EFFECTS OF DIFFERENT DOSES OF TILMICOSIN ON SOME BIOCHEMICAL PARAMETERS AND ANTIOXIDANT STATUS IN SERUM AND CARDIAC TISSUES IN MICE

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Abstract

The objective of this study was to evaluate effects of different doses of tilmicosin on serum and cardiac creatine kinase (CK), creatine kinase-MB (CK-MB), total sialic acid (TSA), and antioxidant status, including the levels of glutathione (GSH) and malondialdehyde (MDA), to determine its cardiotoxic effects and alterations in antioxidant status. Fifty Balb/C mice were divided into 5 groups. The control group received saline. Groups 2, 3, 4, and 5, were injected with a single dose of tilmicosin at 10, 30, 50, and 70 mg/kg, respectively. No differences was found in serum CK and CK-MB activities as well as TSA, GSH, and MDA concentrations at doses of 10 and 30 mg/kg of tilmicosin compared to control. However, doses of 50 and 70 mg/kg of the antibiotic resulted in a significant increase in serum CK and CK-MB activities, TSA and MDA concentrations, with significant decrease in GSH level compared to control group and groups 2 and 3. Increased cardiac MDA, and decreased GSH concentrations were found in groups 4 and 5, compared to other groups, whereas there was dose dependent-increase in CK, CK-MB, and TSA level at 30 mg/kg, and higher doses compared to control and group 2. Tilmicosin administration at doses of 50 and 70 mg/kg appears to alter selected cardiac enzymes and TSA concentration indicating its cardiotoxic and strong pro-oxidant effect in mice.

Key words: mice, tilmicosin, creatine kinase, creatine kinase-MB, sialic acid, cardiotoxicity.

Tilmicosin is a chemically modified macrolide, which is used as a long-acting antibiotic, for the treatment and prophylaxis of bovine respiratory diseases caused by Past. haemolytica, A. pyogenes, H. somnus, and Mycoplasmae. The use of tilmicosin could be fatal in swine after an intramuscular injection, and it is not recommended for use in horses, sheep, and goats due to its toxicity (10). The antibiotic affects mainly the heart, which is the primary target organ (6). Negative inotropy and positive chronotropy in the heart was reported following a single injection of tilmicosin. In humans, accidental injection of a veterinary drug Micotil resulted in alterations in electrocardiogram and cardiac enzymes, including elevation of creatine kinase (CK) and creatine kinase-MB (CK-MB) (14). In animal species, it was demonstrated that tilmicosin induced free radical damage, leading to lipid peroxidation and alterations in cardiac enzymes. The administration of single dose of 25 mg/kg of tilmicosin; resulted in decreased superoxide dismutase and glutathione peroxidase activities in the heart tissue of mice, indicating that tilmicosin could cause oxidative stress by decreasing antioxidant enzymes in the heart (15). It was also reported that tilmicosin resulted in increases in CK, CK-MB, and troponin I levels in the serum of rabbits (16).

Sialic acids are derivatives of neuraminic acids, which are part of carbohydrate residues of glycolipids and glycoproteins in membranes (12). Following the myocardial infarction, sialic acids levels were found to be raised, and the increase is considered associated with the release of membrane bound sialic acid, as a result of cellular damage in the myocardium (5).

The objective of this study was to evaluate effects of different doses of tilmicosin on serum and cardiac CK, CK-MB, TSA, and antioxidant status, including the level of glutathione (GSH) and malondialdehyde (MDA), and to determine dose-response profile of tilmicosin with respect to the assayed parameters.

Material and Methods

Fifty Balb/C mice (weighing 20-25 g) were divided into 5 equal groups. The mice were fed standard pelleted diet, and feed and water were provided ad libitum. The mice in group 1 (control) were given a single subcutaneous injection of saline solution. The mice from group 2 were injected subcutaneously with tilmicosin (Micotil 300® Lilly Elanco, Turkey), at a single dose of 10 mg/kg body weight (b.w.). The mice in
group 3 were injected subcutaneously with tilmicosin at a single dose of 30 mg/kg b.w. The mice from group 4 were injected subcutaneously with tilmicosin at a single dose of 50 mg/kg b.w. The mice in group 5 were subcutaneously injected with tilmicosin at a single dose of 70 mg/kg b.w. Blood samples were collected 6 h after the drug injection. The blood collection was carried out via cardiac puncture under ether anesthesia. The mice were immediately killed by cervical dislocation after collection of blood samples. The heart was immediately removed and washed with cold saline. The cardiac tissues were homogenized in phosphate buffer (pH 7.4) in 0.1 M KCl, and the homogenates were centrifuged at 1500 rpm for 5 min. All the samples were stored at -25 °C until they were analysed. Analyses were carried out by the method of Beutler (et al. 2) and Yoshoiko (et al. 17) for GSH and MDA concentrations, respectively. Serum and tissue CK and CK-MB concentrations were determined using commercially available kit and using an autoanalyser (Olympus Chemistry Analyzer AU 640, Type: 640-03, Japan). The total sialic acid was measured colorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) by the method of Sydow (13).

Statistical differences between the groups were tested by analysis of variance (ANOVA) and Newman-Keuls test using SPSS for Windows version 10.0. The data was presented as mean ± standard errors, and P values less than 0.05 were considered significant.

**Results**

Tables 1 and 2 show blood and cardiac CK, CK-MB, TSA, MDA, and GSH levels, respectively.

**Table 1**

Biochemical parameters and antioxidant status of mouse serum after administration of different doses of tilmicosin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>77.2±4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.5±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.7±4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>197.3±11.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>501.5±12.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>19.8±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.2±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.7±4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.2±7.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>199.5±9.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSA (mg/L)</td>
<td>588.5±18.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>614.3±13.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>642.2±16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>885.0±24.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1156.7±41.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/L)</td>
<td>81.2±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.4±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.1±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.1±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.9±3.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>15.9±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.2±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.0±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.2±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

**Table 2**

Biochemical parameters and antioxidant status of mouse cardiac tissue after administration of different doses of tilmicosin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>CK (U/g)</td>
<td>4905.7±182.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5610.5±218.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6086.0±83.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8390.8±278.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11594.0±431.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CK-MB (U/g)</td>
<td>467.7±25.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>556.5±23.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>770.0±26.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1700.0±75.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3255.5±144.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSA (mg/g)</td>
<td>0.22±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.29±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>0.31±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (µmol/g)</td>
<td>0.29±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>
Discussion

Tilmicosin is recommended for respiratory diseases of cattle at a dose of 10 mg/kg. The dose can be increased to 20 mg/kg, which is slightly more effective than 10 mg/kg. Tilmicosin at the recommended dose is applied using the subcutaneous route in cattle (10). Doses of 5 mg/kg can be fatal for cattle when applied using the intravenous route (6). In human cases, it was reported that small amount of accidental injection of tilmicosin resulted in pain, swelling, erythema, aching, and oedema at the injection site with no systemic complaints (8). On the other hand, unintentional injection at maximum possible dose of 1800 mg led to severe chest pain and agitation. In addition, the injection resulted in alterations in EKG, elevations in CK and CK-MB activities indicating a myocardial damage (14). In conclusion, these results suggest that tilmicosin appears to have no adverse effects at 10 and 30 mg/kg doses, with respect to serum levels of the assayed parameters. However, doses of 50 and 70 mg/kg induced lipid peroxidation and increased CK and CK-MB activities indicating that tilmicosin at these doses is cardiotoxic.

References