Effect of tilmicosin on cardiac muscle and serum creatine kinases activities and serum total protein level in healthy male Balb/C mice

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SUMMARY
In this study, the effect of tilmicosin on cardiac muscle and serum creatine kinases activities and serum total protein level was investigated. Forty male Balb/C mice were used as materials. Ten mice were used as a control group, and thirty mice were injected with tilmicosin (25 mg/kg body weight, SC, single injection) and monitored for 3 days. The results obtained in the present study show that use of tilmicosin caused temporary increases in cardiac muscle creatine kinase activity and serum total protein level in male Balb/C mice.

KEY-WORDS: tilmicosin - creatine kinase - total protein - mice.

RÉSUMÉ
Effet de la tilmicosine sur l’activité des créatinines kinases cardiaques et du sérum et sur les protéines totales sériques chez des souris mâles saines Balb/C. Par. E. YAZAR, V. ALTUNOK, M. ELMAS, B. TRAŞ, A.L. BAŞ et V. ÖZDEMIR.

Dans cette étude, l’effet de la tilmicosine sur les activités sériques et musculaires cardiaques des créatinines kinases et les protéines sériques totales ont été étudiés. Quarante souris mâles Balb/C ont été utilisées. Dix souris sont utilisées comme groupe témoin, et trente souris sont injectées avec la tilmicosine (25 mg/kg de poids corporel, Sc, injection unique), et surveillées pendant 3 jours. Les résultats obtenus montrent que l’utilisation de la tilmicosine peut induire une augmentation passagère des activités musculaires cardiaques de créatinine kinases et des protéines sériques totales chez les souris mâles de souche Balb/C.

MOTS-CLÉS: tilmicosine - protéines sériques - créatinine kinases - souris.

1. Introduction
The macrolide tilmicosin is prepared by chemical modification of desmycosin and interferes with protein synthesis in bacteria. It is used for respiratory diseases in cattle and inhibits growth of Pasteurellae and Mycoplasmae. Micotil 300® (Lilly Elanco, Istanbul, Turkey) contains 300 mg tilmicosin per milliliter and produces therapeutic levels in the lung for 3 to 4 days after a single subcutaneous injection [2].

Gastrointestinal upset, jaundice and liver damage have been commonly reported after administration of macrolides. However, tilmicosin has a different side effect from other macrolides, cardiotoxicity and this effect depends on route of administration and dose [8, 11]. Also, tilmicosin might cause a transient swelling at injection side, severe dyspnea, anaphylaxis, collapse and death [2, 4].

Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine by ATP to form creatine phosphate, the major storage form of high energy phosphate required by muscle [5, 13]. The heart has been selected because it is the target organ of acute tilmicosin toxicity [8, 11].

The aim of this study was to investigate the effect of tilmicosin on cardiac muscle and serum CK activities in healthy mice as a determination of possible cardiotoxic effect of tilmicosin.

2. Materials and methods
Forty male Balb/C (approximately 4 months, 36 ± 4 gram) mice were used in experiment (Selcuk University, Experimental Medicine, Research and Application Center, Konya, Turkey). Mice were fed with standard pellet diet and tap water ad libitum during the experimentation. Ten mice were used as control group. Thirty mice were injected with tilmicosin (Micotil 300®, Lilly Elanco, Istanbul, Turkey) at a single subcutaneous dose of 25 mg/kg bw [12]. The commercial tilmicosin preparation (Micotil 300®) was diluted by using Hamilton microinjector with 100 µl of normal saline.
solution (0.9 %) to achieve a dose of 25 mg/kg. This procedure was performed each of the thirty mice.

After injection of tilmicosin, blood samples were taken from the heart by cardiac puncture under light ether anesthesia at days 1, 2, and 3. Ten mice were randomly selected, and immediately just killed after bleeding at the first, second and third days of experimentation. The same procedure was performed for the ten mice of the control group at the first day of experimentation.

