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Research Article

The antioxidant status in *Trichinella Spiralis*-infected rats, improved by Selenium supplementation

Abstract

Appearance of free radicals and membrane lipid peroxidation are one of the most typical unwelcome effects caused by trichinellosis (a parasite infection). Here the oxidative-antioxidant status of male Wistar rats uninfected and infected with *Trichinella spiralis* (Nematoda) was studied. The purpose of the study was to test substances, significantly diminishing unfavorable peroxidation effects. Selenium was chosen as a suitable defense factor. It was used in the form of the organic compound Sel-plex (Alltech). Blood biomarkers were studied in the experiment. The animals were allocated into four groups. The experiment covered 8 weeks post infection. The results showed that Sel-plex restores antioxidant defense system in Se-supplemented animals. The oxidative marker was malondialdehyde concentration. The antioxidant markers were superoxide dismutase and glutathione peroxidase activities, and concentrations of Se and vitamin E. A mathematical model was firstly proposed for the time course of host body weight. The model solutions were in good agreement with the experimental data. The relative rates of body weight gain were determined as growth kinetic parameters. The supplementation of the rats with dietary Se improved their antioxidant status, as follows: an increase by 10% in SOD activity, 6% in GPx activity, 13% in Vitamin E concentration, 17% in plasma Se concentration, and 19% in liver Se concentration respectively. A decrease by 18% in serum MDA concentration was recorded in the infected and supplemented animals. A reduction 63% in the muscle larvae after Sel-plex application was established. At week 8 the body weight gain in the supplemented rats (both uninfected and infected) was by 30% higher, compared to that in unsupplemented ones. Thus, a selenium diet could be essentially beneficial in the treatment of diseases correlated with considerable oxidative stress.

Introduction

One of the fundamental trace elements for the living organisms is Selenium (Se). This element plays a significant role in the metabolism due to its incorporation into Se-dependent enzymes. Selenium is an important antioxidant element being part of some antioxidant defense systems – enzymes and proteins with antioxidant properties (Se-dependent glutathione peroxidase, thioredoxin-reductase, selenoprotein P), involved in protection against oxidative stress [1-3]. There are plenty of evidences that the Se deficiency in human and animals causes an enhancement of reactive oxygen species (ROS) which disorder organism functions [4]. Some of highly reactive radicals participating in biological processes are in a free form and interact with various organism tissues. They are especially dangerous for the cell membranes. Among the factors leading to the rise of ROS are the parasite infections. Here our attention is drawn on the antioxidant effect of Se supplementation in the context of *Trichinella spiralis* infection.

Trichinellosis is a wide spread parasitic disease in some European countries. The larvae of *Trichinella spiralis* inhabit muscle tissue, where they migrate after their birth in intestine. In trichinellosis, pathological alterations in the epithelium of the small intestine and skeletal muscles appear. The tissue damages most probably are mediated by ROS, generated both by the parasite and by the host as its defense reaction. The formation of oxygen-derived free radicals is an initial, non-specific defense reaction of the host toward parasite infection. Thus, the antioxidant preparations may protect the host against oxidant-mediated damages and against the harmful effects of substances produced because of the host's defense response.

Changes in the activity of some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the host's muscle tissues during experimental trichinelosis have been shown to occur [5-10]. Changes in the levels of SOD and GPx as well as in the nonenzymatic total antioxidant status in the blood from mice infected with *T. spiralis* at different stages of infection have been established [9].

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Selenium exogenous supplementation is very effective in the prevention of oxidative damage (formation of free radicals and processes of lipid peroxidation) induced by parasites [11]. The involvement of Se in the host antioxidative response to parasite infections has been reported for *Trypanosoma crici* [12], *Toxocara canis* [13], and *Heligmosomoides polygyrus* [14], in mice. The results of Se application differed depending on the infection course and the parasite model used. The supplementation with Se did not lead to a general protection of the host during experimental *T. cruci* infection but helped protect the heart against inflammatory damage. Selenium supplementation of mice infected with *T. canis* reduced the number of parasites in the infected tissues but did not change the pathways of the migration of the larvae. Selenium was required for specific IL-4-related changes in intestinal physiology that promote host protection against *H. polygyrus* [14]. In mice infected with *T. spiralis* the antioxidant preparation EVETSEL (sodium selenite plus vitamin E) was applied by Karmanska et al., [15]. These authors have established that the preparation induces rapid expulsion of adult parasites from the small intestine but increases muscular larvae numbers. The mentioned studies comment the effect of Se-supplementation on the parasitaemia and host mortality in the infected animals without any biochemical analyses.

