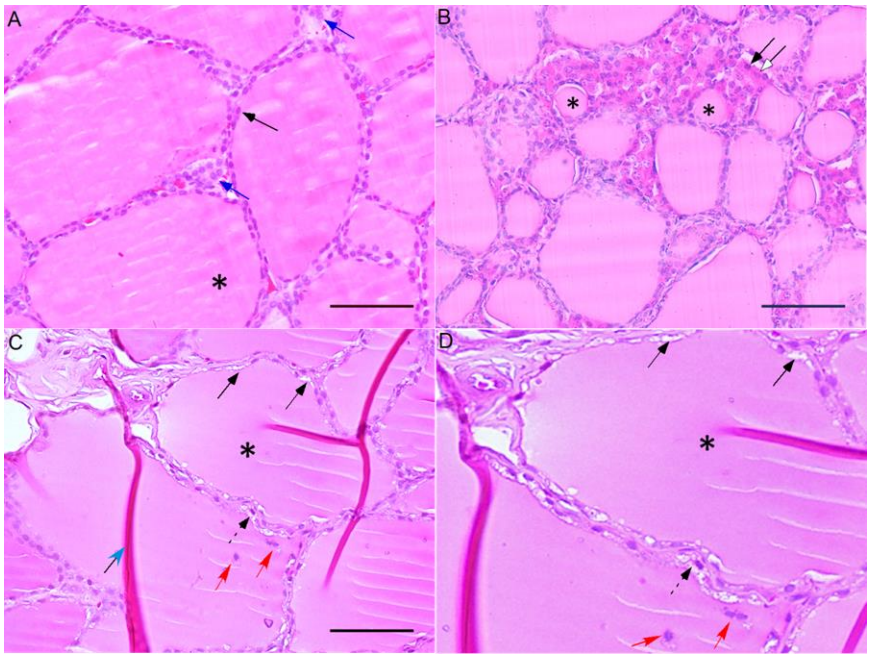


Figure 3. Panels A and B show ultrasound images of a rabbit's thyroid left lobule before and after 4 weeks of oral KI .a) Longitudinal grayscale thyroid gland left lobule sonography of a female rabbit (id=4) before treatment with KI. Left lobule shows an oval shape, homogenous hypoechoic with fine echotexture and smooth surface, measuring 1.17 cm in length and 0.68 cm in width. b) Longitudinal grayscale thyroid gland left lobule sonography of a female rabbit (id=4) 4 week after initial treatment with KI, reveals enlarged thyroid left lobule measuring 1.46 cm in length and 1.02 cm width, and increased echogenicity was noticed, two (2) small circular anechoic structures compatible with thyroid cysts were observed (arrows) together with sub-capsular micro-cystic spaces are also noted (dashed arrow). li=left lobule

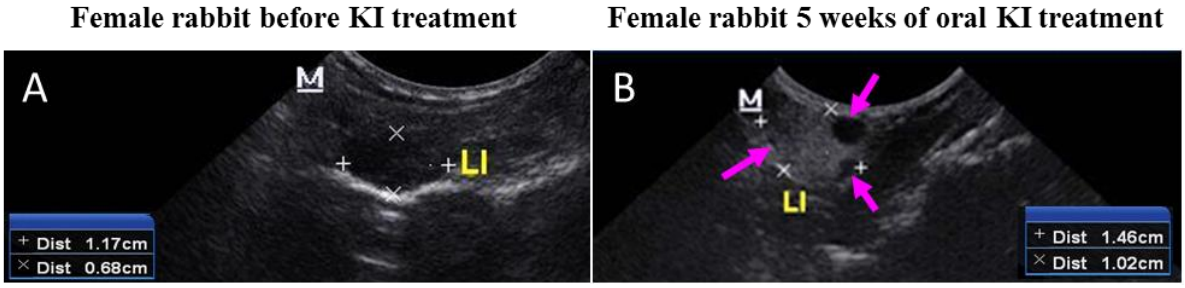
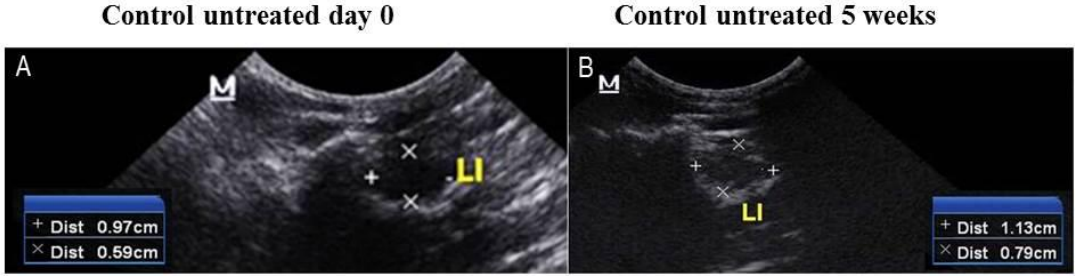


