Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 84 (2008) 95–99
RVS
www.elsevier.com/locate/rvsc

The effect of enrofloxacin on sperm quality in male mice

Faruk Aral [a,*], Füsun Karaçal [b], Füsun Baba [c]

[a] Department of Artificial Insemination and Reproduction, Faculty of Veterinary Medicine, Harran University, Sanliurfa 63300, Turkey
[b] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Harran University, Sanliurfa 63300, Turkey
[c] Department of Pathology, Faculty of Medicine, Harran University, Sanliurfa 63300, Turkey

Accepted 11 April 2007

Abstract

Current study was designed to evaluate toxic effects of enrofloxacin on male mice reproductive system. In the treatment group enrofloxacin was administered subcutaneously to male mice at a fixed dose of 150 mg/kg once daily for 15 days, whereas saline solution was given in the same regimen in the control group for the same period. Mice were sacrificed on day 15 and analyzed for sperm quality.

In addition to routine examination of sperm material, spermatogenetic activity and organization of each animal were graded according to Johnsen's scoring to assess the spermatogenesis relying on seminiferous tubule cross-section scores. A significant decrease in both epididymal sperm count and sperm motility besides abnormal spermatozoa rate were observed in enrofloxacin group compared to controls ($P < 0.01$, for all). Johnsen's score in control mice were better than those in treatment group ($P < 0.01$).

These results suggested that a fixed 150 mg/kg dose of enrofloxacin would lead disruption of spermatogenesis in the testes causing deterioration of motility and content of sperms as well as morphological abnormalities.

Keywords: Enrofloxacin; Johnsen's score; Male mice; Sperm; Toxicity

1. Introduction

Quinolones are a group of antibacterial agents that have a broad spectrum of activities. Quinolones are called fluroquinolones, 4-quinolones or quinolone carboxylic acids. They include ofloxacin, ciprofloxacin, perfloxacin, norfloxacin, enoxacin, difloxacin, fleroxacin, and temofloxacin beside other compounds, which are not of medical use (Abd-Allah et al., 2000a). Enrofloxacin is a member of the family of 6-fluoro-7-piperazinyl-4-quinolones. This antibiotic is highly lipophilic, and the addition of a carboxic acid and tertiary amine contributes to the amphoteric properties of enrofloxacin. Enrofloxacin is bactericidal and has excellent activity against both Gram-positive and Gram-negative pathogens (Mitchell, 2006). Enrofloxacin, a fluoroquinolone marketed specifically for use in veterinary medicine, inhibits DNA synthesis and is rapidly bactericidal against a broad spectrum of aerobic and some facultative anaerobic bacteria, including strains resistant to many other antimicrobial agents (Elmas et al., 2001). Enrofloxacin is well absorbed after oral administration. It is rapidly excreted through the bile and urine, mostly as enrofloxacin and its metabolite, together with relatively small amounts of other metabolites (Emea, 1998). The fluoroquinolones are generally well tolerated with minimal side effects when used appropriately. Specifically, arthropathy and cartilage degeneration have only been shown to occur in immature animals. The most common adverse effects are gastrointestinal in nature. Nausea is the most common complaint, followed by abdominal discomfort, vomiting, and diarrhea. Adverse effects which may be more pressing include the central nervous system toxicity, nephropathy, allergic reactions, and photosensitivity (Anon, 2006). Impaired spermatogenesis and/or testicular damage (atrophy in rats and dog) have been described in long-term toxicity studies with some of the quinolones.

* Corresponding author. Tel.: +90 0 414 312 84 56; fax: +90 0 414 314 41 58.
E-mail address: faral@harran.edu.tr (F. Aral).

0034-5288/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2007.04.007

Neuroendocrine mechanisms may be involved in these effects (Christ et al., 1988).

Ofloxacin, ciprofloxacin and perfloxacin are among the most effective antibacterials for the treatment of bacterial prostatitis and epididymitis. Ciprofloxacin and perfloxacin were detected in the prostatic tissue and seminal fluid in high concentrations. Ciprofloxacin had no apparent effect on spermatogenesis (135 mg/kg/day, for 10 days as measured by DNA flow cytometry in rats). Besides, quinolones were found phototoxic and able to induce singlet oxygen and superoxide anion (Abd-Allah et al., 2000b).

