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Ivermectin acute administration impaired the spermatogenesis and spermiogenesis of adult rats

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ABSTRACT

Ivermectin (IVM) is an antiparasitic agent widely used in agricultural, domestic animals and in human clinical practice. In the present study, the temporal effects of therapeutic doses of IVM in the morphometric and histological assessment of testis were studied to verify if IVM acute administration impaired the spermatogenesis and spermiogenesis of adult rats, if these effects are reversible. The testosterone levels and the plasmatic IVM levels were assessed. The results show: 1) IVM acute exposure, mainly in the higher dose, reduced the testicular volume, the tubular diameter and the germinal epithelium height; 2) no interferences on Leydig cells frequency; 3) histological studies show that tubular sections containing several histological changes indicative of spermatogenesis interruption, such as disorganization of germinal epithelium, vacuolar degeneration of the germ cells and sloughing of cells into the tubular lumen; 4) no differences in testosterone levels; 5) The IVM plasmatic levels were significantly reduced at 72 h after the 0.2 mg/kg. It was concluded that acute IVM impaired the spermatogenesis and spermiogenesis of rats. Probably these effects were not consequence of IVM at the Leydig cells because no effects were observed at this level. Finally, our results suggest that some testicular effects are reversible and correlated with the plasmatic levels of IVM.

1. Introduction

Avermectins are broad-spectrum antiparasitic agents that are widely used in agricultural and domestic animals (Bloomquist, 2003; Casida and Durkin, 2013; Campbell, 2016). In human clinical practice, avermectins is used to treat lymphatic filariasis, onchocerciases, rosacea, scabies, head lice and others (Kircik et al., 2016). Ivermectin (IVM) is an avermectin that belongs to the macrocyclic lactones class of endectocides and consists of a mixture of two homologous compounds, 22, 23-dihydroavermectin B1a (H2B1a; not > 80%) and 22,23-dihydroavermectin B1b (H2B1b; not > 20%). In vertebrates IVM is the first macrocyclic lactones synthesized avermectin (Elgart and Meinking, 2003) and can produce γ-aminobutyric acid (GABA)-mimetic effects by acting as agonists at $GABA_A$ receptors and stimulating GABA release (Dawson et al., 2000; Estrada-Mondragon and Lynch, 2015; Shoop et al., 1995).

In addition to central nervous system (CNS), GABA was found in gonads and accessory reproductive organs, and a direct effect on steroidogenesis and sperm viability and motility has been described (Erdo et al., 1983; Frungieri et al., 1996). GABA can mimic and potentiate the action of progesterone in initiating the acrosome reaction of mammalian sperm, indicating that sperm contain receptors for GABA. The $GABA_A$ (He et al., 2003), $GABA_B$ (He et al., 2001) and $GABA_C$ (Li et al., 2008) receptors were identified in rat testis and sperm. Also, the GABAergic systems may play modulatory roles in spermiogenesis (Kanbara et al., 2005), because expression of glutamate decarboxylase mRNAs, which are GABA synthetic enzymes, has been observed in both round and elongated spermatids. Furthermore, the GABAergic system located in adult Leydig cells in rodent and human testis appears to be linked to the regulation of steroid synthesis by Leydig cells *via* local $GABA_A$ receptors (He et al., 2001; Geigerseder et al., 2003; Hauet et al., 2005).

Data about IVM on reproductive parameters are controversial.

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Histomorphometric evaluation of testicular parenchyma through the daily spermatic production and the efficiency of the spermatogenesis of rats treated with different doses of IVM (0.1, 0.4 and 0.6 mg/kg) did not show deleterious effect (Moura et al., 2006). However, in rats 0.56 mg/kg of IVM induced a variety of side effects on male reproduction as reduction of testes, epididymis, and accessory sex organs weights and change in sperm characters; decrease of sperm count and motility, and increase in sperm abnormalities(El-Sawy et al., 2015). In male rabbits 0.5 or 1.0 mg/kg of IVM induces in rats deleterious effects on kidneys and hepatic functions, oxidative stress, weight loss and increased testosterone and free testosterone (El-Far, 2013). Moreover, at clinical veterinary practice the sperm of recently animals treated with IVM was not considered with good quality to perform fertilization.

In the present study the temporal effects of a low and high therapeutic doses of IVM (Soll, 1989) in the morphometric and histological assessment of tests were performed to verify if IVM acute administration negatively affect adult rat testis and if these effects are reversible. We measured the testosterone serum levels because interferences with this hormone could be responsible for testis injuries (Russell et al., 1981; Meistrich, 1986). The IVM serum levels were evaluated to investigate their relationships with testis injuries and the testosterone levels. Also, the astrocyte behavior was assessed by the glial fibrillary acidic protein (GFAP) expression in the cortex and hypothalamus to examine if the central nervous system are involved in the IVM effects on testes injuries (Chatton et al., 2003).

