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The acute effects of single-dose orally administered doramectin, eprinomectin and selamectin on natural infections of *Syphacia muris* in rats

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ABSTRACT

This study was designed to determine the acute effects of a single-dose of orally administered doramectin, eprinomectin and selamectin on *Syphacia muris* infection in rats. Rats, naturally infected with *S. muris*, were divided into four groups: three different treatment groups ($n = 7$) and one positive control ($n = 7$). Cellophane tape preparations were obtained from the treated rats on day 0 pre-treatment and on days 2, 4 and 6 post-treatment. *Syphacia* sp. eggs were counted. Eprinomectin was found to be 100% effective in eliminating eggs on two post-treatment. However when egg counts on day 6 post-treatment were compared with pre-treatment egg counts, doramectin and selamectin were found to be 99.32 and 98.77% effective in eliminating eggs, respectively. On day 7 post-treatment, blood samples were obtained from all groups, and then the rats were necropsied. Doramectin, eprinomectin and selamectin were found to be 100% effective in eliminating adult *S. muris*, when compared with the positive control group.

1. Introduction

Syphacia muris is an oxyurid nematode, also known as the rat pinworm, which lives in the large intestine of laboratory rodents. Pinworm infection usually does not produce any clinical illness in laboratory rodents, but heavy worm burdens have been associated with rectal prolapse, enteritis, sticky stools and pruritis, which result in biting at the base of the tail (Hendrix, 1998). Parasitized laboratory rodents can also confound animal research results (Fox et al., 1984; Sueta et al., 2002), and so it is clearly important to keep laboratory rodent colonies parasite-free (Sueta et al., 2002).

Control and eradication of pinworm infections are difficult in rat and mouse colonies (Zenner, 1998; Oge et al., 2000). *Syphacia* eggs rapidly embryonate and can survive outside the host, as they are hardy, resistant to environmental extremes, and light enough to aerosolize, making control difficult. Retrograde infection is also possible with *Syphacia* sp. (Owen, 1992; Hendrix, 1998). Consequently, research into effective anthelmintics against pinworm infections is vital in improving infection control.

Doramectin, eprinomectin and selamectin are members of the avermectin family of macrocyclic lactones (Durden, 2007). Although highly beneficial outcomes have been obtained from doramectin treatment of nematode infections (McGregor et al., 2001; Dorchies et al., 2001; Gıcık et al., 2002) and ectoparasite infestations (Logan et al., 1996; Dorchies et al., 2001; Jensen et al., 2002) of farm animals, there has been a limited number of studies on the use of this anthelmintic in laboratory animals (Oge et al., 2000; Kozan et al., 2006). Pour-on application of eprinomectin has been found to be effective against gastrointestinal nematode infections of cattle (Ballweber et al., 2000) and sheep (Cringoli et al., 2003), and *Muellerius capillaris* in goats (Geurden and Vercruysse, 2007). Eprinomectin has also been used for the treatment of ectoparasites of farm animals (Aguirre et al., 2005; Rehbein et al., 2005; Habela et al., 2006). Oral administration of eprinomectin in dogs has been reported to be 100% effective against *Toxocara canis* infection (Kozan et al., 2008). The application of pour-on (Ulutas et al., 2005) or injectable (Baoliang et al., 2006) formulations of eprinomectin has been shown to eliminate ectoparasites in rabbits. However, no studies on the use of eprinomectin against endoparasites of laboratory animals have been reported thus far. The topical application of selamectin has been reported to be effective against psoroptic and sarcoptic mite infestation in rabbits (Kurtdede et al., 2007), while the topical and oral application of selamectin has been found to be effective against ectoparasite, hookworm, ascarid and immature heartworm infections in cats and dogs (Bishop et al., 2000). The topical application of selamectin (0.6 and 6.0 mg/kg) has been determined to be ineffective against *Syphacia* sp. infection in mice and rats (Hill et al.,

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2006) and its effect against *Syphacia obvelata* in infected mice was reported to be a 36.7% reduction via topical application of a dose of 10 mg/kg (Gönenç et al., 2006). No studies investigating the effect of orally administered selamectin against *S. muris* infection in rats have been noted.

In this study, we sought to determine the acute effects of single doses of orally administered doramectin, eprinomectin and selamectin on *S. muris* infection in rats using pathologic and hematologic assessment methods.

2. Materials and methods

2.1. Rats and study design

Twenty-eight Sprague–Dawley rats (males, 6 months, weight range 150–200 g) were selected from a conventional colony naturally infected with *S. muris*. Infections were detected through the use of perianal cellophane impressions. Infected rats were divided into four groups: three treated groups ($n = 7$) and one positive control ($n = 7$). Treated groups were composed of rats treated with doramectin, eprinomectin and selamectin. The treated and control groups were kept at 20–25 °C, with 35–50% relative humidity (Thermohygrometer, Eurofrost – China), in a light/dark cycle of 12 h and 10–15 changes of fresh air per hour. The groups were kept in four different locations under the same environmental conditions. Each group was ventilated separately. The dimensions of the cages were 38 × 22 × 15 cm. Each animal was kept in a separate cage. Cages and implements were scrubbed with anionic detergent (Berilon, Berpa Ltd., İstanbul – Turkey) using hot water (60 °C) by hand and final rinse at 60 °C. The rats were fed a commercial pelleted rodent diet (Tınaztepe – Turkey). The rats were provided acidified water (pH 2.5). Water and food were provided *ad libitum*.

