Theriogenology xxx (2013) 1–7

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Injection of a chemical castration agent, zinc gluconate, into the testes of cats results in the impairment of spermatogenesis: A potentially irreversible contraceptive approach for this species?

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ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 2 September 2013
Accepted 12 September 2013

Keywords:
Zinc gluconate
Chemical castration
Infertility
Spermatogenesis
Cat

ABSTRACT

Male sterilization by chemical agents is a nonsurgical contraceptive approach designed to induce azoospermia and, therefore, infertility. Intratesticular injection of zinc gluconate for sterilization of dogs has been described, but its use in cats remains limited. The objective of the present study was to evaluate, by light and transmission electron microscopy, the efficacy of a single intratesticular injection of a zinc gluconate solution (Testoblock) as a sterilant for male cats. Twelve sexually mature mixed breed cats were allocated at random into two groups (control = 6; treated = 6) and given a single injection into each testis of either isotonic saline or zinc gluconate, respectively. Histopathologic and ultrastructural evaluation was assessed at 120 days postinjection. Histopathologic changes were not detected in the testes from the control group. However, histologic evaluation of the treated group revealed atrophic and dilated seminiferous tubules, a decrease in the number of germ cells, and incomplete spermatogenesis. Sertoli cells had various degrees of cytoplasmic vacuolization. Intertubular tissue revealed active fibroblasts, collagen deposition, and inflammatory cells. The diameter of seminiferous tubules, epithelial height and tubular area were reduced ($P < 0.05$) in the treated group compared with controls. Azoospermia occurred in 8 of the 11 treated cats (73%). Ultrastructural evaluation of Leydig cells revealed loss of nuclear chromatin, increased smooth endoplasmatic reticulum, and mitochondria degeneration. Intratesticular injection of zinc gluconate solution impaired spermatogenesis in cats and has great potential as a permanent sterilant in this species.

1. Introduction

Sterilization by chemical agents is a nonsurgical approach to male contraception. Chemical agents injected into the testis, epididymis, or vas deferens cause infertility by inducing azoospermia. This procedure is not demanding technically, inexpensive, and suitable for mass sterilization programs in both domestic and wild animals [1].

One reason to castrate pets is the elimination of secondary sex characteristics, such as mounting, aggression, and urine marking (spraying) [2]. Additionally, sterilization contributes to pet population control [3]. Traditional methods of contraception require time for the contraceptive to be effective, costs are high, and

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http://dx.doi.org/10.1016/j.theriogenology.2013.09.013

operative approaches involve risks and may induce trauma [4].

There are reports of satisfactory results related to chemical castration in cats. According to Pineda and Dooley [5], chemical castration using intra-epididymal injection of chlorhexidine gluconate induced azoospermia or severe oligospermia in cats. Intratesticular injection of calcium chloride also demonstrated potential for permanent contraception in cats [6].

Previous studies in dogs revealed that intratesticular injection of zinc gluconate impaired spermatogenesis owing to germ cell death [7]. The break down of the Sertoli cell barrier and the subsequent release of germ cell antigens could have been responsible for the observed inflammatory reaction after the use of zinc gluconate [8–13].

Chemical sterilization with zinc gluconate seems to be a feasible alternative method for population control of adult male cats, yet studies using this approach have not been documented. Therefore, the aim of the present study was to evaluate, by light and transmission electron microscopy, the efficacy of a single intratesticular injection of a zinc gluconate solution to promote sterilization in adult male cats.

2. Materials and methods

2.1. Test compound

The test compound was a proprietary zinc gluconate solution for intratesticular injection (Testoblock, Bio-Release Technologies LCC, Birmingham, AL). It contains 0.2 mol/L zinc gluconate (13.1 mg zinc/mL), which is pH-neutralized in a physiologic vehicle.

2.2. Animals

We included 12 intact, short hair, male cats from two private colonies, approximately 9 to 12 months old and weighing 2.0 to 4.5 kg. The study was approved by the Animal Experimentation Ethics Committee of Federal Rural University of Pernambuco (Protocol 008/2010). All cat owners were given detailed information regarding the experiment and signed a research consent form. The work was undertaken between January and November in Recife, PE, Brazil (8°040 South; 33°550 West). At this location, there is approximately 12 hours of light per day and a mean temperature of 26.4 °C. There were no clinical abnormalities in any of the cats based on a physical, hematology, and clinical chemistry examinations. All cats produced ejaculates with 80% progressively motile sperm and sperm counts were within normal limits. All males displayed sexual interest toward an estrous queen.

Cats were randomly assigned to two groups, controls ($n = 6$) and treated ($n = 6$), and each cat was given a single injection into each testis of either isotonic saline or zinc gluconate, respectively.

2.3. Intratesticular injection

Cats were anesthetized for intratesticular injections and semen collection. Access to food and water was withheld for 12 and 7 hours, respectively, before anesthesia, which was induced using xylazine (0.5 mg/kg, im; Rompun 2%, Bayer, Tarrytown, NY) followed by ketamine (5.0 mg/kg, IM; Ketalar, Pfizer, Rye Brook, NY). If needed, an additional treatment (approximately 25% of the original dose of each product) was given.

Before intratesticular injection the scrotum was cleaned (10% povidine iodine, SLF, Gama, DF, Brazil). The width of each testis was measured with calipers, and a single injection of isotonic saline or Testoblock was given into each testis (1 mL of solution for every 27 mm of testis width; adapted from Wang 2002 [11]). The volume injected per testis ranged from 0.44 to 0.51 mL. Injections were performed using a 0.5-mL U100 insulin syringe with a 28-ga, 12-mm needle (a separate needle was used for each testis). The needle was inserted in a plane parallel to the testis, into the dorsal-cranial area of each testis, near the caput epididymis (close to the rete testis and efferent ducts). Cats were observed for approximately 1 hour after injections. Owners were instructed to observe their animals and to contact the researchers if there were changes in cat behavior or appearance.

2.4. Testicular weight evaluation

At 120 days after intratesticular injection of saline or zinc gluconate all cats were castrated. After orchiectomy, testes were weighed and 19% (concerning to tunica albuginea) was subtracted to calculate the liquid weight of the testes, as described by França and Godinho (2003) [14].

2.5. Testicular histopathology

Testes were cut in half and immersed in 4% glutaraldehyde in phosphate buffer (0.1 mol/L and pH 7.3) for at least 2 days. Then, testis were trimmed, dehydrated in a series of graded alcohol solution, and embedded in plastic resin of glycol methacrylate (Historesin, Leica, Wetzlar, Germany). Histologic sections (4 μm) were stained with 1% toluidine blue/sodium borate and analyzed morphologically and morphometrically [15]. Seminiferous tubule diameter and area, as well as epithelial height were analyzed according to the method of Tenorio, et al. [16].

2.6. Transmission electron microscopy

Testicular fragments were processed for electron microscopy according to Cavalcanti, et al. [17]. Ultrathin sections were stained with uranyl acetate (3%) and lead citrate, imaged and photographed in a transmission electron microscope (Jeol JEM 100CX, Tokyo, Japan).

2.7. Clinical assessments

General attitude of the cats, body weight, ability to walk, scrotal changes, and rectal temperature were evaluated on Days 0 (immediately before treatment), 60, and 120. Concurrently, testes width was measured and