Pro-oxidant effects of Mebendazole in albino rats experimentally infected with *Trichinella spiralis*

V. A. Tolstoj • A. Lytvynets • L Langrova

Abstract Trichinellosis treated with Mebendazole often leads to complications m the course of the disease in humans and animals as a result of intoxication and hyper-sensitization of an organism due to the massive destruction of parasites. This study was conducted to research Mebendazole incidence on lipid peroxidation processes (LPP) in rats' blood in *Trichinella spiralis*-infected and parasite-free albino rats. The research was conducted to evaluate erythrocyte superoxide dismutase (SOD) activity and malonic dialdehyde (MDA) concentration in blood serum. The parameters of the SOD-MDA system in infected albino rats, which were treated or untreated with Mebendazole were analyzed. It was concluded that Mebendazole amplifies the LPP in the blood of both infected and parasite-free animals: in a dose of 150 mg/kg anthelmintic causes disturbances of biochemical homeostasis in the SOD-MDA system, thus working as a pro-oxidant

V. A. Tolstoj
Department of Biology, Belarus State Medical University,
Dzerzhinskogo Street 83,
220116 Minsk, Belonissia

A. Lytvynets
Department of Laboratory Animal Breeding and Hygiene..
Institute of Physiology,
Academy of Sciences of the Czech Republic,
Videnska 1083,
142 20 Prague 4-Krc, Czech Republic

A. Lytvynets (✉) I. Langrova
Department of Zoology and Fisheries, Faculty of Agrobiology,
Food and Natural Resources,
Czech University of Agriculture in Prague,
Kamycka 129.,
165 21 Prague 6-Suchdol, Czech Republic
e-mail: litvinec@biomed .cas.cz

Introduction

Mebendazole is one of the most effective anthelraintics from the imidazole group and widely applied in medicine and veterinary science for the treatment of trichinellosis. Mebendazole effectiveness is attributed to its immunomodulatory properties (Astaf'ev 1987; Burak and Ozeretskovskaia 1990) and also to its ability to suppress the processes of glycolysis and reproductive functions in trichinells (Bekish et al. 1979; Kharkevich 1999).

With regard to our observation results and to the facts noted by other authors (Bekish et al. 1979; Britov 1982; Kociecka 1996; Tolstoj 2005), Mebendazole application as an experimental treatment for trichinellosis often leads to the expressed symptoms of intoxication with the subsequent death of a significant number of experimental animals. The effects discussed earlier are associated with the hyper-sensitization of the organism by antigens and matabolites released from

the destroyed parasites (Britov 1982; Senutaite 1990; Bogoiavlenskii et al. 1994). However, considering the chemical nature and pharmacological characteristics of Mebendazole, it is possible to assume that it can have toxic effects not only on the parasites, but also on the host organism.

It was determined by other researchers (Hadaas and Gustowska 1995; Boczon et al. 1996; Derda et al. 2004) that while the organism is infected with trichinellosis, lipid peroxidation processes (LPP) are activated. Superoxide dismutase (SOD) is a basic enzyme that regulates the processes of free radical lipid oxidation. Enzyme activity depends on the concentration of the superoxide anion radicals initiating chain reactions of the LPP (Vladimirov and Archakov 1972).

The LPP products, malonic dialdehyde (MDA) in particular, possess expressed cytotoxic incidence (Vladimirov and

Archakov 1972) that allows them to assume their essential value in the development and the course of trichmellosis infection. Information about the dynamics of LPP indicators during trichineilosis treated with anthelmintics is very scarce in literature.

We have not discovered any data on the influence of Mebendazole on lipid peroxidation processes in parasite-free animals.

The purpose of the present study is to investigate the effect of Mebendazole on the erythrocyte superoxide dismutase (RBC-SOD) activity and malonic dialdehyde level in the blood serum of the control and *Trichinella spiralis*-infected albino rats.

Materials and methods

Housing/animal care

For our study, we used 160 males of outbred Wistar rats with an average body weight of 200 g. The rats came from the Belarus State Medical University breeding facility and underwent an acclimatization period of 10 days.

During the experiment, the rats were housed in a conventional animal room. The animals (in groups of 2-4 per cage) were kept in type K5 clear styrofoam cages. Wood shavings were used for bedding. The room was maintained at $20\pm2°C$ and $50\pm10\%$ relative humidity with natural lighting.

The air was automatically ventilated 10-15 times per hour. Pellet rodent food and tap water in drinking bottles were given ad libitum. Cages, bedding, steel wire tops and bottles were changed twice a week.

