EFFECTS OF DIFFERENT DOSES OF TILMICOSIN ON ERYTHROCYTE CATALASE ACTIVITY AND PLASMA MALONDIALDEHYDE LEVELS IN CHICKS

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Abstract

Three-day-old 105 Ross race female broiler chicks were used. The chickens were divided into 5 groups comprising 1 control and 4 experimental groups. Animals in the control group (group 1) were provided with normal drinking water, whereas the experimental groups, namely, group 2, group 3, group 4 and group 5 were administered with tilmicosin at doses of 20 ppm, 40 ppm, 80 ppm and 160 ppm (approximately, 5 mg/kg/b.w./d, 10 mg/kg/b.w./d, 20 mg/kg/b.w./d, and 40 mg/kg/b.w./d), respectively, via drinking water for a period of 3 d. Seven animals were euthanized from each group, on the 1st d (first period), 4th d (second period) and 7th d (third period) of the experiment for the assessment of blood malondialdehyde (MDA) levels and catalase (CAT) activity. Statistically significant differences in comparison with the control group were observed in plasma MDA levels of all experimental groups in the first period, groups 2 and 5 in the second period, and group 4 in the third period. With regards to CAT activity, a statistically significant decrease was found in all experimental groups only in the first period.

Key words: chicken, tilmicosin, catalase, malondialdehyde.

Tilmicosin, belonging to antibiotics used for the treatment of poultry diseases, is a semi-synthetic macrolide antibiotic (5, 13). It is used particularly for the treatment of respiratory diseases of poultry species (5, 13, 23). Tilmicosin that passes into the systemic blood circulation accumulates in phagocytes (macrophages, monocyctic macrophages, and heterophils) and is named intra-cellular tilmicosin (14, 20). Thereby, antibiotic transfer from intra-cellular to extra-cellular occurs. The tendency to accumulate is generally observed to occur at higher levels and more rapidly in tissues, organs, and media with lower pH values (5, 21, 22). Microorganisms that affect the respiratory system, in particular Mycoplasma sp.; are highly susceptible to this antibiotic (11, 12, 22, 23).

Biological membranes possess low electrical conductivity, and peroxidation of the two-layered lipid membrane increases the conductivity. As a result, reciprocal change in the location of phospholipids layers with each other accelerates, mobility of membrane proteins becomes restricted, and protein and lipid layers integrate at an advanced level (1-3, 18). Therefore, the general definition of lipid peroxidation encompasses the oxidative damage of membrane lipids (24). Numerous chemical reactions are known to occur in the body. Many compounds are formed during these reactions as intermediate or end products. These compounds are transformed into less effective and harmless compounds via enzymatic or non-enzymatic reactions (7, 8). Essentially, as long as they remain at certain levels, molecules of free radicals play an important role in the defence of the organism against foreign substances and infectious agents. Since they have oxygen groups, the term “reactive groups” corresponds to free-active oxygen groups such as the superoxide anion group, hydroxyl group, and singlet oxygen (7-10).

The purpose of the study was to determine whether tilmicosin administered in different doses causes lipid peroxidation in chicks. No previous study on the capacity of tilmicosin to cause oxidative stress in poultry has been reported as yet.

Material and Methods

A total of 105 three-day-old female Ross race broiler chicks were used in the study. The chicks were divided into 5 groups comprising of 1 control and 4 experimental groups. Group 1 was allocated as the control group, whereas groups 2, 3, 4, and 5 were administered with tilmicosin (Pulmotil®AC) at doses of 20 ppm, 40 ppm, 80 ppm, and 160 ppm (approximately, 5 mg/kg/b.w./d, 10 mg/kg/b.w./d, 20 mg/kg/b.w./d, and 40 mg/kg/b.w./d), respectively, with drinking water for 3 days. Seven animals were killed from each group on the 1st, 4th, and 7th d of the experiment, and blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 3000 rpm. The remaining
erythrocytes were washed three times and haemolysed (25). Plasma was used for the assessment of plasma malondialdehyde (MDA) levels, whereas the haemolysate was used for the determination of haemoglobin levels and catalase (CAT) activity. Assessment of MDA levels was performed in accordance with the method reported by Yoshioka et al. (28), whereas CAT activity and haemoglobin levels were determined according to methods reported by Luck (15) and Fairbanks and Klee (6), respectively.

SPSS for Windows package programme was used for statistical analyses, and one-way analyses of variance and the Duncan test were performed. Data was calculated in arithmetical mean values and standard deviations.

Results

An increase in plasma MDA levels, in comparison with the control group, was observed in all groups. This increase was found to be statistically significant (P<0.05) in comparison with the control group, in all experimental groups in the first period, in groups 2 and 5 in the second period, and in group 4 in the third period. A decrease in CAT activity was observed in all experimental groups compared to the control group. This decrease was statistically significant (P<0.05) only in the first period in all experimental groups when compared to the control group (Tables 1-2).

Discussion

Today, there are few valid methods used in the assessment of the severity of oxidative stress. Amongst these methods, the most widely used is the assessment for the level of end products formed as a result of lipid peroxidation reactions. This provides direct information on the severity of oxidative stress. The most widely used parameter is the level of MDA. In addition, the assessment of the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) also provides information on oxidative stress (1, 7-10, 26).

