J Toxicol Pathol 2018; 31: 147–150

Case Report

Testicular microlithiasis in a clinically healthy cynomolgus monkey (*Macaca fascicularis*)

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Abstract: The present article describes an occurrence of testicular microlithiasis in a cynomolgus monkey from a routine regulatory toxicology study. The monkey was from a negative control group. Microscopically, the lesion was characterized by multiple extracellular mineralized calculi within seminiferous tubular epithelia of both testes without any tissue reaction or abnormal condition such as cryptorchidism, testicular neoplasm, or hypogonadism. The present case is remarkable in that there is a paucity of reports on spontaneous testicular microlithiasis in nonhuman primates. It is hoped that this case report will help to facilitate the differentiation of spontaneous changes from induced changes in nonhuman primate toxicology studies that are designed to use limited numbers of animals. (DOI: 10.1293/tox.2017-0065; J Toxicol Pathol 2018; 31: 147–150)

Key words: testis, microlithiasis, monkey

A healthy adult (approximately 4 years old) male cynomolgus monkey (*Macaca fascicularis*), supplied by Charles River BRF, Inc. (Houston, TX, USA), belonging to a vehicle (purified water) control group in a 2-week regulatory toxicology study underwent necropsy. This animal had been dosed orally once daily by gavage, fed Certified Hi-Fiber Primate Diet 5K91 (PMI Nutritional International), and given water *ad libitum*. Acclimation, veterinary care, housing and environmental conditions, study conduct, euthanasia, and post-life procedures were performed in accord with Good Laboratory Practices, the study protocol, and relevant standard operating procedures, and all procedures performed on the animal were in accordance with regulations and established guidelines reviewed and approved by an Institutional Animal Care and Use Committee.

A complete necropsy was performed at scheduled termination of the study.

The necropsy findings were unremarkable. Terminal body weight was 3.70 kg. Organ weights obtained during necropsy for the liver, spleen, heart, thymus, adrenal glands, kidneys, and brain were within the reference ranges. The testes (weighed together) weighed 7.173 g, which was considered to be within the reference range. The eyes were fixed in Davidson's solution, testes and epididymides were fixed in modified Davidson's solution, and all remaining tissues were fixed in 10% neutral buffered formalin. Protocol-required tissues were then conventionally processed to paraffin blocks, sectioned at a thickness of 5 microns, stained with hematoxylin and eosin (H&E), and examined with light microscopy. Special histologic stains or other analyses were not done.

Microscopic tissue examination of the testes revealed seminiferous tubules with adequate sperm and typical sperm stages, which was consistent with sexual maturity. Although some dilated seminiferous tubules were present, they were not generally associated with microliths and were considered to be an incidental background finding. Interstitial areas were unremarkable. All other tissues examined were not remarkable or had common background findings for this species. Microscopic examination of both testes revealed multiple extracellular, basophilic, non-birefringent, round-to-oval mineralized foci (microliths) within lumens of seminiferous tubules. The microliths ranged from 12 to 60 µm (more commonly 20–40 µm) in diameter and were randomly and diffusely distributed across the parenchyma (Fig. 1). There were approximately 3 to 8 microliths per low-power field. Individual microliths were composed of concentric layers, were often surrounded by an adjacent rim of seminiferous tubular epithelium (Fig. 2 and 3), and appeared either singly or less commonly as a cluster of either two or three individual microliths. Neither tissue reaction nor luminal tubular dilatation was seen in areas of these concretions. Microliths were not present in vascular, interstitial, or adventitial areas of the testis, in the epididymis, or

Received: 14 November 2017, Accepted: 26 January 2018
Published online in J-STAGE: 18 February 2018
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in any other tissues examined.

Testicular microliths have been described as intratubular bodies containing a calcified center with concentric laminations of collagen fibers. They are theorized to arise from defective phagocytosis of degenerate tubular cells by Sertoli cells[1]. The cells that encircled microliths in the current case were most likely Sertoli cells. Microliths have been reported in retained testes of several species, including the goat, cat, rabbit, and horse[2]. The condition has been seen unilaterally in a 5-year-old Siberian Husky dog with contralateral cryptorchidism[3], in which the undescended testis had concomitant seminoma and intratubular germ cell neoplasia.

