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THE DEVELOPMENT OF ENCEPHALITOZOONOSIS IN RABBITS AFTER INFECTION BY *ENCEPHALITOZOON CUNICULI* AND TREATMENT WITH ALBENDAZOLE.

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The influence of albendazole on the development of encephalitozoonosis induced experimentally by E. cuniculi in adult female rabbits of the New Zealand white breed was studied with the aim of observing the development of clinical symptoms of the disease in relation to serological prevalence.

The animals were infected by E. cuniculi microsporidia with a single intraperitoneal dose of $5 \cdot 107 \cdot ml^{-1}$. Albendazole (Aldifal 2.5% susp. a.u.v.; Mevak, Slovakia) was administered by orogastric intubation in a dose of 5 mg · kg^{-1} of body weight. It was given twice a week for 11 weeks from day 7 after inoculation. The titre of antibodies was determined by the IFAT method (test of indirect immunofluorescence according to Chaloupsky et al. (1971) on day 0 (before infecting the animals) and then on days 7, 14, 21, 30, 45, 60, 90, and 120 of the experiment after infecting the animals. The detected low titre of specific serum antibodies corresponded with the course of the disease. From the middle of the experiment, conjunctivitis and lacrimation were recorded. Eye symptoms were most probably induced by sporular infection from the environment.

Key words: rabbits, encephalitozoonosis, albendazole therapy, titre of antibodies

INTRODUCTION

During evolution, microsporidia have completely adapted to the parasitic way of life inside the host cell. As they do not have mitochondria they are dependent on the energy metabolism of the host cell and they develop only intracellularly.

From the epizootologicaland epidemiological points of view *Encephalitozoon cuniculi* (*E. cuniculi*) and *Encephalitozoon hellem* have an important position with in the *Encephalitozoon species.* During 1964-1970. *E cuniculi* was called *Nosema cuniculi* because Weiser (1964) and Lainson et

al.(1964) concluded that it belonged to the *Nosematidae* family and not to *Encephalitozoonidae*. At the beginning of the 70's the original name, *Encephalitozoon cuniculi*, started to be used again on the basis of Cali's studies (1970). *E cuniculi* is the only known microsporidian, which is an obligate intracellular parasite of mamamals (rodents, rabbits, carnivores, primates) and birds. Typical mammal hosts of *E.cuniculi* are rodeents and rabbits, However, in the large-scale breeding of rabbits encephalitozoonosis takes a chronic asymptomatic-latent form. It can cause significant economic losses because of the loss of body weight (Flatt and Jackson, 1970; Pattison et 1971; Vavra and Chaloupsky, 1980). As a result of various immunosueppressive factors, the latent form can develop into the clinical one (Balent et al., 1995). In that case the economic losses are multiplied. Because of this and since encephalitozoonosis has a zoonotic character, prevention and therapy are most appropriate courses. The rules of preventive measures proposed by Cox et al., (1977) end Bywater and Kellet (1978) for the breeding of rabbits are accepted in general. Works concerning the problems of pharmaceutical studies of *Encephalitozoon cuniculi in vitro* conditions demonstrate that the drugs examined had a higher or lower antiicrosporidial effect, but that in vivo they were not very effective or had a high toxicity (Shadduck, 1980; Pakes and Gerrity, 1989). In conditions *in vivo* albendazole seems to be the most efficacious. This is supported by the studies of van Gool (1993) in people suffering from AIDS with disseminated encephlitozoonosis. Taking all this into account we describe here the influence of albendazole on the development of encephalitozoonosis experimentally induced by *E. cuniculi* in rabbits with the aim of observing the development clinical symptoms of the disease in relation to serological prevalence of the parasite.

MATERIALS AND METHODS

In the experiment we used 7 adult female rabbits of the New Zealand white breed, 6 months old, with a mean body weight of 2.8 $\pm$ 0.4 kg. The animals were kept in cages under standard zoohygienic conditions according to the rules of the European Convention on the Protection of Animals (1989). They were fed a standard feed mixture (KK) and their access to feed and water was ad libitum.