2. Material and methods

2.1. Animals

Male Wistar rats from the Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ/USP), were used. The animals were housed in groups of four in polypropylene cages with a metal cover (40 cm × 50 cm × 20 cm) under controlled room temperature (22 °C ± 2 °C), humidity (45–65%), and artificial lighting (12 h/12 h light/dark cycle, lights on at 7:00 h a.m.). The animals received free access to Nuvilab® rodent chow (Nuvital Company, São Paulo, Brazil) and filtered water. Sterilized residue-free wood shavings were used as bedding. Animals were divided randomly into control and experimental groups. All of the procedures were reviewed and approved by the Animal Care Committee of Paulista University (UNIP) (protocol no. 333/15) and FMVZ/USP (protocol no. 1892040315) and conformed to the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA (Committee, 2011). All efforts were made to minimize animal suffering.

2.2. Drugs

Ivermectin (IVM – 1% Ivomec® injectable, Merial Animal Health Ltda., Paulínia, SP, Brazil) was dissolved in a saline solution (NaCl 0.9%) plus a drop of Tween 80 and was administered subcutaneously (SC) at a dose of 0.2 or 1.0 mg/kg. Saline solution plus a drop of Tween 80 was also administered as control solution. All solutions were administered in a volume of 1.0 mL/kg. The 0.2 mg/kg IVM dose was chosen because it is the standard therapeutic dose used in several animals' models (Dadarkar et al., 2007; Almeida et al., 2017) The 1.0 mg/kg dose is considered high, and we previously observed that this dose reduced male and female sexual behavior (Bernardi et al., 2011; Moreira et al., 2014).

2.3. Groups and treatments

Male Wistar adult rats (90 day old) were distributed among seven groups. A group of animals received control solution (control group). Six groups received IVM; three of them received a 0.2 mg/kg dose and other three received 1.0 mg/kg. All experimental procedures were made 24, 48 and 72 h after IVM administration. After each time of IVM administration, the rats were euthanized (decapitation) and used to evaluate the testes morphometric and stereological examination, testosterone levels and ivermectin plasmatic level.

2.4. Procedures

2.4.1. Testicular weight and testis volume

The rats 24, 48 and 72 h after the treatments were euthanized (decapitation) and their brains, blood, and testes were collected.

The testes were weighed, and were calculated as the proportion of the gonad mass relative to total body mass: Testicular weight = (gonad weight/body weight) × 100.

The volume of each testis (V) was calculated: $V = 4/3 \cdot p \cdot a \cdot b^2$, where a is the semiprolate axis and b is the semioblate axis (Miraglia and Hayashi, 1993; Botelho Cabral et al., 1997).

2.4.2. Histological examination

The testes were fixed by immersion in Bouin's liquid fixative for 48 h. The specimens were processed and embedded in Paraplast Plus (Sigma Chemical Co., St. Louis, MO). Cross sections were obtained from the fragments of the gonad, allowing an adequate morphometric analysis (Gundersen et al., 1988). To obtain a better identification of all phases of spermatogenesis, 6 µm-thick sections were stained with the Hematoxilin-Eosin (HE) method (right testis). A periodic acid-Schiff method, counterstained with Harris'Hematoxylin (PAS + H), was also processed as a histochemical method to determine interstitial cells as well as elongate spermatids (left testis).

2.4.3. Morphometric examination

2.4.3.1. Tubular diameter and germinal epithelium height. The analysis of the linear morphology of seminiferous tubules to determination of tubular diameter sizing was made measuring the distance between the basal lamina of a tubule to the basal lamina in the opposite side. To determine the height of the germinal epithelium the distance from the basal lamina of a tubule until the beginning of the tubular lumen was measured, using a computerized image analysis program, ImageJ software. Ten fields were selected from the histological testicular sections to each animal and photomicrographs were taken using a 40 × objective. For each field, five tubules were measured and the distance of tubular diameter and the height of the germinal epithelium were automatically calculated in pixels using ImageJ software totalizing 50 tubules per animal.

2.4.3.2. Interstitial cells frequency. Ten fields were selected from the histological testicular sections to each animal. The frequency of interstitial cells per field was estimated using a 40 × objective directly at the microscope.

2.4.4. Testosterone levels

Serum testosterone levels were assessed using commercially available enzyme-linked immunosorbent assays (testosterone kit, Cayman Chemical, Ann Arbor, MI, USA; catalog no. 582701). The procedure was performed according to the manufacturer's instructions.

2.4.5. IVM serum levels

The IVM serum levels were assessed using liquid chromatography technique coupled with tandem mass spectrometry (LC-MS/MS analysis). The IVM was analyzed by a Agilent Technologies 1260 Infinity system with a binary pump, degasser, automatic sampler and column oven with interface for the triple quadrupole mass spectrometer (3200 QTRAP) Applied Biosystem MDS Siex with TurboIonSpray. The LC-MS/MS control software was Analyst V1.5.1. The column used was Agilent Zorbax XDB C18 (50 mm × 2.1 mm and 3 um diameter). The column oven was maintained at 40 °C. The following gradient elution with methanol and 20 mM aqueous