

THE DYNAMICS OF CREATININE AND UREA CONCENTRATIONS IN THE BLOOD SERUM
OF RABBITS INFECTED BY *ENCEPHALITOZOOM CUNICULI* MICROSPORIDIUM AND
TREATED WITH ALBENDAZOLE

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In this work the concentrations of creatinine and urea in the blood serum of rabbits infected by Encephalitozoon (E) cuniculi and treated with albendazole was investigated. A significant decrease in mean creatinine concentration compared to the initial level (day 0) was observed from days 7 to 45. A marked decrease in mean urea concentrations was recorded only on day 60. The above-mentioned changes in observed biochemical parameters were not clinically manifested. Albendazole administered twice a week at 5 mg kg⁻¹ created a sufficiently protective shield for animals from days 7 to 78 of the experiment biochemical parameters were not.

Key words: encephalitozoonosis, creatinine, urea, rabbits, albendazole

INTRODUCTION

Encephalitozoonosis is a dangerous invasive disease of rabbits. It is caused by the intracellular protozoal parasite *Encephalitozoon (E) cuniculi* (*Nosema cuniculi*), which was first described by Levaditi, Nicolau, and Schoen in 1923 as a microsporidial parasite occurring in rodents, lagomorphs, carnivores, and primates (Canning and Lom, 1986). The disease usually has a subclinical-latent course, accompanied by lesions in the kidneys, brain and also in other organs (Scharmann et al., 1986; Koudela et al., 1993). However, the preferred site for the parasites is the kidney. Clinical symptoms of the disease are motor paralysis of the rabbit (Jurišek et al., 1993).

Important indicators of renal dysfunction are changes in creatinine and urea concentrations in the blood serum of animals (Schirmeister et al., 1969; Reder and Hartman, 1995). Recently many papers have drawn attention to

the considerable antimicrosporidal activity of albendazole (Beauvais et al., 1994; Koudela et al., 1994).

On the basis of this knowledge we decided to carry out an experiment to observe the effect of albendazole in rabbits infected by microsporidia *Encephalitozoon cuniculi* including the dynamics of creatinine and urea concentrations in the blood serum

MATERIALS AND METHODS

A total of 7 female rabbits (New Zealand white) were used in the experiment with a mean body weight of 2.8 ± 0.4 kg at the age of 6 months. They were negative for coccidiosis, pasteurellosis, viral haemorrhagic disease of rabbits and encephalitozoonosis. The animals were placed in cages under standard zoohygienic conditions, in keeping with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (1989). A commercially produced feed mixture (KK; Čečejevce, Slovak Republic) was provided. Ad libitum access to feed and water was allowed.

Animals were infected by microsporidia feed *Encephalitozoon cuniculi* with a single intraperitoneal dose of $5 \cdot 10^7$ ml⁻¹. From the 7th day after inoculation, albendazole in a pharmaceutical preparation known as Aldifal 2.5% susp. (produced in Mevak-Nitra, Slovak Republic) was administered to animals *per os* at a dose of 5 mg kg⁻¹ body weight two times a week for 11 weeks. Blood was taken from the *v. auricularis marginalis* at the beginning of the experiment, before infecting the animals (day 0) and then on days 7, 14, 21, 30, 45, 60, 90, 120 and 150 of the experiment. Concentrations of creatinine and urea in the blood serum were determined using the BIOLA test (Lachema Brno). Results were evaluated by the computer programme Excel 5. Separate measurement data were compared by the two choice t-test with the values for day 0 of the experiment.

RESULTS

The average values for creatinine concentrations in the blood serum of rabbits during the experiment are given in Figure 1. On day 0 this value was 177.15 mmol/l with which the data of later measurements were compared. A significant decrease ($P < 0.05$) in mean serum creatinine was found on days 7 (139.39 mmol/l), 21 (128.56 mmol/l) and 45 (131.99 mmol/l) of the experiment. The concentration of creatinine in serum showed a greater decrease on day 14 (109.53 mmol/l; $P < 0.01$).