Assessment of superoxide dismutase activity

Superoxide dismutase activity was detected by the method of Kostiuk et al. (1990). which is based on the inhibition of quercetin auto-oxidation. SOD activity was measured in diluted hemolysate (1:1,000) by the spectrophotometer CF-46 (LOMO, St. Petersburg, Russia). Experimental tests contained 0.05 ml of hemolysate; the control tests contained the same volume of distilled water. The percentage of inhibition of quercetin auto-oxidation in the experimental and control samples in 20 min (T_{sod-20}) was calculated on the basis of the formula:

$A_{sod} = T_{sod-20}$ x $1,000/C_{Hb}$

A_{sod} — superoxide dismutaseactivity {*units/mg Hb*)

1,000 — hemolysate diluting

C_{Hb} — hemoglobin volume in hemolysate (mg)

Malonic dialdehyde concentration

The concentration of MDA was determined by a speetrophotometric method (spectrophotometer CF-46), using thiobarbituric acid (Gavrilov et al. 1987). It was expressed in nmol/ml.

Study design

After the acclimatization period, the rats were randomly divided into four groups. The first one was the control (C) group and included *Trichmella*-free rats. The second group (C+M) included

Trichinella-fiee rats, which were administered with Mebendazole. The third group (T) included rats experimentally infected with T. spiralis without treatment. The fourth group (T+M) included rats experimentally infected with T. spiralis with Mebendazole treatment.

Larvae of T. *spiralis* were recovered from the infected rats by the acid-pepsin digestion method. Groups T and T+ M were infected with larvae of T. *spiralis* in a dose of 20 larvae/g body weight of the rat (Bekish et al. 1980).

The Mebendazole (Vermox, Gedeon Richter, Hungary) dose was 50 mg/kg of body weight. It was given per os by a single application for 3 days on days 8. 9 and 10 of the experiment The infected and control rats were killed by administration of an overdose of Thiopental Natricum anaesthetic (Thiopental ICN, ICN Czech Republic a.s., Czech Republic) on days 14, 21, 30 and 45 post-infection and their blood was taken for examination. Each experimental group consisted often rats for one period of the experiment

Statistics

Statistical analysis was performed using Student's t test for unpaired data. A P value of less than 0.05 was considered statistically significant.

Results and discussion

The results of our study showed that SOD activity (Table 1) in the erythrocytes of untreated animals with trichineilosis decreased on days 14-21 and was approximately 66.35% (P<0.05) of the control level. On day 30, it reached a maximum value (184.5%; P<0.05) and on day 45, it still remained high (123.8%; P<0.05) compared with the levels of the control group.

In the T+M group, the maximum activity of SOD was observed on the 14th day of infection from the control values (137%; P<0.05), but then on day 21, a significant decrease of levels to 40.4% (P<0.001) of the control values
was noticed. During the subsequent periods of observation, a strong increasing trend of levels reaching the control level was monitored.

A reliable decrease of SOD activity in erythrocytes to 68.5% on day 14 of the experiment was observed in the C+ M animals group.

Examination of MDA concentration in the blood serum of rats yielded the following results (Table 1). The levels of the T group animals were reliably increasing during the first three periods of observation, whereas a maximum MDA level was noticed on the 21st day of infection (204% compared to the control).

Table 1 SOD activity (units/mg Hb) in the erythrocytes and MDA concentration (nmol/ml) in the blood serum of laboratory rats

Days	Groups	C	T	T+M	C+M
14	SOD	114.71±11.82	71.86±9.59*	157.14±15.07*	78.57±6.86*
	MDA	1.99±0.12	2.89±0.34*	3.77±0.19**	3.52±0.36*
21	SOD	108.43±11.51	76.00±8.55*	43.86±5.61**	89.43±5.42
	MDA	2.02±0.09	4.12±0.28**	4.80±0.39**	2.71±0.20*
30	SOD	104.29±6.09	192.43±17.76*	84.29±4.41*	101.57±7.52
	MDA	2.08±0.06	3.06±0.31*	2.40±0.11*	1.95±0.08
45	SOD	109.71±5.95	135.86±8.71*	106.14±10.17	112.86±9.88
	MDA	1.93±0.08	2.26±0.23	1.84±0.13	2.09±0.19

**P<0.001; *P<0.05 compared to the control group levels.

The same trend remained in the T+M group, but on days 14-21 the MDA concentration was higher and on day 30, lower than in the T group animals. This can be explained by the trichinellosis effect. The MDA level of the C+M group on day 14 of the experiment was 176.3% and on day 21, it was 134.3% of the control group level. During the subsequent periods of observation this data did not significantly differ from group C.

Reliable differences in the determined levels were also noted between C+M and T+M groups,

which allows us to determine the degree of toxic effect of the medicine and parasitic products produced with *Trichinella* deaths as a result of Mebendazole treatment. Statistically reliable differences of SOD activity were found on days 14-21 (P<0.001) and of MDA level on days 21 (P<0.001) and 30 (P<0.05) of the experiment. During the analysis of results obtained, it was found that SOD activity on day 14 was lower in the T and C+M groups, but animals from the T+M group had higher levels than the control group.

