CHANGES IN MALONDIALDEHYDE LEVEL AND CATALASE ACTIVITY AND EFFECT OF TOLTRAZURIL ON THESE PARAMETERS IN CHICKS INFECTED WITH *EIMERIA TENELLA*

GOKHAN ERASLAN¹, YUCEL CAM², MERYEM EREN³ AND BILAL CEM LIMAN¹

¹Department of Pharmacology and Toxicology, ²Department of Internal Medicine, ³Department of Biochemistry, University of Erciyes, Faculty of Veterinary Medicine, Kayseri, Turkey

e-mail: geraslan38@hotmail.com

Received for publication February 26, 2004.

Abstract

Ninety six 3-day-old Bowans White strain chicks were divided into a control and 7 experimental groups. Group 1 (control) received normal drinking water for 7 d. Chicks from groups 2, 3 and 4 received 50 ppm of toltrazuril at the beginning and on days 3 and 6 of the experiment. Groups 5, 6, 7 and 8 were infected with a single dose of *Eimeria tenella* oocysts and then groups 6, 7 and 8 received additionally 50 ppm of toltrazuril on days 0, 3 and 6 after the infection. At the end of the 7th d, all the animals were sacrificed, their blood were collected into heparinised tubes and the plasma malondialdehyde (MDA) level and the erythrocyte catalase (CAT) activity were examined. The obtained results revealed that *E. tenella* oocysts caused lipid peroxidation in chicks and the administration of toltrazuril relieved the oxidative damage. Therefore, in case of infection, the detected level of plasma MDA and the activity of erythrocyte catalase may be taken into consideration as references with other parameters, in order to determine the severity of infection and the effectiveness of treatment.

Key words: chicks, *Eimeria tenella*, catalase, malondialdehyde, toltrazuril.

*Eimeria tenella*, the most pathogenic strain of coccidium in chicken, is usually located in cecum and leading to cecal coccidiosis (2, 3). There have been applied several drugs to minimise the possible negative effects of infection (6). Toltrazuril has been introduced recently as effective drug in the treatment of various *Eimeria* infections. It is a triazinon derived compound and its chemical structure is as follows: 1-methyl-3-(3-methyl-4- (4-(trifluoromethyl)thio)phenoxy) phenyl-1, 3, 5-triazine 2,4,6(1H,3H,5H)-trione (5, 18). This compound is efficient against schizonts and microgametes of coccidium strains (*E. tenella*, *E. acervulina* and *E. maxima*) in poultry (15). In the present study, it will be shown that in the case of coccidiosis, caused by *E. tenella*, the changes occur in MDA level and catalase (CAT) activity which are one of the end-products of peroxidation as well as lipid peroxidation parameters and the latter is an antioxidant enzyme. There have been few studies concerning the effect of various *Eimeria* strains on the oxidative damage in animal species (1, 8). However, we did not come across any study with respect to the effect of *E. tenella* infection on oxidative damage in chicks and the treatment of this damage by toltrazuril. It will be determined in this study whether lipid peroxidation plays a role in the pathogenesis of the disease, and whether the anticoccidial drug changes these parameters and in which direction it would be. In addition, it will be shown whether these parameters can be taken as references with other parameters in order to make diagnosis and to determine the severity of the infection, as well as the efficiency of treatment.

Material and Methods

Ninety six 3-day-old Bowans White strain chicks were divided into 8 equal groups. Group 1 was chosen as control; groups 2, 3 and 4 received with drinking water ad libitum 50 ppm of toltrazuril at the beginning and on days 3 and 6 of the experiment, respectively. Each chick from groups 5, 6, 7 and 8 was infected with 40 000 of sporulated oocysts of *Eimeria tenella*. Moreover, chicks from groups 6, 7 and 8 received with drinking water 50 ppm of toltrazuril on days 0, 3 and 6 after the infection, respectively.

In order to maintain and increase the pathogenicity of the oocysts, they were suspended in potassium dichromate and centrifuged at 2000 rpm for 10 min. Following that the supernatant was discarded and the remain collected. Later, oocysts were washed out by centrifugation with distilled water to remove the residues of potassium dichromate. After that, the oocysts were counted using Mc Master microscope slide and...
diluted with distilled water to receive 40,000 of sporulated oocysts per ml.