Hearts were immediately removed and washed with cold saline solution after killing. Whole heart was homogenized (Kontes) with 500 μl of cold (+ 4 °C) homogenate solution (0.25 M sucrose (Sigma) + 10 mM Tris (Sigma) + 1 mM EDTA (Pharmacia Biotech), pH 7.4) into ice [20, 21]. The homogenates were centrifuged (10,000 rpm, 15 minutes, + 4 °C) and the supernatants were carefully removed and stored for analysis (- 80 °C). Cardiac muscle and serum CK (Randox) activities, and cardiac and serum total protein (Sigma) level were assayed by using commercially available kits using spectrophotometry (Schimadzu UV 2100). Cardiac muscle CK activities were expressed as U g\(^{-1}\) tissue protein.

All values are expressed as mean ± SE. The results were analyzed by Tukey HSD multiple range test (SPSS for windows, release 6.0). In all cases, probability of error of less than 0.05 was selected as the criterion for statistical significance from the control values.

### 3. Results

Cardiac and serum CK activities and total protein level were given in Table I. At the 1\(^{st}\) day, cardiac muscle CK activity was found higher than control values (p < 0.05) and, at the 2\(^{nd}\) day, total protein level was found higher than control level (p < 0.05).

### 4. Discussion

Tilmicosin is used for treatment of respiratory diseases in cattle, but recent studies suggest that it may be used in swine [16], sheep [6], goat [14], rabbit [12] and turkey [9]. Gastrointestinal upset, jaundice, liver damage and transient swelling at injection side are reported after administration of macrolides [2, 4]. In addition to these side effects, tilmicosin has a cardiotoxic effect. The toxic and potentially fatal doses of tilmicosin depend on animal species and route of administration. The median lethal dose for parenteral administration is 97 mg/kg (S.C.) in ICR mice [8]. The results of tilmicosin administration to laboratory animals and domestic livestock suggests that cardiovascular system is the target of acute tilmicosin toxicity. However, only a weak not significant increase of serum CK was obtained in this study. Surprisingly, significant increased cardiac muscle CK activity was found one day after administration of tilmicosin.

Although this effect cannot be exactly explained, it may be linked to positive chronotropic effect of this antibiotic, previously reported in human [8, 11]. This result is of interest because of the central role of CK in the energetic metabolism of excitable cells [8, 11, 15, 19]. It is concomitant with high level of cardiac CK activity in Balb/C mice in comparison to rat and rabbit (respective activities of 644 and 1600 U/g [3]).

Two days after administration of tilmicosin, serum total protein level was statistically different from control values. High doses of tilmicosin (150 mg/kg, SC) caused a decrease serum protein and albumin levels in calves (200 kg) and this effect was attributed to tissue damage and edema at the injection side [8]. Effects of macrolides on enzymes activities and total protein level depend on animal species, doses and administration route of macrolides. Tylosin (10000 U/kg, P.O., thrice daily) caused an increase serum protein level in calves [18], erythromycin caused an increase serum CK activity, but did not effect protein level in broilers [10], and erythromycin lactobionate (600 mg/kg, I.V., thrice daily) caused an increase total protein level in human beings [7].

It was concluded that when tilmicosin was used at recommended doses and administration route, tilmicosin had no negative effect serum CK activity in male Balb/C mice. Nevertheless, tilmicosin caused temporary increases cardiac muscle creatine kinase activity and serum total protein level in male Balb/C mice. However, further studies are necessary to confirm this increase and determine which mechanism is involved.

### Bibliography


<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1(^{st}) day</th>
<th>2(^{nd}) day</th>
<th>3(^{rd}) day</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n:10</td>
<td>n:10</td>
<td>n:10</td>
<td>n:10</td>
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<tr>
<td>Serum CK U/L</td>
<td>61 ± 9.9</td>
<td>68 ± 11</td>
<td>56 ± 7.6</td>
<td>45 ± 9.8</td>
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<tr>
<td>Cardiac CK U/g tissue</td>
<td>7696 ± 1095</td>
<td>10883 ± 1533*</td>
<td>7843 ± 671</td>
<td>6564 ± 564</td>
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<tr>
<td>Total protein g/dL</td>
<td>5.17 ± 0.07</td>
<td>5.37 ± 0.13</td>
<td>5.47 ± 0.25*</td>
<td>5.14 ± 0.07</td>
</tr>
</tbody>
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* p < 0.05 significantly different from control values.

Table 1. — Effect of tilmicosin on cardiac muscle and serum creatine kinases (CK) activities and total protein level in Balb/C mice (mean ± SE, n:40).