Gabrashanska et al., [16], applied dietary Se in the form of organic compound Sel-plex to broiler chickens infected with *Ascaridia galli*. The antioxidant status of the host was tested via GPx activity, liver Vitamin E, and plasma and liver Se levels. Selenium supplementation did not influence the mortality in the infected chickens but decreased the detrimental effects of ascaridiosis, improved the antioxidant status of the broilers, and showed beneficial effects on the host growth performance.

Here we first applied Se alone in trichinellosis. The objective of the present investigation was to examine the effect of Se in the organic form of Sel-plex (Alltech, Inc. Nicholasville, KY) on the oxidative and antioxidant status, mortality, and body weight in rats experimentally infected with *T. spiralis*. As criteria for the oxidative and antioxidant status of the host, were used the biochemical data indicating the responses of the oxidative biomarker, serum malondialdehyde (MDA), as well as antioxidant biomarkers: plasma and liver Se, plasma Vitamin E, and activities of Cu/Zn-SOD and GPx in blood to the parasite attack.

For the estimation of the host growth, the mathematical model elaborated by us [17], for quantitative investigation of parasite-host systems was applied. The time course of body weight was obtained and the kinetic parameters of the growth in unsupplemented and supplemented animals were calculated.

Material and Methods

Experimental settings

The experiment was conducted according to approved protocols, and in compliance with the requirements of the European Convention for Protection of Vertebrate Animals used for experimental and other Specific Purposes and the current Bulgarian laws and regulations.

Thirty two male Wistar rats, 60-day-old (weighting 100 ± 10 g), were allocated into four groups each of 8 specimens, as follows: group 1, control (healthy and unsupplemented rats); group 2, healthy and supplemented with Sel-plex (Alltech); group 3, experimentally infected with *T. spiralis* and unsupplemented; group 4, experimentally infected with *T. spiralis* and supplemented with Sel-plex (Alltech). The mortality and the body weights of the rats were recorded in each group. In parallel to groups 1, 2, 3 and 4, separate groups 1p, 2p, 3p and 4p, each of five rats, were used for determination of parasite counts, and biochemical parameters.

The rats were housed individually in cages constructed of polypropylene and glass. All animals were kept under conditions of controlled lighting with alternative dark (18.00-6.00 h) and light (6.00-18.00 h) cycles. The control and infected (non-supplemented) rats were fed with a commercial, Se adequate diet (with concentrations 0.12 mg/kg Se). Rats were allowed access to deionized water ad libitum.

Each rat from groups 3, 3p, 4 and 4p was orally infected with 1000 *T. spiralis* muscle larvae. The larvae (code ISS03) were obtained from the International Trichinella Reference Centre, Rome, Italy. After the method of Wakelin and Lloyd [18], the *T. spiralis* infective larvae were isolated by digestion of skeletal muscles from eviscerated, skinned, and minced carcasses of mice in 0.5% HCl and 0.5% pepsin at 37°C. These mice had been infected about 4 months ago.

The number of muscle larvae in the experimental rats was determined recording the larvae isolated from diaphragm. This organ alone was used because it is the usual predilection site of the *Trichinellae* according to literature data [19,20].

Treatment with Sel-plex of groups 2, 2p, 4 and 4p initiated two weeks before the infection and continued three weeks after the infection. Daily dose selenium of 0.5 mg/kg body mass in the form of Sel-plex was administrated to each rat from these groups.

Selenium, Vitamin E concentration, GPX and Cu/Zn-SOD activities (markers of the antioxidant status), and MDA concentration (index of endogenous lipid peroxidation) as well as the body weights and mortality were determined at weeks 1, 2, 4, 6, and 8 post infection (pi). The number of *T. spiralis* larvae was established at week 8 pi.