At the end of the experiment (day 7), all the birds were sacrificed and their blood samples were collected into heparinized tubes. The samples were then centrifuged and obtained plasma was transferred into other tubes. The plasma malondialdehyde levels were detected according to the method of Yoshiiko et al. (20). The erythrocyte suspension, located as the lower phase, was washed by Na-phosphate buffer 3 times and the erythrocytes were transferred into separated tubes. The erythrocyte haemoglobin level as well as its CAT activity was performed by the methods of Fairbanks and Klee (10) and Luck’s (14), respectively. The results were shown as nmol/ml for MDA and k/mgHb for CAT activity. The data were evaluated in terms of arithmetic means and standard deviation. Non-parametric Kruskal Wallis test was used in order to evaluate the data statistically.

### Results

The MDA level showed an increase in the groups infected with *E. tenella* oocysts (groups 5, 6, 7 and 8), compared to control. This numerical increase was also observed in the groups, which received toltrazuril (groups 2 to 5) at specified periods only. These increases were seen as well in the groups infected and treated with toltrazuril (groups 6 to 8), however, they were not as high as in chicks from the group 5 which were only infected. On the contrary, the values intended to be closer to the value of the control group (group 1). The changes similar to those which occurred in plasma MDA level also were observed in erythrocyte CAT activity. The statistical evaluation showed that there were significant differences, regarding the plasma MDA level in the group infected by *E. tenella* and receiving no treatment (group 5), compared to control (P<0.05). In erythrocyte CAT activity, all of the experimental groups (groups 2, 3, 4, 5, 7 and 8) showed the significant changes (P<0.05), compared to control (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Groups*</th>
<th>MDA (nmol/ml)</th>
<th>CAT (k/mgHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>13.200±2.856a</td>
<td>0.544±0.310a</td>
</tr>
<tr>
<td>Group 2</td>
<td>14.140±8.134a</td>
<td>0.301±0.062b</td>
</tr>
<tr>
<td>Group 3</td>
<td>14.726±3.238a</td>
<td>0.325±0.060b</td>
</tr>
<tr>
<td>Group 4</td>
<td>10.762±4.141a</td>
<td>0.388±0.246b</td>
</tr>
<tr>
<td>Group 5</td>
<td>21.460±6.989b</td>
<td>0.198±0.088b</td>
</tr>
<tr>
<td>Group 6</td>
<td>10.820±3.774a</td>
<td>0.336±0.112b</td>
</tr>
<tr>
<td>Group 7</td>
<td>11.610±3.308a</td>
<td>0.347±0.104b</td>
</tr>
<tr>
<td>Group 8</td>
<td>19.166±7.051a</td>
<td>0.235±0.049b</td>
</tr>
</tbody>
</table>

*a,b*Values followed by the different letters in the same columns are statistically significant (P<0.05), according to Kruscal Wallis non-parametric test.

*Group 1: control; group 2: toltrazuril, day 0; group 3: toltrazuril, day 3; group 4: toltrazuril, day 6; group 5: infection with *E. tenella*, day 0; group 6, infection with *E. tenella*, day 0 + toltrazuril, day 0; group 7: infection with *E. tenella*, day 0 + toltrazuril, day 3; group 8: infection with *E. tenella*, day 0 + toltrazuril, day 6.

### Discussion

The presence of unsaturated fatty acids in cell membrane makes the membrane susceptible to peroxidation. The peroxidation of cell membrane leads to the impairment in its semi-permeability and triggers the series of reactions what may result in the cell death (7, 11). There are several intracellular defense mechanisms to prevent the potential oxidative damage. These defense systems are classified as enzymatic and non-enzymatic. The enzymatic defense systems include the antioxidant enzymes. These enzymes protect the cell membrane against peroxidation by converting the reactive compounds to less harmful or harmless metabolites. The determinations of MDA level and antioxidant enzyme activity are major criteria concerning the severity of possible peroxidation, which takes place in cell membrane (4, 12, 16). The researches on this point revealed that parasites usually do not cause lipid peroxidation on individual basis, but following infestation, the synthesis and secretion of some compound in order to build up the immune response trigger off the oxidative stress (1). As it was mentioned above, the compounds, which are the direct cause of peroxidation, have an endogenic origin.