Animals were infected by microsporidia of *Encephalitozoon cuniculi* with a single intraperitoneal dose of $5 \cdot 10^7$ ml^{-1}.

Albendazole in the form of the pharmaceutical preparation Aldifal (2.5% susp. a.u.v., Mevak, Slovakia) was administered *per os* with tube in a dose of 5 mg kg^{-1} body weight in a volume of 0.2 ml. It was given twice a week for 11 weeks from day 7 after inoculation. Each animal received it 21 times.

The titre of antibodies was determined by the IFAT method (test of indirect immunofluorescence of antibodies) according to Chalupsky et al., (1971) on day 0 (one day before infecting the animals) and then on days 7, 14, 21, 30, 45, 60, 90, and 120 of the experiment. During the experiment, food intake and

general health status were observed twice a week to detect any possible signs of disease in a clinical form. Body weight was recorded before infecting the animals, in the middle, and at the end of the experiment. Changes of body weight were evaluated by Student's t-test.

RESULTS

An antibody response to *E. cuniculi* antigens (before albendazole was administered) was observed in 5 out of 7 rabbits (rabbits No. 1, 4, 5, 6, and 7) on day 7 after infecting the animals (71,42%. The titre of antibodies was low (1:32) and not considered positive. (Table 1.) In the other two animals no antibody response was recorded.

Table 1. The titre of antibodies in the course of encephalitozoonosis in rabbits under albendazole therapy from day 7 to 87 of the experiment

Rabbit No.	Inicu-lation	Titre of antibodies							
	day 0	day 7	day 14	day 21	day 30	day 45	day 60	day 90	day 120
1	–	1:32	–	1:128	–	1:64	1:64	1:64	1:64
2	–	–	–	–	–	–	1:64	1:64	1:64
3	–	–	–	–	1:64	1:64	–	–	1:32
4	–	1:32	–	–	–	1:64	1:64	–	–
5	–	1:32	–	1:64	1:64	1:64	1:64	1:64	1:64
6	–	1:32	1:32	1:64	1:64	1:64	1:64	1:64	1:64
7	–	1:32	–	–	–	–	–	–	–

On day 14 after inoculation (and after 2 subsequent doses of albendazole) there was no antibody response in 6 rabbits (85.71%) and the same low titre of antibodies remained in one rabbit (No.6).

Positive results (an increase in antibody titre from 1:32 to 1:64) in two animals (rabbits No. 5 and 6) and an increase to titre 1:128 in rabbit No. 1 were observed on day 21, in rabbit No. 3 on day 30, in rabbit No. 4 on day 45, and in rabbit No 2 only on day 60 after inoculation.

A titre of antibodies (1:64) was also registered in four animals (No. 1,2,5, and 6) on days 90 and 120 of the experiment, when albendazole was no longer administered.

On these days negative titres were recorded in three animals (No.3,4, and 7). In rabbit No. 7 negative samples of serum were registered from day 14 to the end of the experiment.

The body weight of the rabbits during the course of encephalitozoonosis after repeated administration of albendazole is shown in Figure 1. It is evident that mean body weight slightly decreased in the middle and at the end of experiment compared to initial figures. The differences were not statistically significant.