The dynamics of mean urea concentrations in blood serum is shown in Figure 2. A slight decrease was observed starting on day 30 of the experiment. A statistically significant difference was found only on day 60 of the experiment (4.13 mmol/l⁻¹, $P < 0.05$) compared to day 0 (6.97 mol/l⁻¹). The increase in mean urea concentration on day 120 (7.7 mol/l⁻¹) compared to that on day 0 was not statistically significant.

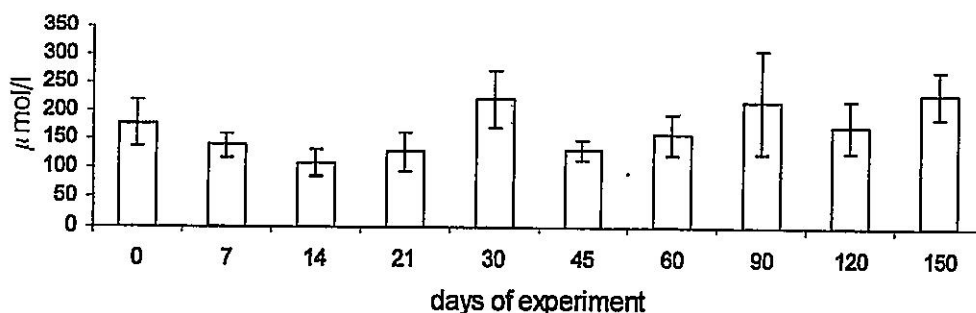


Fig 1. The concentration of creatinine ($\mu\text{mol/l}$) in the blood serum of rabbits infected by *E. cuniculi* during albendazole therapy in the dose of 5 mg/kg b.w.

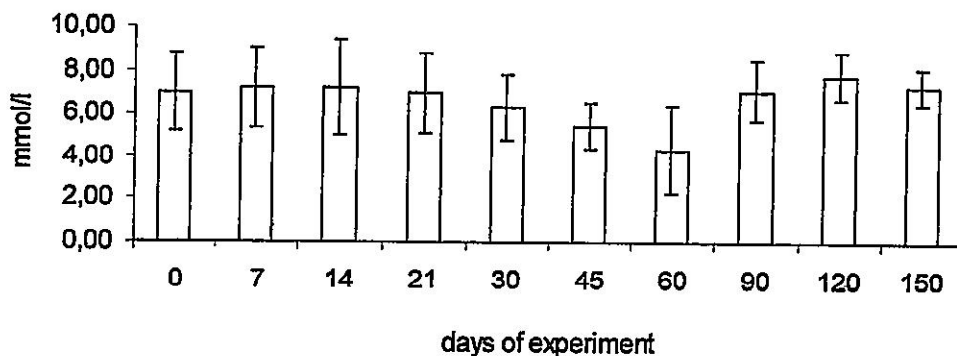


Fig. 2. The concentration of urea (mmol/l) in the blood serum of rabbits infected by *E. cuniculi* during albendazole therapy in the dose of 5mg/kg b.w.

DISCUSSION

Pharmaceuticals based on albendazole are used in veterinary medicine for the treatment or prevention of various parasitic diseases of animals. The minimum inhibitory concentration in which albendazole showed significant activity in the test in vitro against *E. cuniculi* was 0.015 mg/ml^{-1} (Ditrich, 1994). However, a 90% inhibition of *E. cuniculi* growth had already been observed at 0.005 mg/ml^{-1} concentration of albendazole (Beauvais et al., 1994). The