In our opinion, this effect can be explained by the following chain of events: Metabolites produced by T, *spirallis* larvae have a suppressive effect on SOD in erythrocytes in infected animals. Mebendazole exhibits the same effect when administered to intact animals (group C+ M). Treatment with Mebendazole in group T+M causes the death of larvae and eliminate them as an SOD-inhibitive factor. SOD negative effect of Mebendazole was also eliminated (probably as a result of the chemical interaction between the drug and parasitic products). As a result, the SOD activity in the erythrocytes of infected animals (groups T+M) increases after the administration of Mebendazole.
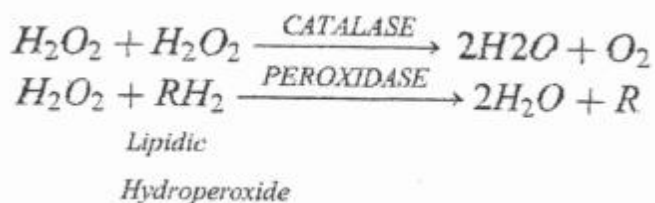
However, this process has more adaptive characteristics as a result of the acceleration of peroxidation processes caused by parasitic metabolites and treatment with medicine (Table 1). We also observed a sub-compensated condition of the host's SOD system because the MDA level of the T+M group remains high on day 14. The increased burden on the fermentative SOD system exhausts it, which is confirmed by the significant decrease of ferment activity level and the maximum MDA level of the T+M group on day 21 of the experiment.

Complex analysis of biochemical parameters revealed the discrepancy of a degree of SOD activity to MDA content in rat blood in some experimental groups. It was theoretically possible to expect that an increase of SOD activity will lead to a decrease in concentration of MDA because SOD neutralizes high aggressive superoxide anion radical in the reaction of dismutation (Vladimirov and Archakov 1972):

$$\cdot O_2^- + \cdot O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$
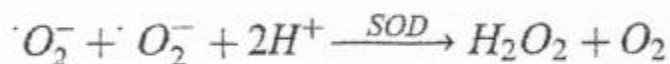
The dismutation of the anion radicals hinders the excessive initiation of free radical chain reactions in the LPP and therefore should not lead to an increase in MDA concentration. In our experiment, however, for the rats of group T on day 30 and for the rats of group T+M on the day 14 of the experiment, the concentration of MDA considerably exceeded the normal parameters despite high activity of SOD, (Table 1). The abovementioned disparity is explained in the following paragraph.

The above-stated reaction forms toxic hydrogen peroxide, which is neutralized (reduction up to water) by catalase and peroxidase enzymes and, also, by using different compounds as reducing agents.

$$H_2O_2 + H_2O_2 \xrightarrow{CATALASE} 2H2O + O_2$$
$$H_2O_2 + RH_2 \xrightarrow{PEROXIDASE} 2H_2O + R$$

Lipidic
Hydroperoxide

SOD and catalase, as a rule, function in coordination and their joint action prevents the formation of hydroxyl radicals in the reaction:

$$\cdot O_2^- + H_2O_2 \rightarrow HO^\cdot + HO^\cdot + O_2$$
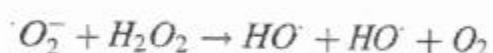
Hydroxyl radicals are powerful stimulators of the LPP and cannot be eliminated by a specific

fermentative method (Miroshnichenko 1992).

However, the catalase activity can be oppressed by an over-abundance of its own substratum-hydrogen peroxide (Miroshnichenko 1992) that leads to functional decomposition in the fermentative "SOD-catalase" system (the high activity of the SOD is sometimes accompanied by catalase inhibition). As a consequence of the decomposition of the SOD and catalase activity; the content of the hydroxyl radicals LPP stimulant is raised, which leads to a considerable increase in the MDA levels.

Thus, at certain stages of trichinellosis (and Mebendazole deworming), the host's tissues form biochemical conditions that lead to the decomposition of anti-oxidant enzymatic systems and to the expressed disturbance of metabolite homeostasis in the form of LPP hyper-activation.

For the T group it is day 30 (the height of the clinical presentations of *trichinellosis*) and for the T+M group it is day 14 (immediately after the completion of the deworming course, when perished *Trichinella's* larvae are lysed and large volumes of antigens penetrate into the animals' blood). The SOD hyper-activity in these groups at specified terms can be considered as an attempt of a macro-organism to correct its own biochemical homeostasis.

The above-described processes apparently have significant effects on the disease severity and pathogenesis of trichinellosis because the death of experimental rats treated with Mebendazole occurred particularly on days 16-23 of the experiment (four rats of the T-+-M group died; no animals died in the other groups). We noticed the gradual stabilization and balance of the SOD-MDA system in the experimental groups during later periods.

Therefore, the use of Mebendazole treatment for trichinellosis results in the significant activation of LPP in infected organisms. This is one of the reasons for trichinellosis disease complications and death of the weakest animals. Parasite-free animals receiving Mebendazole treatment showed a decrease in erythrocyte SOD activity to 68.5% and an increase in MDA concentration in the blood serum to 155.3% on the average.

The present study has demonstrated that the anthelmintic Mebendazole in 150 mg/kg doses may cause the destruction of biochemical homeostasis in the SOD-MDA system of the blood of albino rats, thus acting as a prooxidant

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