Blood samples were taken after anesthesia with ketamine, collected in heparinzed plastic tubes, and then centrifuged at 3000 g for 15 min. Plasma and erythrocytes were separated. Liver samples were collected immediately after the killing of the animals. They were placed in ice-cold 0.15 M NaCl solution and perfused with the same solution to remove blood cells. They were homogenized under standardized conditions in 9 ml ice-cold M NaCl containing 6 mg 250 mM butylated hydroxytoluene in ethanol to prevent formation of new peroxides. Of 10% homogenates were centrifuged at 10,000 *g* for 15 min at 4°C, and the supernatant was kept on ice until assayed.

For biochemical assays, Cu/Zn-SOD (EC 1.15.1.1) activity in washed erythrocytes was determined by the epinephrine

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method according to Fridovich [21] and GPx (EC 1.11.1.9) activity in full blood samples was measured spectrophotometrically according to Beutler [22]. Enzyme activities were expressed in units per milliliter blood. Plasma vitamin E concentration was determined by HPLC using a fluorescence detector [23]. Lipid peroxidation was measured by serum MDA concentration as malondialdehyde-thiobarbituric acid adductor [24]. Plasma and liver Se contents were analyzed by the fluorimetric method of Watkinson [25], with 2,3-diaminonaphthalene (DAN) as a complex reagent. The fluorescence of DAN complex was measured using a Hitachi-F-4010 fluorescence spectrophotometer (Tokyo).

For the statistical analysis, data were expressed as mean ± SD and processed by one-way analysis of variance. The differences were considered significant when P values were less than 0.05.

Mathematical model

For a quantitative description of the growth of a single eukaryote we established the use of logistic equation $dP/dt = \mu P (C - P)/C$ in an interpretation different towards this in population kinetics [17]. The quantity P is not biomass (or number) of a population. It is the specimen's body weight. The term $\mu P/C$ describes not a negative density-effect as it is in case of population development but reflects the cell genetic program determining the organism growth. The parameter μ ($[\mu] = [\text{day}^{-1}]$) is the relative rate of the increase of body weight.

The experimental data allow consider two stages of rat's growth within the experimental period. No statistically significant differences were observed between groups 1 and 3, and groups 2 and 4, respectively. Thus, the following ordinary non-linear equations for the kinetics of mean body weight P ($[P] = [\text{g}]$) of the host may be written:

$$\frac{dP_i}{dt} = \mu^1_i P_i \frac{C^1_i - P_i}{C^1_i} + \mu^2_i P_i \frac{C^2_i - P_i}{C^2_i} i = 1, 2 \quad (1)$$

under initial conditions:

$$t_0 = 0, \quad P_i(t_0) = P_0 \quad (2)$$

where dP/dt is the change of P with time. We assume that the development of rats takes place under the condition of substrate saturation because they take in a sufficient amount of food, i. e. μ^i = const; (i = 1, 2). Index i = 1 corresponds to first and second groups and index i = 2 corresponds to third and fourth groups. The parameters C^i and C^2 are the maximal possible values of the rat's weight for the first and second stages, respectively. The parameters μ^1 and μ^2 correspond to the first and second stages of the rat's growth. They are binary variables and have the form:

$$\mu^1 = \mu^1 \frac{-\text{sign}\,(t-T) + 1}{2}; \mu^2 = \mu^2 \frac{\text{sign}\,(t-T) + 1}{2};$$

$$[-\text{sign}\,(t-T)+1] / 2 = 1 \text{ if } t < T \quad [\text{sign}\,(t-T)+1] / 2 = 0 \quad \text{if } t < T$$
$$[-\text{sign}\,(t-T)+1] / 2 = 0 \text{ if } t > T \quad [\text{sign}\,(t-T)+1] / 2 = 1 \quad \text{if } t > T.$$

T ([day]) is the moment of "switching" from the first to the second regime of rat growth. (The transfer between two development stages does not take place simultaneously for all rats so that the moment T is related to the most likely day this transfer occurred).

For equation (1) under initial condition (2) the following analytical solution was obtained:

$$P_i = P_0 \frac{\mu^1_i + \mu^2_i}{P_0(\frac{\mu^1_i}{C^1_i} + \frac{\mu^2_i}{C^2_i}) + [\mu^1_i + \mu^2_i - P_0(\frac{\mu^1_i}{C^1_i} + \frac{\mu^2_i}{C^2_i})]e} - (\mu^1_i + \mu^2_i)t \quad (3)$$

Equation (3) can be written for one of two stages in the following form:

$$\frac{1}{P_i} = \frac{1}{C_i} + (\frac{1}{P_o} - \frac{1}{C_i})e^{-\mu_i t} \quad (4)$$

After taking in a logarithm we have:

$$-\ln(\frac{1}{P_i} - \frac{1}{C_i}) = -(\frac{1}{Po} - \frac{1}{C_i}) + \mu_i t \quad (5)$$

The relative growth rates μ^1 and μ^2 for two stages may be determined as angular coefficients of straight lines (5), using the values of P_i, measured in the experiment.

