Experimental and Toxicologic Pathology 65 (2013) 1121–1125

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Short communication

Retrograde ejaculation associated spontaneous sperm cystolithiasis in four rhesus macaques (*Macaca mulatta*)

Sanjeev Gumber[a,*], Cynthia L. Courtney[a], Karen R. Strait[b], Prachi Sharma[a], Julie E. Freebersyser[c], Maria M. Crane[d]

[a] Division of Pathology, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road NE, Atlanta, GA 30329, USA
[b] Division of Animal Resources, School of Medicine, Emory University, Whitehead Biomedical Research Building, Atlanta, GA 30322, USA
[c] Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[d] Yerkes National Primate Research Center, Emory University, 2409 Taylor Lane, Lawrenceville, GA 30043, USA

ARTICLE INFO

Article history:
Received 8 January 2013
Accepted 6 May 2013

Keywords:
Calculi
Ejaculation
Retrograde
Rhesus
Sperm

ABSTRACT

Retrograde ejaculation (RE) has been reported in humans and animals but RE with subsequent sperm calculi has rarely been reported. This report documents clinical and pathological findings of spontaneous sperm cystolithiasis in four rhesus macaques. While this condition has been associated with repeated electroejaculation, spontaneous sperm cystolithiasis is highly unusual. The animals presented with either stranguria, dysuria, hematuria, distended abdomen or lethargy. Ultrasound examination revealed several hyperechoic masses within the lumen of the urinary bladder. The animals were euthanized due to poor prognosis or study end points. Postmortem examination revealed multiple angular, amorphous, soft to firm, pale yellow to greenish-brown and variably sized calculi in the lumen of the urinary bladder or prostatic/penile urethra. Histologically, the calculi were composed of numerous sperm embedded in abundant brightly eosinophilic matrix. Based on gross and histologic findings, RE associated sperm cystolithiasis was diagnosed, with ulcerative urethritis as the major primary apparent etiology. To the authors' knowledge, this is the first report of four spontaneous cases of sperm cystolithiasis in rhesus macaques.

1. Introduction

In non-human primates, the routine collection of sperm is often required for scientific investigations. Sperm are produced in the testis and stored in the caudal part of the epididymis. Ejaculation of sperm occurs either during natural mating, masturbation or loss through normal urination (Newman et al., 1982). Electroejaculation was first developed for primates in the early sixties. It provides an expedient, high volume, high quality sample of sperm compared to other methods (Mastroianni and Manson, 1963). Electroejaculation can be performed by direct penile or rectal probe stimulation. Rectal probe stimulation is routinely used in non-human primates; however, this procedure has been reported to induce retrograde flow of sperm into urinary bladder (Schaffer et al., 1989b). In rats sperm urolithiasis can be induced by inseminating sperm into the neck of the urinary bladder or ventral prostate. The retention of sperm in the urinary bladder can cause coagulation and subsequently urinary obstruction (Stein-Werblowsky and Ablin, 1994). Mineral urolithiasis is uncommon in non-human primates and has rarely been reported in cynomolgus macaques (Faltas, 2000; Lees et al., 1995; O'Rourke et al., 1995; Renlund et al., 1986; Stephens et al., 1979). While rare reports exist in the literature of retrograde ejaculation (RE) with subsequent sperm mass or calculi in monkeys that have been electroejaculated (Chandolia et al., 2007; Frisk et al., 1974), this is the first report to the authors' knowledge of four spontaneous cases in rhesus macaques. This report describes the clinical and pathological features of spontaneous sperm cystolithiasis caused by RE in four rhesus macaques, and the underlying etiopathogenesis is discussed in comparison to humans and other species.

2. Materials and methods

2.1. Case no. 1

A 7-year old, male rhesus macaque presented to the Yerkes National Primate Research Center (YNPRC) Veterinary Service with acute hematuria and stranguria. The monkey was enrolled in a research protocol that studied modulation of HIV immunity with dendritic cells in Simian immunodeficiency virus (SIV) infected animals. The subject was SIV positive, infected in with SIVmac251

* Corresponding author. Tel.: +1 404 727 7022; fax: +1 404 727 4531.
E-mail addresses: sgumber@emory.edu, gumber77@gmail.com (S. Gumber).

0940-2993/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.etp.2013.05.003

intra-rectally. The viral load after 12 weeks of infection was 238421.2 copies/ml of blood.

2.2. Case no. 2

An 11.5-year old, male rhesus macaque presented to YNPRC Veterinary Service with the history of chronic weight loss, diarrhea, dysuria and a distended abdomen. The monkey was SIV negative and assigned to a study examining the role of hormones in sex differences in adaptability.

2.3. Case no. 3

A 7.5-year old, male rhesus macaque developed severe lethargy. The animal was SIV negative, an alpha male and was not assigned to any research protocol.

2.4. Case no. 4

A 10.5-year old, male rhesus macaque presented with an enlarged prepuce, stranguria, distended urinary bladder, swollen and dark purple distal penis. The animal was SIV negative and was not assigned to any research protocol.