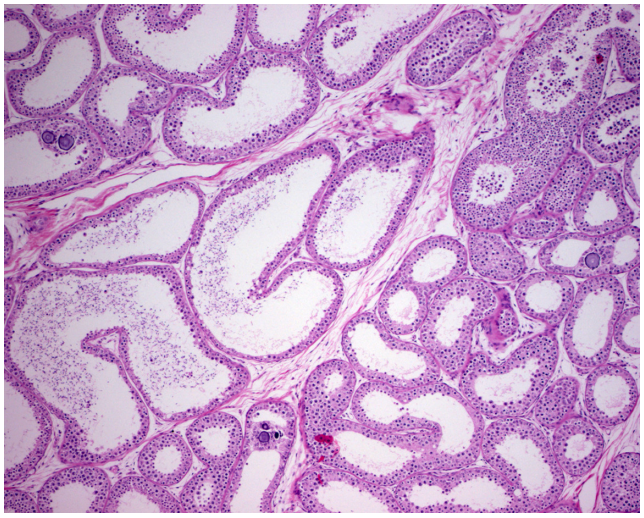

Fig. 1. Testicular microlithiasis. Multiple extracellular, basophilic, non-birefringent, round-to-oval mineralized foci within lumens of seminiferous tubules distributed randomly across the parenchyma. H & E stain. Original magnification 40×.

Microscopic concretions have been documented in several tissues from various species. For example, pulmonary alveolar microliths have been reported in birds[4], mice[5], and dogs[6]; intraglandular prostatic concretions are a common age-related change in primates[7, 8]; and corpora arenacea occur in the human brain, especially along midline structures[9]. These concretions, unlike nephroliths and uroliths, are usually associated with minimal or no local tissue reaction. Human subjects with coexisting pulmonary and testicular microlithiasis have been reported, some of whom had a mutation of a sodium-phosphorus cotransporter gene[10]. However, most cases of testicular microlithiasis are of unknown cause.

The prevalence of human testicular microlithiasis has been reported to range from 0.68%[11] to 5.6%[12] depending on the characteristics of the cohort examined and has been detected from 10 months[13] to at least 74 years of age[14]. It has been associated with hypogonadism, testicular neoplasia, cryptorchidism, subfertility, or other conditions[11, 14–16] but has also been reported as an incidental finding in a healthy human patient[17]. Testicular microlithiasis typically occurs bilaterally but can be unilateral[18].

There is controversy about the relationship of testicular microlithiasis with future development of gonadal neoplasia and therefore to the clinical recommendations given to patients. Some evidence suggests that the presence of microliths in an otherwise normal human testis heralds development of intratubular germ cell tumor, the germ cell counterpart to carcinoma *in situ*, and that frequent and periodic sonographic surveillance of human subjects with testicular microlithiasis is considered essential[11, 14]. However, other evidence indicates that testicular microlithiasis is far more common than testicular neoplasia, suggesting that rigorous clinical monitoring of patients with testicular microlithiasis may be unnecessary[12] and does not offer improved results

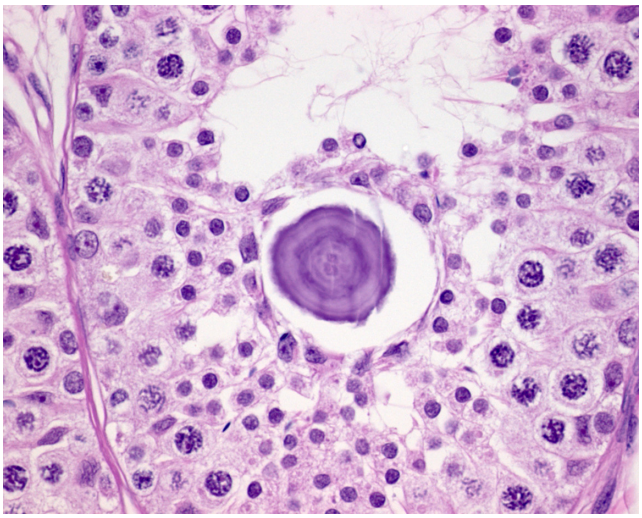

Fig. 2. Testicular microlithiasis. A microlith composed of concentric layers. H&E stain. Original magnification 400×.