antimicrosporidal effect of albendazole in immunodeficient mice inoculated with *E. cuniculi* was studied by Koudela et al. (1994). In groups which were treated with albendazole in doses of 5 or 50 mg/kg⁻¹ for 14 days neither histopathological changes nor microsporidia were found at necropsy. Both doses caused the elimination of *E. cuniculi* after two weeks, but when the therapy was interrupted microsporidiosis regenerated again and mice perished within three weeks. It is not clear if this was caused by a persistent infection or if the mice were reinfected by a contaminated environment. Albendazole can also be used in the treatment of microsporidiosis in people positive for AIDS (van Goll et al., 1993) including invasions caused by microsporidia belonging to or morphologically similar to the genus *Encephalitozoon* (Lecuit et al., 1994, Molina et al., 1998). Its effect on the parasite consists in blocking of cell division by binding to the colchicine-sensitive site of tubulin with consequent inhibition of its polymerization into microtubules (Orenstein, 1991). After 16 weeks of albendazole therapy, samples of body excreta taken from an immunodeficient patient, were found not to contain the causative agent of the disease (Lecuit et al., 1994). In our experiment, we chose a longer variant as 14 days therapy seemed less effective. We can say that the chosen therapeutic programme was successful. After finishing albendazole therapy on day 78 none of the experimental rabbits perished before the end of the experiment (day 150). This positive result was expected as therapy started early (on day 7 after parasite inoculation) and also because the animals included in the experiment were not immunodeficient and so their organism responded well to the pathogen.

Histo-pathological findings, especially in the kidneys and brain, confirms the diagnosis of encephalitozoonosis (Scharmann et al., 1986; Koudela et al., 1993). Fewer lesions were observed in the liver, lungs and heart, but these were evaluated as part of the general immune response of the organism (Scharmann et al., 1986). On the basis of these data, we concentrated our attention on the observation of creatinine and urea in the blood serum of rabbits as indicators of renal dysfunction. The test for determining creatinine in blood plasma is used when acute or chronic types of renal disease are suspected, and also to screen renal damage. It aids the assessment and diagnosis of renal diseases (Meško et al., 1988). Botha et al., (1986) confirmed progressive irreversible lesions of the kidneys in dogs infected by *E. cuniculi* by biopsy. At the same time they found changes of creatinine and urea levels in the blood serum of dogs. Although at the beginning of the disease, there was a reduction, with the development of the disease a slight increase was recorded. An increased concentration of urea was also detected by Mc Innes and Stewart (1991) in one of two female dogs with a positive titre against *E. cuniculi*. Spores of the causative agent of the disease were detected in the renal tubules.

During our experiment, statistically significant increases in these two parameters were not observed. The apparent decrease in creatinine from day 7 to 45, excluding day 30, had no negative influence on the health status of animals. Similarly a significant decrease in urea in the blood serum of rabbits on day 60 was not clinically manifested. We suppose that this effect could be

caused by albendazole. It is known that some drugs interfere in the biochemical interactions of living organisms (Katzung, 1995, Meško et al., 1998). However we have not confirmed this hypothesis in relation to albendazole yet.

We suppose that our present results could contribute to an increase in the knowledge about encephalitozoonosis and its therapy. It would be favourable to discuss also the medication of feed mixtures by benzimidazole drugs, especially in large-scale breedings of fur animals, where according to Persin and Dousek (1986) microsporidiosis causes significant economic losses.

A c k n o w l e d g e m e n t s

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**DINAMIKA PROMENA U KONCENTRACIJI KREATININA I UREE U SERUMU KUNIĆA
INFICIRANIH SA MIKROSPORIDIJOM *ENCEPHALITOZOON CUNICULI* I TRETIRANIH
ALBENDAZOLOM**

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SADRŽAJ

U ovom radu su izneti rezultati određivanja koncentracije kreatinina i uree u krvnom serumu kunića inficiranih sa *Encephalitozoon cuniculi* i tretiranih albendazolom. Uočeno je značajno smanjenje srednje vrednosti koncentracije kreatinina u serumu kunića od 7. do 45. dana ogleda u poređenju sa početnim vrednostima (0 dan). Značajno smanjenje srednje vrednosti za koncentraciju uree primećeno je samo 60. dana ogleda. Navedene promene u vrednostima biohemijskih parametara nisu bile klinički manifestne. Albendazol, aplikovan dva puta nedeljno u dozi od 5 mg/kg pružao je zadovoljavajuću zaštitu kunićima od 7. do 78. dana ogleda.