Animals 1 and 2 were born at YNPRC and enrolled in research protocols that were approved by the Institutional Animal Care and Use Committee of Emory University. Animals 3 and 4 were acquired from Oregon National Primate Research Center and Mannheimer Foundation/Haman Ranch (Florida, USA), respectively for breeding and genetic diversification in the Yerkes colony. All animals were fed a standard diet of commercial primate chow (5037 Old World Primate Diet, PMI International, Brentwood, MO) supplemented daily with various fresh fruits and vegetables. Water was provided ad libitum by means of lixits. Room conditions included a 12:12 h light: dark cycle, temperature of 24.6–29 °C and 10–15 air changes per hour. A complete physical examination, complete blood cell count (CBC), serum biochemical panel, urethral catheterization, survey radiographs or double contrast cystography and abdominal ultrasonography were performed on these animals. Due to the poor prognosis or because of study end points, the animals were euthanized. A complete necropsy was performed on these animals. For histopathologic examination, various tissue samples were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin. Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections of urinary bladder and prostatic urethra of Case 1 was performed for SIV (SIVgag mAb) (clone FA2; AIDS Reagent Repository Program) using the streptavidin–biotin complex peroxidase method as described by the manufacturer (Dako North America Inc., Carpinteria, CA).

3. Results

3.1. Case no. 1

Physical examination revealed an enlarged, firm urinary bladder in the caudal abdomen. A sterile 5 French urinary catheter was successfully passed through the urethra to rule out an obstruction. Urinalysis revealed severe hematuria and proteinuria, and 3–5 white blood cells (WBC)/high power field (hpf). No bacteria were isolated on bacteriological culture. No significant findings were noted on the CBC and serum biochemical panel. Survey radiographs of abdomen were within normal limits and no urinary calculi were seen. A double contrast cystogram (Omnipaque, GE Healthcare) revealed numerous, irregularly shaped, radiolucent filling defects in the lumen of the urinary bladder. An abdominal ultrasound examination (8C transducer; GE Medical Systems Co., Ltd., Jiangsu, China) showed a distended urinary bladder containing hyperechoic masses in the bladder lumen not associated with the urinary bladder mucosa.

Postmortem examination revealed a thickened urinary bladder wall and the lumen contained multiple angular, amorphous, soft, and greenish masses measuring approximately 2–5 cm in diameter (Fig. 1A). Similar calculi were present near the ejaculatory duct opening in the proximal urethra (Fig. 1B). Pinpoint ulcers were present on the trigone area of the urinary bladder mucosa (Fig. 1B). Seminal vesicles, epididymis, and testes were unremarkable. No other significant macroscopic findings were observed. Histologically, the urinary bladder calculi were composed of numerous sperm embedded in abundant brightly eosinophilic matrix (Fig. 1D). The submucosa of the urinary bladder was moderately edematous and multifocally infiltrated by moderate numbers of neutrophils. The prostatic urethra proximal to the seminal vesicles was multifocally ulcerated and the underlying submucosa was expanded with moderate edema, numerous neutrophils, macrophages, lymphocytes, plasma cells, and semen matrix in the lumen (Fig. 1E). The prostatic urethral section directly over the ejaculatory duct and distal to the site of mucosal ulceration had marked mucosal hyperplasia with submucosal edema and acute inflammation. Other histological findings included moderate multifocal neutrophilic prostatitis and renal interstitial lymphoplasmacytic inflammation. Immunohistochemical staining for SIV was negative on the urinary bladder and prostatic urethra.

3.2. Case no. 2

On physical examination, the animal had enlarged/swollen tip of the penis extruding from the foreskin. The urinary bladder was enlarged and firm. Attempts at urethral catheterization were unsuccessful but produced two large (2–4 cm in length) pale yellow to white casts upon flushing with sterile saline. Gritty material was blocking approximately 6 cm of the urethra. Urinalysis revealed severe hematuria, moderate proteinuria, and 2–4 WBC/hpf but bacterial cultures remained negative. The CBC results revealed moderate leukocytosis (WBC count, $17.4 \times 10^3/\mu l$, reference (ref.) interval: $5.8–10.4 \times 10^3/\mu l$) characterized by moderate neutrophilia ($15.7 \times 10^3/\mu l$, ref. interval: $2.2–6.3 \times 10^3/\mu l$). The other hematological and serum biochemical panel findings were within normal limits. The hematological and serum biochemical panel reference intervals were used as described by Association of Primate Veterinarians (Lee and Doane, 2012). Abdominal ultrasound examination revealed a large amount of hyperechoic material in the dorsal (dependent) aspect of the bladder.

On necropsy, the abdominal wall adhered to the dorsal wall of the markedly distended urinary bladder. The urinary bladder contained 40–50 ml of slightly brown-tinged urine and two 1–2 cm, angular or dumbbell shape, greenish-brown calculi. The urinary bladder wall was slightly thickened and the tip of penis was markedly swollen. No other remarkable gross findings were noticed. Histologically, the calculi were consistent with sperm cystoliths as described above with multifocal areas of calcification (data not shown). Additional histological findings were moderate prostatic urethral ulceration with overlying semen matrix, marked fibrinosuppurative pyelonephritis, and moderate necrosuppurative inflammation of the penis.

3.3. Case no. 3

On physical examination, the rhesus macaque was weak, lethargic and dehydrated. Urethral catheterization revealed hematuria. The CBC results revealed moderate leukocytosis (WBC count, $15.4 \times 10^3/\mu l$, ref. interval: $5.8–10.4 \times 10^3/\mu l$) characterized