The experimental protocols were approved by the Animal Care and Use Committee at Afyon Kocatepe University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Parasitological procedure

Cellophane tape preparations were obtained from the treated rats on day 0 pre-treatment. *Syphacia* sp. eggs present in each rat were counted separately. A single-dose of doramectin (Dectomax®, Pfizer) 0.4 mg/kg, selamectin (Stronghold®, Pfizer) 10 mg/kg, and eprinomectin (Eprinex®, Topkim) 15 mg/kg, was diluted in 0.8 ml sesame oil and administered by gavage (16 × 7.5 Gug Curved 7.4.0 IMS dosing canulla Harwart). Nothing was administered to the positive control group.

Cellophane tape preparations were obtained from the treated rats on days 2, 4 and 6 post-treatment. *Syphacia* sp. eggs present in each rat were counted separately. In order to remove residual Syphacia eggs after each cellophane tape impression, the perianal region was washed out with 2% creolin. Egg count percent reduction (ECR) of the rats in the treated groups was calculated by comparing pre-treatment (day 0) egg counts with egg counts on days 2, 4 and 6 post-treatment, calculated separately for each day using a modified formula from the literature (Oge et al., 2000)

$$\text{ECR } (\%) = (a - b/a) \times 100$$

ECR = a is the geometric mean pre-treatment egg count per cellophane tape, b is the geometric mean post-treatment egg count per cellophane tape.

Treated groups and the control group were anesthetized on day 7 post-treatment (Ketamine 21.2 mg/kg; Xylazine 4.2 mg/kg intramuscular). Blood samples from rats in all groups were obtained via intracardiac puncture into heparinated tubes for hematologic examination. Subsequently, the rats were euthanized by cervical dislocation and necropsied to determine the effects of the treatments. Organs (heart, liver, kidneys, stomach and intestines) were dissected for pathologic examination.

The large intestine was separated from the small intestine by ligature and placed into a Petri dish. Subsequently, the cecum and the colon were separated from each other by ligature, and both were opened longitudinally with a pair of scissors in different Petri dishes, followed by removal of the cecal and colonic contents. The cecal and colonic mucosae were washed with normal saline (0.9% w/v of NaCl) with a fine-tip brush. Cecal and colonic contents were obtained in small portions and examined under stereomicroscope (SZ2-ILST, Olympus) (4× objective). The parasites found were placed into normal saline (0.9% w/v of NaCl) in a separate Petri dish with a pair of fine-tipped forceps. Parasites were placed between thin glass covers, identified under light microscope (CX21FS1, Olympus, 4× objective) (Hendrix, 1998; Anderson, 2000), and counted. In treated groups, the efficacy of doramectin, eprinomectin and selamectin in reducing adult *S. muris* infection was calculated using the following formula (Oge et al., 2000; Gönenç et al., 2006)

$$\text{Efficacy } (\%) = (a - b/a) \times 100$$

Efficacy = a is the geometric mean number of adult *S. muris* in positive control rats, b is the geometric mean number of adult *S. muris* in treated rats.

2.3. Pathological procedure

Tissue samples obtained from heart, liver, kidneys, stomach, cecum and colon tissues were fixed using tamponated 10% formaldehyde. Tissue samples were processed using routine methods and 4–5 μm thick cross-sections were taken. Tissues were stained with hematoxylin and eosin and examined under a light microscope.

2.4. Hematological procedure

Red blood cell (RBC) and white blood cell (WBC) counts, hematocrit value (PVC), hemoglobin quantity, and percentage of WBCs were determined according to the method of Feldman et al. (2000).

2.5. Statistical analysis

The collected data were analyzed using SPSS. Data were analyzed using Friedman's test for repeated measures, and Tukey HSD multiple comparison tests and Mann–Whitney U-tests with statistical significance set at $\alpha = 0.05$.

3. Results

3.1. Parasitological result

Cellophane tape samples obtained from treated rats on days 0, 2, 4 and 6 were evaluated (Figs. 1–3). On day 2 of treatment, eprinomectin was found to be 100% effective in eliminating the observed eggs, and this effect was observed to last through days 4 and 6 (Fig. 2). Eprinomectin was found to be more effective than doramectin and selamectin on day 2 post-treatment (Table 3). However, both doramectin and selamectin treatments were also able to reduce egg counts until day 6 (Figs. 1 and 2). When compared with pre-treatment, the reduction of egg counts of the doramectin-treated group was found to be 44.80% on day 2, 79.30% on day 4, and 99.32% on day 6 (Table 3), while the reduction in egg counts of the selamectin-treated group was found to be 66.49% on day 2, 89.63% on day 4, and 98.77% on day 6. The observed