MDA is a product formed as a result of the oxidative damage of certain macromolecules, and is found in either free form or bound to various structures in tissues (8, 9, 24). CAT is an enzyme that contains protoporphirin, known to have a high molecular weight. It is found in all cells. In mammalian cells, most of the enzyme is found within peroxysomes in which the concentration of stable H₂O₂ is very high (10⁻⁴). The enzyme causes 2H₂O₂ molecules to transform into 2H₂O and O₂ (17, 18). The intracellular resources of H₂O₂ are the mitochondria. Consumption of glutathione leads to an increase in the intracellular concentration of H₂O₂. CAT plays an important role in the destruction of this increasing radical. Intracellular CAT activity is directly related to the extra-cellular concentration of H₂O₂ (3, 7-10, 18).

**Table 1**

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Periods</th>
<th>1 (on day 1)</th>
<th>2 (on day 4)</th>
<th>3 (on day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.270±0.766²</td>
<td>2.195±0.408²</td>
<td>2.323±0.554²</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td>3.710±0.816²</td>
<td>3.085±0.584²</td>
<td>2.909±0.538²</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td>3.632±0.699²</td>
<td>3.001±0.842²</td>
<td>2.473±0.783²</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td>3.804±0.981²</td>
<td>2.719±0.923²</td>
<td>3.640±0.779²</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td>3.688±0.692²</td>
<td>3.359±0.672²</td>
<td>2.508±0.453²</td>
</tr>
</tbody>
</table>

* Group 1 (control); group 2 - 5 mg/kg/b.w./d of tilmicosin; group 3 - 10 mg/kg/b.w./d of tilmicosin; group 4 - 20 mg/kg/b.w./d of tilmicosin; group 5 - 40 mg/kg/b.w./d of tilmicosin.

²,³ Means within the same column with different letters are statistically significant (P<0.05).

**Table 2**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Periods</th>
<th>1 (on day 1)</th>
<th>2 (on day 4)</th>
<th>3 (on day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td>4328.094±983.86²</td>
<td>4471.362±1687.038</td>
<td>4405.022±1455.068</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td>2210.949±1157.99²</td>
<td>4004.374±1097.059</td>
<td>3373.602±1126.906</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td>1209.338±263.32²</td>
<td>3351.285±1189.753</td>
<td>3188.760±1796.601</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td>2242.572±931.28²</td>
<td>4014.835±1195.539</td>
<td>3694.136±1089.922</td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
<td>2004.980±1037.99²</td>
<td>3949.860±1740.192</td>
<td>4095.091±1689.708</td>
</tr>
</tbody>
</table>

²,³ Means within the same column with different letters are statistically significant (P<0.05).
In this study, an increase was demonstrated in plasma MDA levels of the experimental groups in comparison with the control group. However, a difference directly proportional to the increase in dose did not exist among groups. This increase was found to be statistically significant in all groups on the first day of the study. On the other hand, no statistically significant difference was observed in the MDA levels of the experimental groups compared to the control group in the other periods excluding the second period in groups 2 and 5, and the third period in group 4. A statistically significant decrease was observed in the CAT activity of all experimental groups compared to the control group in the first period. No statistically significant difference was found between the groups in the second and third periods. Observation of statistically significant differences between groups with respect to both parameters in the first period (1st d) suggests that the oxidative impulse is triggered in the inception period of the experiment. The presence of statistically significant differences between MDA levels of some groups and the CAT activity of none of the groups in the following days (the second and third period), despite antibiotic administration until the end of the 3rd d, points out the involvement of cellular antioxidant defence systems and decrease in the severity of lipid peroxidation. As a matter of fact, observation of an increase in MDA levels and decrease in CAT activity compared to the control group, in all periods, suggests, in spite of insignificance of these differences, the oxidative impulse, and free radical to form at levels that are tolerated by the body. On the other hand, since the differences observed at all dose quantities were not directly proportional to the increase in doses the free radicals, which formed as a result of the administered doses were considered to be compensated by the antioxidant mechanism. Yazar et al. (27) administered 25 mg/kg/b.w. of tilmicosin as a single dose to mice and found a decrease in cardiac SOD and GSH-Px activity. Mezes et al. (16) administered 50 mg/kg/b.w.of tiamulin or 140 mg/kg/b.w. of salinomycin to 28-day-old broiler chickens, reared on feed containing 60 ppm of salinomycin, and determined MDA and glutathione levels, as well as GSH-Px and CAT activities in the liver. They reported hepatic MDA levels and CAT activity to increase, and glutathione levels and GSH-Px activity to decrease in the both groups treated with salinomycin and tiamulin. Salyi et al. (19) administered monensin to broiler chickens at a dose of 150 mg/kg/b.w. and found no alteration of blood and muscle oxidative stress parameters. However, they observed a decrease in the hepatic GSH-Px activity, and increase in MDA levels and CAT activity. Carreras et al. (4) administered dietary enrofloxacin to broiler chickens without any difference in tissue antioxidant enzymes upon examination of the oxidative effect on various tissues. Despite the presence of many studies on the capacity of antibiotics to cause lipid peroxidation in other animal species, the number of studies carried out in poultry species is quite low. Since so far no study was carried out on oxidative stress caused by tilmicosin in poultry species, it is difficult to compare the results of this study with any similar investigations in poultry. In this respect, this study bears significance with regard to being a reference for future studies in poultry.

In conclusion, tilmicosin administered via drinking water for 3 d to chickens below treatment doses (5mg/kg/b.w./d), at treatment doses (10-20 mg/kg/bw/d), and above treatment doses (40 mg/kg/b.w./d) did not form very high free radical level from aspect of MDA level and CAT activity.

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References