The moment T, which indicates the transfer from the first to the second stage, can be determined from the equation:

$$\frac{\mu^1}{P_0 \frac{\mu^1}{C^1} + (\mu^1 - P_0 \frac{\mu^1}{C^1})e^{-\mu^1 t}} = \frac{\mu^2}{P_0 \frac{\mu^2}{C^2} + (\mu^2 - P_0 \frac{\mu^2}{C^2})e^{-\mu^2 t}} \quad (6)$$

Results

The resulting average intensity of the worm burden at week 8 pi was 4435.8 ± 131.2 muscle larvae per gram tissue in rats without Se supplementation and 1638.7 ± 76.8 muscle larvae per gram tissue in rats with Se supplementation. As we seen a larvae reduction of 63% after Sel-plex application was realized!

For clearness and for the sake of brevity, further we shall use the following symbols for the Se concentrations: Se_1^{plasma}, Se_2^{plasma}, Se_3^{plasma}, Se_4^{plasma}, Se_1^{liver}, Se_2^{liver}, Se_3^{liver}, and Se_4^{liver}, where the indices 1,..,4 correspond to the number of the respective groups. In addition, other antioxidant biomarkers as well as the oxidative biomarker MDA will be denoted in a similar way.

The plasma and liver Se contents are presented in figures 1,2. The Se levels in the control group i. e. Se_1^{plasma} and Se_1^{liver} were quite stable during the experiment. In the healthy supplemented rats Se_2^{plasma} was 1.2 and 1.4 times higher compared to Se_1^{plasma} at the ends of weeks 1 and 8, respectively. The infected rats exhibited significantly lower Se levels compared to the control ones from week 1 to week 6 ($P < 0.05$; 0.001). At week 4 the following ratio was established: $Se_1^{plasma} / Se_3^{plasma} = 1.4$. At the end of experiment, Se_3^{plasma} was restored to the normal value ($P > 0.1$). In the infected and supplemented rats, Se_4^{plasma} after sharp decrease up to week 2 reached normal values at week 6 and remained at that level. In the infected animals Se^{liver} decreased sharply up to week 4 and then remained almost constant. At week 4 $Se_1^{liver} / Se_3^{liver} = 1.15$. Thus, the drop of Se concentration in the infected rats was about 20% less in the liver then in the plasma. During the experiment, Se_2^{liver} and Se_4^{liver} slightly increased over the level in the control group

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($P < 0.02$). Both Se_4^{plasma} and Se_4^{liver} were significantly higher than Se_3^{plasma} and Se_3^{liver}, respectively ($P < 0.01$). At week 4 we established the following relations:

$$Se_4^{plasma} / Se_3^{plasma} = Se_4^{liver} / Se_3^{liver} = 1.2 \quad (7)$$

The relations (7) show that Se levels in group 4 increased (compared to group 3) in similar pattern in plasma and liver.

MDA serum levels, indicating the lipid peroxidation induced by ROS, in control, supplemented, *T. spiralis*-infected and *T. spiralis*-infected and supplemented with Sel-plex rats, are presented in figure 3. MDA level in the control rats remained stable during the experiment. In the healthy supplemented animals, this marker showed significantly lover values. MDA concentration sharply increased up to week 4 in the infected rats from group 3 compared with the controls ($P < 0.001$). After week 4, up to the end of experiment MDA level in this group remained without changes. MDA concentration in group 4 was lower compared to that in group 3 ($P < 0.001$) and higher compared to that in the control group ($P < 0.001$). At week 8: $MDA_1 = 1.34\ MDA_2$; $MDA_3 = 1.65\ MDA_1$; $MDA_3 = 2.2\ MDA_2$; $MDA_4 = 1.37\ MDA_1$; $MDA_4 = 1.83\ MDA_2$; $MDA_3 = 1.2\ MDA_4$.