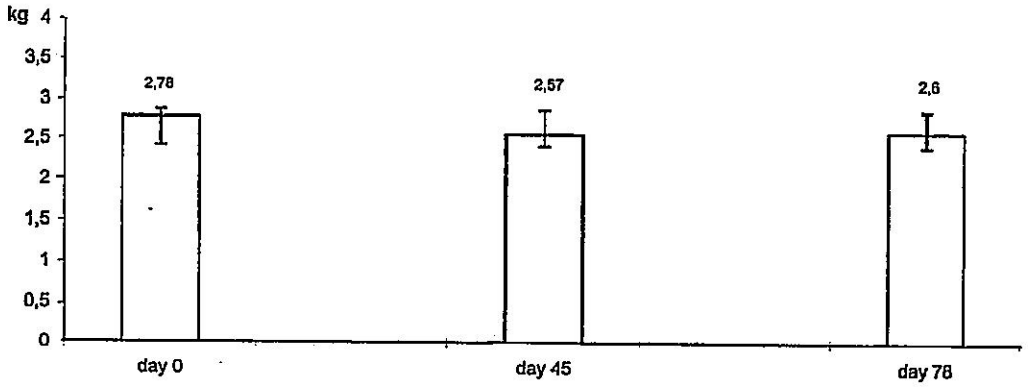

Figure 1. The body weight of rabbits in the course of encephalitozoonosis undergoing repeated albendazole administration (21 times) at a dose of 5 mg. kg^{-1} body weight.

During the experiment no symtpoms of the clinical form of encephalitozoonosis were registered. Conjunctivitis and lacrimation of both eyes were recorded in most rabbits from about the middle of the experiment. For 2-3 weeks after this time mild hair loss was recorded in the rabbits, when handled.

DISCUSSION

Encephalitozoon cuniculi is able to survive in the organism of the host despite the latter's active immune response. Production of antibodies activated by microsporidia signifies a latent course of encephalitozoonosis. To prevent the activation of the latent to the symptomatic form of the disease, an important role is played not only by antibodies but also by cell responses. Vavra (1996) states that, in people, the cell response is the determining agent in the prevention of the symptomatic form of the disease. Thus a decrease in CD4 lymphocytes in blood under the level of $100/mm^3$ is the onset factor of microsporidiosis in patients in the terinal stage of AIDS. Latent asymptomatic infection lasts only while microsporidia multiplication and the immune responses of the host are in balance. From this aspect it is evident that pharmacotherapy can upset this balance with a negative influence on microsporidia multiplication. Fumagilin (Schadduck, 1980), chlorochin and oxytetracyclin (Pakes and Gerrity, 1989) had a considerable effect in suppressing the growth and multiplication of microsporidia in cell cultures. Sulfafurazole potentiated by trimethotrim also had a certain antiprotozoal effect (Waller, 1979).

Studies suggest that albendazole may be the most advantageous preparation in conditions *in vitro* and *in vivo*. Thus, an antimicrosporidal effect of albendazole in vitro against *E. cuniculi* was recorded at a concentration of 0.015 mg/ml^{-1} (Ditrich, 1994). A concentration of 0.005 mg. ml^{-1}, led to a 90%

growth inhibition of *E. cuniculi* (Beuvais et al. 1994). Albendazole in vitro inhibits the growth of various protozoal parasites, reduces the number of microsporidia, and causes growth deformities in *Encephalitozoon spp.* spores (Canning et al., 1992, 1993). Koudela et al. (1994) studied the microsporicidial effect of albendazole in immunodeficient mice, which were experimentally infected by *E. cuniculi*. Neither histopathological changes nor *E. cuniculi* were found after albendazole administration in doses of 5 and 50 $mg \cdot kg^{-1}$ body weight for 21 days. After two weeks administration, elimination of *E. cuniculi* was observed, but when therapy was interrupted, and exacerbation of the disease occurred with the consequent loss of animals for 3 weeks. The drug was also tested with encephalitozoonosis *(E. cuniculi, E. hellen)* in people with AIDS (van Gool, 1993, Lecuit et al., 1994).

The results of both studies suggest an improvement in the patient' clinical status and the elimination of microsporidia. Orestein (1991) explains the antimicrosporidial effect of albendazole by the way that it binds to colchicine-sensitive areas of tubulin resulting in inhibition of its polymerization in to microturbules, which leads to the blocking of cell division.