Figure 4. Panels A and B ultrasound images of control (untreated, R1) rabbit's thyroid left lobule at day 0 and after 4 weeks period. Longitudinal grayscale thyroid gland left lobule sonography of a female control rabbit (R5). At day 0, left lobule shows a fusiform shape, homogenous hypoechoic with fine echotexture and smooth surface, measuring 0.97cm in length and 0.59 cm in width. b) Longitudinal grayscale thyroid gland left lobule sonography of a female control rabbit 5 week later. left lobule possess a fusiform shape, is homogenous hypoechoic with slight echogenic decrease and smooth surface, measures 1.13 cm in length and 0.79 cm in width. li=left lobule



DISCUSSION

The present study addresses clinical manifestations and histopathological changes in target tissues caused by an excess of potassium iodide, and ultrasound changes in the thyroid glands of New Zealand rabbits. The toxicity was established by administration of potassium iodide (KI) at a concentration of 200 ppm in drinking water over a 4-week period. Iodide is an essential micronutrient for the synthesis and metabolism of thyroid hormones T3 and T4; however, when ingested in excess, thyroid dysfunction may result [8]. Once ingested, iodide is totally absorbed in the digestive tract and is almost exclusively acquired by the thyroid, and is incorporated into thyroglobulin; however, it may also be found to a lesser extent in the stomach, mammary glands and placenta [9]. In farm animals, food consumption containing "safe" levels of iodide for a long period of time, lead to clinical signs such as weight loss, anorexia, lethargy and tearing [9]. In our experiment, administration of iodide, in the form of potassium salt of iodide (KI) at a concentration of 200 ppm during 4 weeks in rabbits, produced weight loss, distress, and signals of toxicity in thyroid and target tissues. Diagnosis of the thyroid condition was assessed by ultrasound and histopathological studies. Ultrasound analysis revealed enlargement, cystic formations and increased echogenicity (Figures 3 and 4). The histopathological feature of depletion of parafollicular cells (PC) is similar to that reported by Soliman et al., who induced a severe hypothyroidism in male New Zealand rabbits by administration of KI at higher concentration (400 ppm) in drinking water [6]. Vacuolar degeneration of follicular cells (FC) was detected and nuclei degenerative changes such as pyknosis, karyorrhesis and karyolisis indicate FC were undergoing necrosis (Figure 2 panels C and D). Together, these results suggest that oral administration of KI at a concentration of 200 ppm over a 4-week period, induced thyrotoxicity. Human cytological studies of the thyroid frequently reveal the presence of Hürthle cells (HC), which are follicular-derived epithelial cells with oncocytic characteristics, associated with diverse thyroid pathological conditions [1]. This study utilized rabbits from a research facility that provided animals raised in a controlled environment. However, an unexpected histopathological finding of HC in thyroid tissue sections was observed in these otherwise healthy specimens. Because of this finding, we propose using rabbits as a model to develop and study thyroid-associated disorders. While further studies with different approaches are needed to elucidate the clinical significance of neutropenia observed in this study, it is, nonetheless, well known that thyroid hormones regulate proliferation and differentiation of diverse cells, therefore controlling cell growth and homeostasis [10]. Therefore, we believe that the clinical signs observed in this study may be associated with a down-regulation of immunological responses due to dysregulation of hematopoiesis. Although pharmacokinetic analyses and a thyroid function tests need to be performed, the ultrasound study, together with the histopathologic evidence and clinical manifestations, strongly suggest that KI at a concentration of 200 ppm triggers toxic injuries in thyroid parenchyma and results in neutropenia. The results of the present study suggest that potassium iodide causes direct cytotoxicity to follicular epithelial cells. Although we must increase animal numbers, include male rabbits and optimize the potassium iodide dose, the toxicological model presented in this work represents an inviting opportunity to conduct further studies to assess toxicological disorders affecting the thyroid gland.

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