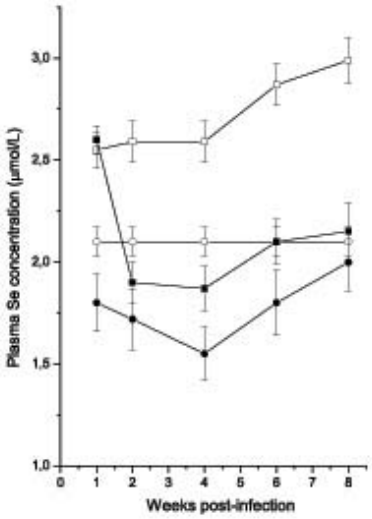

Figure 1: Changes with time of selenium concentration in the plasma of Wistar rats. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, supplemented).

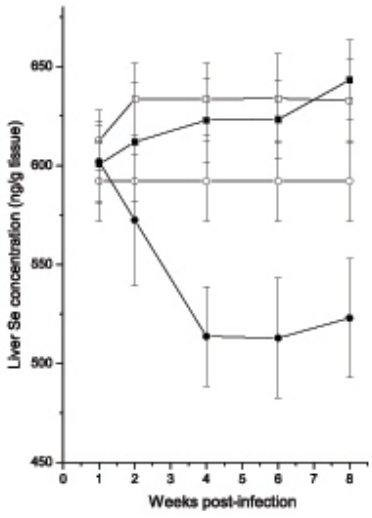

Figure 2: Changes with time of selenium concentration in the liver of Wistar rats. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, supplemented).

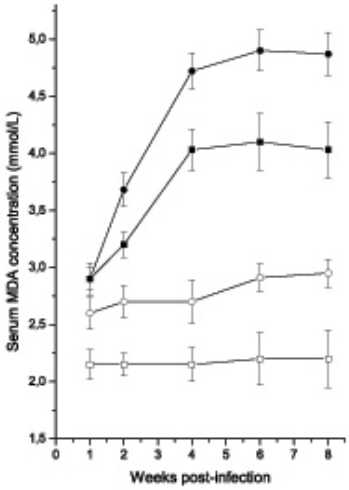

Figure 3: Changes with time of MDA concentration in the serum of Wistar rats. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, supplemented).

The activity of the antioxidant enzyme Cu/Zn-SOD in washed erythrocytes is presented in figure 4. SOD_1 and SOD_2 activities remained almost unchanged during the experiment. SOD_2 was higher than SOD_1 activity: $SOD_2 = 1.3\ SOD_1$ ($P < 0.001$). At week 1 $SOD_3 = SOD_4$ were significantly lower compared to SOD_1 ($P < 0.05$). Then they sharply increased and at week 6 and 8 they were significantly higher towards SOD_2 activity ($P < 0.01$). SOD_4 differed significantly from SOD_3 only at week 8. At this time, the following ratios take place:

$$SOD_3 / SOD_1 = 1.5 \text{ and } SOD_4 / SOD_1 = 1.7 \quad (8)$$

The activities of the blood antioxidant enzyme GPx in groups 1 and 2 were constant throughout the experiment (about 900 U/ml) and no statistically significant differences were established between these groups. GPx activities in the infected rats from groups 3 and 4 increased significantly over GPx activity in control animals. Only GPx_3 at week 1 was under the control: 820.1 ± 90.33 U/ml. The maximum GPx_3 was measured at week 6: 1070.3 ± 120.45 U/ml. At weeks 6 and 8 GPx_4 was respectively: 1100.6 ± 96.42 and 1100.3 ± 89.4 U/ml. We have the ratios:

$$GPx_3 / GPx_1 \gg GPx_4 / GPx_1 = 1.2 \quad (9)$$

The levels of GPx_3 and GPx_4 differed insignificantly one from other during the experiment ($P > 0.1$).

The concentration of vitamin E in rat plasma is displayed in figure 5. The vitamin E level in the control group was quite stable during the experiment. In group 3 the vitamin E levels

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fell under the control value. At week 8 Vitamin E_1 = 1.2 Vitamin E_3. The Se supplementation (group 4) weakly enhanced the vitamin E level. Vitamin E_4 = 1.15 Vitamin E_3 ($P < 0.05$). Vitamin E_2 = 1.37 Vitamin E_3 ($P < 0.02$).