Our results suggest that the administration of albendazole decreases antibody responses to *E. cuniculi* antigens by inhibiting microsporidia multiplication. This is indicated by the fact that the antibody response (titre of antibodies = 1:32) found in 5 animals on day 7 after inoculation with *E. cuniculi* was so weak after two doses of albendazole (on day 14), that specific antibodies were not detected, apart from one animal. It can also be seen from the finding that positive samples of serum with a low titre of antibodies (1:64) were regularly recorded from day 21 up to the end of the experiment or from the fact that we detected three negative samples of sera on days 90 and 120 of the experiment. The low titre of specific serum antibodies corresponded with the course of the disease. Encephalitozoonosis ran in a latent from. After finishing albendazole administration (78^{th} day), the disease did not break out into a clinical form. The latent course lasted until the end of the experiment, i. e. to day 150. We did not record signs of the clinical form in rabbits (motor paresis especially of the hind quarters, torticollis, convulsions, tremors, and coma) described by Innes et al., (1962), Wright and Craighead (1992) and others. Nevertheless, we noted conjunctivitis and lacrimation from the middle of the experiment. These ophthalmic signs recorded during the course of the asymptomatic, latent form of the disease were most probably caused by the eyes being infected with spores from the environment. We do not suggest that it was an allergic reaction or contamination of the inoculum e. g. by mycoplasmas and so on.

In connection with the above-mentioned ophthalmic findings, the observation of Bjerkas (1990) is interesting. He found lesions in the cornea and crystalline lens with a gradual deterioration of sight or even blindness in mink bred on farms in the course of chronic encephalitozoonosis (induced by *E. cuniculi*). Typical eye infections in people *(E. hellem, E. cuniculi)* result from direct contamination by spores from the environment.

The mild decrease in body weight in rabbits registered in the middle and at the end of the experiment comapred to initial body weight had no connection with encephalitozoonosis. However, it was related to oesophagus irritation (long-lasting and frequent orogastric intubation) and stress. The tendency of the rabbits to lose a litle hair when they are handled lasted about 2 weeks and was probably caused by a temporary increase in the temperature of the rabbit rooms due to the failure of the thermostat.

Our results concerning the influence of albendazole on the development of encephalitozoonosis in rabbits indicate its inhibitory effect on the development of the disease, and suggest that it is very well tolerated by rabbits. Changes of the biochemical parameters, serum urea and creatinine concentrations (Čonkova et al. 1993), also testify to its positive influence on encephalitozoonosis development.

If our results pointing out the suppressant influence of albendazole on encephalitozoonosis development in rabbits be confirmed, consideration about the possible medications of feed mixtures by albendazole in rabbit breeding would be well-founded. The above-mentioned considerations could be supported by the fact that albendazole was very well tolerated by rabbits despite the long period of administration. The current problems of the whole range of encephalitozoonoses require more target studies with therapeutical possibilities in mind.

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RAZVOJ ENCEPHALITOZOONOZE KOD KUNIĆA POSLE INFEKCIJE SA *ENCEPHALITOZOON CUNICULI* I TRETMAN SA ALBENDAZOLOM

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SADRŽAJ

U radu je ispitivan uticaj albendazola na pojavu encefalitozonoze, eksperimentalno izazvane sa *E. cuniculi* kod odraslih ženki kunića novozelandske bele rase u cilju praćenja kliničke stike i serološke prevalence.

Životinje su inficirane sa mikrosporama *E. cuniculi*, jednom dozom $5 \cdot 10^7$ m^{-1}. Albendazol (Aldifal 2,5%. Mevak, Slovakia) je aplikovan želudačnom sondom u dozi od 5 mg/kg^{-1} telesne mase, dva puta nedeljno, tokom 11 nedelja, počev od 7. dana po inokulaciji. Titar antitela je određivan IFAT metodom (test indirektne imunoflorescence) prema Chatoupsky (1971), nultog dana, 7, 14, 21,21, 31, 45, 60, 90 i 120 dana eksperimenta. Ustanovljen niski titar specifičnih antitela bio je u skladu sa tokom bolesti. Kod životinja se pojavilo suzenje i konjuktivitis sredinom eksperimenta. Ovi simptomi su bili indukovani infekcijama sporama iz spoljašnje sredine.