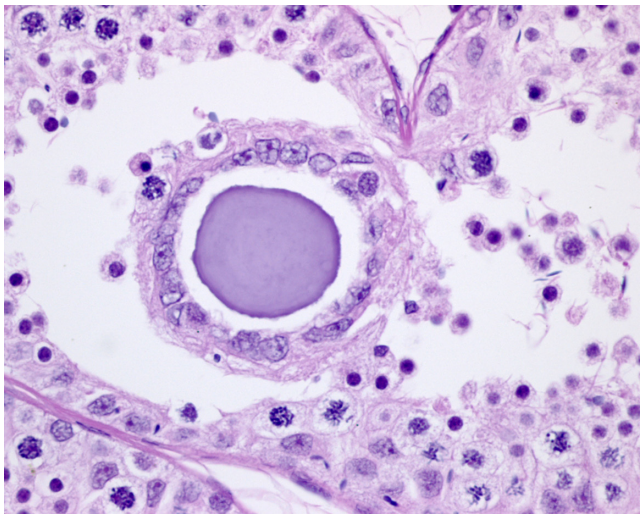

Fig. 3. Testicular microlithiasis. A microlith surrounded by an adjacent rim of seminiferous tubular epithelium. H&E stain. Original magnification 400×.

over testicular self-examination. Interestingly, patients with unilateral germ cell tumors are at increased risk for developing a tumor in the contralateral testis[19].

The advent of ultrasonography has contributed to increased awareness, detection, and reporting of testicular microlithiasis since the first description of its sonographic image[20]. Diagnostic criteria for human microlithiasis are based on quantification of individual concretions from sonograms. While arbitrary, the observation of five or more microliths in a human testis is considered sufficient for diagnosis[21]. Sato *et al.* reported an image of one concentric laminated eosinophilic body as a corpora amylaceum in the testis of a cynomolgus monkey[22]. This would differ from the current case given the multiplicity and basophilic staining property of the change. As mentioned earlier, there is controversy about the relationship of testicular microlithiasis with future development of gonadal neoplasia. However, limited evidence suggests that the predisposition for development of neoplasia is greater as the density of testicular microliths increases[21].

Because nonhuman primate regulatory toxicology studies are designed to use minimal numbers of animals, the characterization and documentation of an uncommon, incidental finding from a vehicle-only control animal are imperative for maximal utilization of diminishing laboratory animal resources and for appropriate interpretation of study-specific pathology data. Moreover, rare histopathologic changes in reproductive tissues in nonhuman primates can represent some of the most challenging findings for toxicologic pathologists to interpret. Inappropriate interpretation of uncommon histopathologic observations, when present in non-control animals, can result in unneeded repeat studies or in other delays.

While uncommon findings in preclinical regulatory toxicology studies used for drug development should always be interpreted in the context of a given study, in our experience testicular microlithiasis is a rare, incidental finding in cynomolgus monkeys. In this case, no association could be made between testicular microlithiasis and either gonadal neoplasia, hypogonadism, ectopia/maldescent, or other conditions, as has been suggested in other species. Testicular ultrasound screening may be a useful pre-study adjunct in those nonhuman primate experiments in which gonadal effects are of increased concern.

Disclosure of Potential Conflicts of Interest: The authors are employees of Pfizer Inc. and own Pfizer's stock. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflicts of interest.

Acknowledgements: We thank Kayla Nguyen for her superb assistance and Dr. Winston Evering (Pfizer, Inc., San Diego, CA, USA) for useful discussions.

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