The number of survived animals up to the end of experiment was 7 in groups 1 and 2, and 6 in groups 3 and 4. The mortality in control animals is a normal state. In our study, it was in physiological limits for young rats.

The time courses of the mean body weight of the rats from all groups are displayed in figure 6. The values of body weights of the animals from groups 1 and 3 did not differ significantly ($P > 0.1$) in the all-experimental period. The rats body weights, determined in groups 2 and 4 also did not exhibit significant differences. However, the gain of body weight of the supplemented rats from groups 2 and 4 was significantly higher compared to that of the unsupplemented animals from groups 1 and 3 ($P < 0.01$). At the end of the experiment, the body weights of the rats in condition of supplementation with Sel-plex were about 30% higher than the rat body weights without Se supplementation.

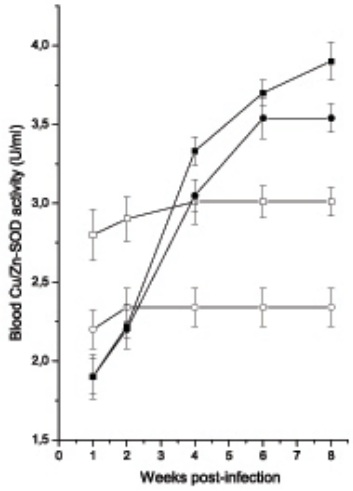

Figure 4: Changes with time of Cu/Zn-SOD activity in the blood of Wistar rats. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, supplemented).

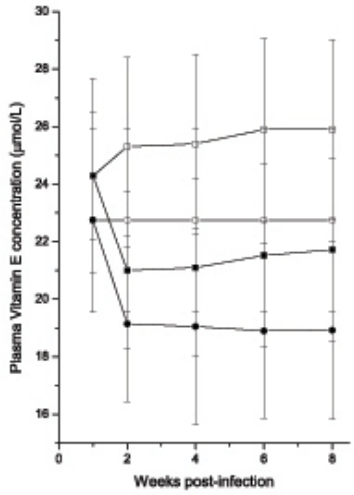

Figure 5: Changes with time of vitamin E concentration in the plasma of Wistar rats. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, supplemented).

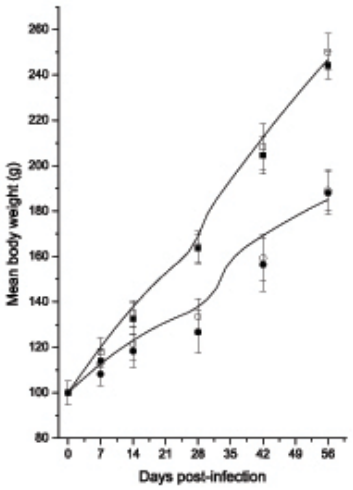

Figure 6: Time course of the mean body weight of Wistar rats up to week 8 post-infection (experimental points and model solutions). Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected, unsupplemented); filled square group 4 (infected, -*+supplemented).

In figure 6 are presented also the time courses of the host growth, obtained using the mathematical model. Via the model, the kinetic parameters of host growth, i. e. the relative rate constants μ^1 and μ^2, representing the two stages of host development, were calculated. On the basis of experimental data and using the equation (5) the plots were constructed (Figure 7) and the angular coefficients (μ- constants) of the straight lines were determined:

$$\mu^1_1 = \mu^1_3 = 0.059,\ \mu^2_1 = \mu^2_3 = 0.033; (10)$$

$$\mu^1_2 = \mu^1_4 = 0.062,\ \mu^2_2 = \mu^2_4 = 0.033.$$

The moments $T_i (i = 1, 2, 3, 4)$, which indicate the transfer from the first to the second stage, were determined from equation (6):

$$T_1 = T_3 = 32 \text{ day p.i.} \qquad T_2 = T_4 = 28 \text{ day p.i.} \qquad (11)$$

The theoretical curves in Fig. 8 were calculated on the basis of the initial condition $P_0 = 100$ g, determining of $C^1_1 = C^1_3 = 150$ g, $C^2_1 = C^2_3 = 220$ g, $C^1_2 = C^1_4 = 190$ g, and $C^2_2 = C^2_4 = 340$ g, based on an estimation of the trends, indicated by the experimental data, and values presented in (10) and (11).