Although many paper reported about the toxic effects of quinolones on different organs and systems, there is a lack of data concerning the effects of quinolones on the reproductive system of male mice considering pathologic changes in seminiferous tubules and sperm quality characteristics. As far as we know, current study is the first paper in the literature concentrating on the toxic effects of enrofloxacin on sperm quality and spermatogenesis in male mice.

The aim of this study was to investigate toxic effects of enrofloxacin on male mice reproductive system and to determine some biological indices of sperm in toxic enrofloxacin dose.

2. Materials and methods

2.1. Chemicals and drugs

Enrofloxacin antibiotic, Enroksin inj. (100 mg enrofloxacin in 1 ml) was purchased from İbrahim Ethem Co/İstanbul.

2.2. Animals and treatment

In this study, 20 healthy male swiss albino mice (12 weeks old weighing 23 ± 3.2 g) were used. Ethic committee of University of Harran, Faculty of Veterinary Medicine approved the experiment protocol. The animals were kept under standard laboratory conditions (12-h light:12-h dark and 23 ± 2 °C, 60–65% humidity) and fed with a diet consisting of 88% dry matter, 24% crude protein, 2600 kcal/kg, 7% crude cellulose, 8% crude ash, 1% calcium, 0.9% phosphorus, 0.5% sodium, 1.0% NaCl, 0.6% methionine. Food and water were provided ad libitum.

Mice were randomly divided into two groups as the control ($n = 10$) and enrofloxacin treatment ($n = 10$) groups. Subacute experiments are held usually in a variable period of time between 14 and 30 days. The investigated material is applied 3–4 times of the normal dose and given continuously along the study (Kaya and Bilgili, 2002). In the light of general tendency (Kaya and Bilgili, 2002), treatment dose for mice was calculated according to the doses needed for the dogs and determined toxic dose of 150 mg/kg which is four times the therapeutic dose for mice. Toxic dose of enrofloxacin (Enroksin inj) was injected once daily for 15 days subcutaneously in treatment group. Control males were given saline solution in the same regimen for the same period.

2.3. Epididymal sperm concentration, motility and abnormal sperm

The mice were sacrificed by cervical dislocation. The epididymes were excised and placed in a prewarmed petri dish containing 1 ml phosphate buffered saline (PBS, pH 7.4) at 37 °C and placed in a 37 °C incubator for 15 min, prior to determining sperm motility. The suspension was stirred, one drop was placed on a warmed microscope slide, and a 22×22 mm coverslip was added. Microscopic fields were observed at 400× magnification using a standard light microscope, and the percentage of motile sperm was determined. Five microliters of the sperm suspension was transferred into an Eppendorf tube and diluted with 95 μl of PBS. After mixing, the sperm suspensions were counted. Sperm counts were made using a Thoma counting chamber and expressed as $\times 10^6$/ml. A drop of sperm suspension was smeared onto a slide and stained with Papanicolaou stain. The percentages of sperm with normal and abnormal morphologies were estimated in five random fields in each slide selected at random.

2.4. Histopathological examinations

Testes were fixed in Bouin's fixative (0.2% picric acid/2% (v/v) formaldehyde in PBS) for histological evaluation. Bouin's fixed testes were processed by routine automatic tissue processor, embedded in paraffin, sectioned into 4 μm slices, and stained with Hematoxylin-Eosin. The prepared slides were examined under light microscopy by using Johnsen's Scoring System (Johnsen, 1970) for the status of spermatogenesis. Spermatogenetic activity and organization were graded, and Johnsen's score was given for each animal. Johnsen's score for assessing the spermatogenesis depends on scoring each seminiferous tubule cross-section. The criteria are as follows: 10, complete spermatogenesis; 9, many spermatozoa present but disorganized spermatogenesis; 8, only a few spermatozoa present; 7, no spermatozoa but many spermatids present; 6, only a few spermatids present; 5, no spermatozoa or spermatids present but many spermatocytes present; 4, only a few spermatocytes present; 3, only spermatogonia present; 2, no germ cell present; and 1, no germ cell or Sertoli cell present. Mean score count was given to each animal. From each testis group five seminiferous tubules were randomly selected.

2.5. Statistical analyses

All values were given as mean ± standard error of measurement (SEM). The values in the sperm motility, concentration, abnormal sperm rate and Johnsen's score were compared with the Mann–Whitney test by using the MINITAB® software package program (version 12.1).