In this study, while the level of MDA showed an increase, the significant decrease was detected,
regarding CAT activity in the chicks infected with *E. tenella*. These changes indicated that *E. tenella* led to lipid peroxidation during the specified period. The decrease in CAT activity proved that the formation of reactive compounds was much more higher than the level which could be compensated by the cellular defense systems, thus, these compounds may not be converted to less harmful or ineffective metabolites at the sufficient level. Another reason of a decreased activity might be the formation of reactive compounds in the body, versus the strain, which inhibited the enzyme. Thirdly, the decrease in the antioxidant vitamin level, which was involved in the non-enzymatic mechanisms, resulted in the occurrence of oxidative damage. There have been data that the parasitic infections may lead to a decrease in the blood level of antioxidant vitamin (8). The groups which received only the drug (groups 2 to 4) showed the significant changes with respect to their enzyme activities and MDA levels. These changes revealed that the drug also inhibited CAT activity. In addition, it was understood that the severity of oxidative damage depended on the exposure time of toltrazuril, what was consisted with the obtained data. Therefore, in the group 4, which had the shortest exposure period, the MDA levels were lower than the others; in contrast, the CAT activities were higher. Following the administration of the coccidia strain, in the group which received the drug immediately (group 6) and in the groups which received the drug on the 3rd and 6th d of the experiment (groups 7 and 8), the enzyme activities showed an increase, compared with the group 5 which was only infected. This increase proved that the drug was effective against the strain of the coccidia. On the other hand, these values did not reach the level of infections may lead to a decrease in the blood level of antioxidant vitamin (8). The groups which received only (group 5). This decrease was found to be related with respect to their enzyme activities and MDA levels. These changes revealed that the drug also inhibited CAT activity. In addition, it was understood that the severity of oxidative damage depended on the exposure time of toltrazuril, what was consisted with the obtained data. Therefore, in the group 4, which had the shortest exposure period, the MDA levels were lower than the others; in contrast, the CAT activities were higher. Following the administration of the coccidia strain, in the group which received the drug immediately (group 6) and in the groups which received the drug on the 3rd and 6th d of the experiment (groups 7 and 8), the enzyme activities showed an increase, compared with the group 5 which was only infected. This increase proved that the drug was effective against the strain of the coccidia. On the other hand, these values did not reach the level of control group. Toltrazuril showed its best effect in the group which received it, following the administration of coccidium oocysts (group 6). The MDA level showed a decrease in the group which received treatment following infection with *E. tenella* strain (group 6), compared to the group which received *E. tenella* strain only (group 5). This decrease was found to be related with the administration time of the drug. With the respect to the similar studies which were performed on several animal species and humans, Allen (1) reported that nitro-L-arginin was effective to restore oxidative damage in chickens with *E. maxima* infection. Dede et al. (8) reported that the parasites (*Trichostrongylylidae* sp.+*Eimeria* sp.+*Babesia* sp.), which were detected in infected goats, induced the lipid peroxidation. Pabon et al. (17) reported that malaria, a protozoal disease, increased the MDA level, but caused the decrease in the antioxidant enzyme levels in humans. Similarly, Erel et al. (9), showed that the antioxidant enzyme level decreased and in contrast, the LPO level increased in the patients with vivax malaria. The data obtained by the present study were consistent with the results of the studies mentioned above. Eventually, *E. tenella* oocysts provoked the lipid peroxidation at the specified period and quantity. The occurrence of oxidative damage may be contributed directly to the mechanism of negative effects of the disease. Toltrazuril itself as well led to the oxidative damage at the specified period and dosage. The intensity of this damage was not as strong as the damage induced by the coccidium strain itself. On the other hand, the drug administrations were effective against this oocyst, regarding the obtained results. Therefore, the investigated parameters, both the MDA level and CAT activity, may be taken into consideration with other parameters in the case of *E. tenella* infection, in order to determine the severity of the infection and, if the drug was applied, the prognosis of the disease. In this context, the results of present study are prominent.

**References**