Discussion

Essential alterations in the serum MDA level as well as in the activities of blood antioxidant enzymes Cu/Zn-SOD and GPx, plasma vitamin E concentration, and plasma and liver Se stores in *T. spiralis*-infected rats were observed during the experiment. These findings clearly show a development of

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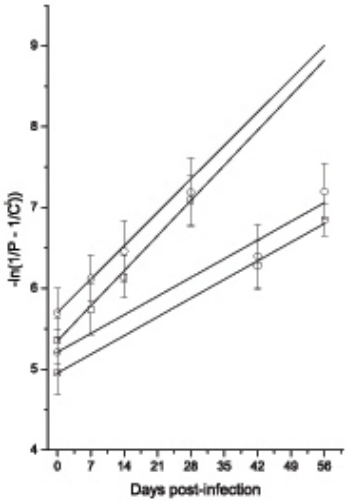

Figure 7: Determination of experimentally determinable constants μ^1 and μ^2 (relative growth rates) on the basis of Eq. 5 using experimental measures of the rat body weight. Unfilled circle group 1 (healthy, unsupplemented); unfilled square group 2 (healthy, supplemented); filled circle group 3 (infected unsupplemented); filled square group 4 (infected supplemented).

antioxidant imbalance in the infected host. They agree with the results of Derda et al., [9], who have investigated the changes in the antioxidant levels in *T. spiralis*-infected mice. Thus, our data confirm that *T. spiralis*-infection enhances ROS production. Trichinellosis may induce oxidative stress by producing hydrogen peroxide, hydroxyl radicals, and nitric oxide. ROS generation is also a defense mechanism of the host and it is realized in phagocytosis [5,26]. The production of free radicals leads to a significant increase of lipid peroxidation – one of the main manifestations of oxidative damage playing an important role in the pathogenesis of many parasitizes [27].

The increase of MDA concentration, indicating lipid peroxidation extent, is a reliable marker of radical-induced damages in host organism. This was demonstrated also by the results of Boczon et al., [28]. Similar increase of serum MDA level in the *T. spiralis*-infected rats was established by Tolstoj et al., [29], with a maximum concentration on day 21 pi. Increased MDA content in infected chicken implies that the infected organism may not have sufficient amount of antioxidant enzymes to cope with the increased oxidative stress [30]. Our data also give grounds about such an interpretation. Up to week 4 MDA concentrations in the infected rats increased sharply. At week 4 its increase stopped (Figure 3), however, MDA retained the high levels also after this time. Although the oxidative stress status in the rats continued during the experimental period, a further increase of MDA was not observed because of the development of defense reactions (expressed by the significant rise of the antioxidant enzymes GPx and Cu/Zn-SOD). Thus, the oxidative stress was not overcome, however, it worsened not further.

Changes in the activity of some antioxidant enzymes such as SOD, CAT, GPx and GST in the host's blood and muscle tissues during experimental trichinellosis have been shown to occur [5-9]. These enzymes work as a "team" to scavenge ROS. SOD is considered as the first line defense against superoxide radicals. It converts superoxide into hydrogen peroxide. CAT participates in the reduction of hydrogen peroxide to water and oxygen. GPx converts hydrogen peroxide into harmless molecules before they form free radicals [31,32]. Thus, SOD, CAT, and GPx are the most important enzymes in attenuating of free radical induced oxidative damages.

SOD and GPx activities in our experiment demonstrated a relatively similar pattern of changes with time. At the opening stage of the infection, metabolites produced by *T. spiralis* larvae exerted a suppressive effect on the enzymes SOD and GPx. This is the reason for the early depletion of erythrocyte SOD activity and blood GPx activity in the infected animals. Derda et al., [8], noted that in trichinellosis, the host tissues form biochemical conditions leading to decomposition of antioxidant enzymatic systems and expressed disturbance of metabolite homeostasis in the form of lipid peroxidation hyperactivation. After week 2, we observed an increase of the activities of these enzymes due to the development of defense reactions. At week 4, SOD and GPx activities in the infected rats (groups 3 and 4) were significantly higher compared to those in the control group. They remained at elevated levels until week 8. The stopping of MDA increase after week 4 (Figure 3) suggests a synergistic action of both enzymes. Derda et al., [9], observed maximum SOD and GPx activities in mice with trichinellosis at weeks 5 – 7 pi. This agrees with our results.

The fact that there is not statistically significant difference between GPx activity in groups 3 and 4 may be due to the low dose of Se, used for supplementation in our experiment. Probably in such doses, Se is not yet sufficient to elevate significantly the GPx level in infected and supplemented animals.

The rise of SOD and GPx activities is not sufficient to prevent lipid peroxidation. In addition, Vitamin E is an essential component of the antioxidant defense. It is a major free radical chain-breaking antioxidant, which can also interfere with the initiation and progression of parasite-induced oxidative damage. As a primary lip soluble antioxidant, it has an important role in scavenging of ROS and stabilizing the cell membranes, thus maintaining their permeability [33]. It is well known that Se and vitamin E show compensative effects and that their deficiency may cause massive injury [34].

De Souza et al., [35], have been studied the effect of low Se supplementation on mice experimentally infected with *Trypanosoma cruzi*. Mice treated with 4-16 ppm Se showed a dose dependent decrease of parasitaemia. The same authors reported that the rats' survival has not changed significantly. Thus, their results imply that Se supplementation does not lead to a general protection during infection. Selenium may help protect the heart from inflammatory damage. Huang and Yang [35,36], have been done studies with Se application in *Cryptosporidium parvum*-infection. An anticryptosporidium effect of Se was evaluated in a bovine fallopian tube epithelial cell culture system. Parasite number in cell culture was significantly reduced. This result is in accord with our finding regarding the reduction of *T. spiralis*-infection in Se supplemented rats (52% reduction of the infection in group 4).

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The antioxidant defense system and active immune response in *T. spiralis*-infected rats may be the possible reason for the significant reduction in the number of the muscle larvae [37]. The intensification of the antioxidant processes is related to the presence of the larvae, which enhance the phagocyte production, leading to ROS generation [38]. The immune status is positively influenced by Se application [39,40]. A positive effect on the immune response and an improvement of the antioxidant status was established by Kumar et al., [41], when Se as sodium selenite was administrated to lambs.

We see also a strong difference between the patterns of the time course of the body weight in *F. hepatica*-infected and *T. spiralis*-infected rats. In *F. hepatica*-infected rats, a significant body weight reduction was observed compared to healthy rats [42]. The possible reason for that could be a worsened digestion due to the oxidative stress, and disturbed absorption of food ingredients including many biogenic microelements [43]. The growth of the *T. spiralis*-infected rats displayed almost the same time course as in the control rats (Fig. 6). Similarly, the values of body weights in the supplemented with Sel-plex groups 2 and 4 were almost equal but by 30% higher (at week 8) compared to those in non-supplemented groups. A positive influence of Se supplementation on the growth performance has been reported also by other authors. The amount of Se (inorganic and organic) present in the feed significantly increased feed intake [44].

The model solutions for the mean body weight P of the rats were in good agreement with the experimental data (Figure 6). This type of model, constructed to determine the main trends in eukaryote growth, is based on an original hypothesis about the action of the genetic program as a limiting factor [17]. The model allows a quantitatively description of development stages in eucaryote organisms as well as prediction of growth rate pattern in wide range situations.

In conclusion, the results here discussed confirm that Se and especially the organic selenium, plays an important role in the context of parasitoses and suggest a promising approach for treatment of animals with trichinellosis. One could state that the development of oxidative stress due to trichinellosis was attenuated by Se supplementation in the form of Sel-plex. Selenium at low doses contributes to antioxidant response and improvement of the antioxidant status in host. In general, this element could be considered as an important tool for antioxidant-dependant protective mechanisms in the infected mammals. Sel-plex treatment reduces also in high degree *T. spiralis* muscle larvae. Further experimental studies are needed to investigate the effect of Se supplementation on the antioxidant system through the later stages in the same animal-parasite model [45].

Declaration

The experiment was approved by the Committee on Animal Experimentation at the Institute of Experimental Pathology and Parasitology – Bulgarian Academy of Sciences, Sofia, Bulgaria. It was performed according to the Bulgarian Animals Protection, Act of 2006 (protocol No 11130006) and recommendations of the Directive 86/609/EC of November 24, 1986.

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