

Effects of Different Doses of Tilmicosin on Malondialdehyde and Glutathione Concentrations in Mice

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Abstract

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The aim of this study was to follow the effects of different doses of tilmicosin on malondialdehyde and reduced glutathione levels of heart and liver, and on selected haematological indices. Forty male Balb/C mice were used throughout the experiment. They were divided into four groups (n = 10), and injected subcutaneously as follows: Group 1 (control), with isotonic saline solution, Group 2 with 25 mg/kg body weight of tilmicosin, Group 3 with 50 mg/kg, and Group 4 with 75 mg/kg of tilmicosin in single injections. After three days, plasma, cardiac and hepatic malondialdehyde and reduced glutathione levels were measured with spectrophotometer. Red blood cell, white blood cell, platelet, haemoglobin, packed cell volume and percentage of leukocytes were also determined.

At the end of the experiment, tilmicosin did not cause any statistically significant ($P > 0.05$) changes in haematological parameters such as red blood cells, white blood cells, platelet, haemoglobin, packed cell volume and percentage of leukocytes. Hepatic malondialdehyde and reduced glutathione levels increased ($P < 0.05$) only at the highest dose of tilmicosin. The results indicate that tilmicosin did not cause lipid peroxidation in the heart.

Tilmicosin, heart, liver, lipid peroxidation, mouse

Antibiotics are the most widely used drugs in veterinary medicine. Antibacterials may cause different adverse or side effects. Adverse drug reactions are classified according to their etiology, and biochemical side effects are generally accepted as indicators of pathological side effects (Kayaalp 1994).

Tilmicosin has been prepared by chemical modification of desmycosin. It has been used in therapy of respiratory disease in cattle. It inhibits growth of *Pasteurella* spp. and *Mycoplasma* spp. Micotil 300® (Lilly Elanco, Istanbul, Turkey), contains 300 mg tilmicosin per milliliter, produces therapeutic levels in the lungs for 3 to 4 days after a single subcutaneous injection (Barragry 1994; Modric et al. 1999).

Gastrointestinal upset, jaundice and liver damage have been reported after administration of macrolides. In addition to these, tilmicosin has cardiotoxic effect. On the other hand, a transient swelling at the injection site, severe dyspnea, anaphylaxis and collapse after tilmicosin treatment (Jordan et al. 1993; McGuigan 1994; Barragry 1994) may occur. Tilmicosin decreased cardiac superoxide dismutase and glutathione peroxidase activities (Yazar et al. 2002).

Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals (Valenzuela 1990), which is accepted as an indicator of lipid peroxidation (Neilsen et al. 1997). Glutathione peroxidase catalyses the reduction of hydrogen peroxide or lipid peroxides with reduced glutathione (GSH) (Chan and Decker 1994).

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The purpose of this study was to investigate the effects of different doses of tilmicosin on plasma, cardiac and hepatic MDA and GSH levels to determine the possible cardiotoxic and hepatotoxic effects, and investigate the effect of tilmicosin on haematological parameters.

Materials and Methods

Forty clinically healthy male Balb/C mice (aged approximately 3-3.5 months, body mass 28-36 g) were used throughout the experiment (Animal Research Institute, Konya, Turkey). Mice were fed on a standard pellet diet and tap water *ad libitum*. They were divided in four groups of ten animals each. Mice were injected subcutaneously with: (Group 1 = control) isotonic saline solution, (Group 2) 25 mg/kg, (Group 3) 50 mg/kg and (Group 4) 75 mg/kg, body weight, tilmicosin (Micotil 300®, Lilly Elanco, Istanbul, Turkey), single injection. Micotil 300® was diluted by propylene glycol (Merck, Darmstadt, Germany) to achieve 25, 50 and 75 mg/kg doses.

At the end of the experiment, blood samples were taken from the heart by cardiac puncture under light ether anesthesia. Mice were then immediately killed by cervical dislocation. Liver and heart were removed immediately. Plasma, cardiac and hepatic MDA and GSH levels were determined by previously reported methods (Mihara and Uchiyama 1978; Ellmann 1959; Draper and Hadley 1990) with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Cardiac and hepatic MDA and GSH levels were expressed as nmol/g and $\mu\text{mol/g}$ tissue protein, respectively.

Red blood cell (RBC) and white blood cell (WBC) counts were obtained with a haemocytometer. Hemoglobin (Hb) and packed cell volume (PCV) were determined by Sahli haemometer and microhaematocrit methods, respectively. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels were calculated.

All values were expressed as mean \pm SE. Tukey multiple range test (SPSS for Windows, release 11.0) was used to determine statistical differences between control and experimental groups. In all cases, probability of error of less than $P < 0.05$ was selected as the criterion for statistical significance from the control values.

Results

Effects of different doses of tilmicosin on plasma, cardiac and hepatic MDA and GSH levels, and haematological parameters are given in Table 1.

Table 1
Effects of different doses of tilmicosin on malondialdehyde and glutathione levels, and haematological parameters (mean \pm SE)

Parameters	Control (n = 10)	25 mg/kg (n = 10)	50 mg/kg (n = 10)	75 mg/kg (n = 10)
MDA _{plasma} nmol/ml	4.04 \pm 0.26	3.96 \pm 0.17	3.88 \pm 0.15	4.15 \pm 0.16
MDA _{hepatic} nmol/g	24.08 \pm 2.39	28.13 \pm 3.97	22.55 \pm 1.10	42.21 \pm 2.06*
MDA _{cardiac} nmol/g	19.10 \pm 1.76	19.43 \pm 0.70	17.93 \pm 1.01	17.19 \pm 0.97
GSH _{hepatic} $\mu\text{mol/g}$	14.90 \pm 1.88	14.78 \pm 1.01	11.42 \pm 0.76	22.43 \pm 2.61*
GSH _{cardiac} $\mu\text{mol/g}$	4.45 \pm 0.21	4.07 \pm 0.18	4.07 \pm 0.50	3.34 \pm 0.18
RBC ($\times 10^6/\mu\text{l}$)	8.46 \pm 0.4	7.60 \pm 0.31	7.98 \pm 0.94	7.71 \pm 1.09
WBC ($\times 10^3/\mu\text{l}$)	4.28 \pm 0.27	4.17 \pm 0.32	4.29 \pm 0.51	4.17 \pm 0.50
Platelet ($\times 10^3/\mu\text{l}$)	992.1 \pm 38.78	942.2 \pm 60.86	1032 \pm 134.22	1005 \pm 43.43
Haemoglobin (g/100 ml)	11.89 \pm 0.16	11.10 \pm 0.23	11.25 \pm 1.06	10.96 \pm 0.20
PCV (%)	40.25 \pm 0.67	38.89 \pm 0.82	38.01 \pm 1.04	39.70 \pm 0.76
Monocytes (%)	2.14 \pm 0.55	3.85 \pm 0.56	3.14 \pm 0.46	2.86 \pm 0.45
Neutrophils (%)	34.85 \pm 3.93	28.57 \pm 3.68	21.71 \pm 5.96	21.55 \pm 2.14
Lymphocytes (%)	62.86 \pm 3.90	66.14 \pm 3.97	74.71 \pm 1.86	73.43 \pm 13.36
Basophils (%)	0.43 \pm 0.20	0.29 \pm 0.18	0.00 \pm 0.00	0.14 \pm 0.14
Eosinophils (%)	1.00 \pm 0.58	1.14 \pm 0.69	0.42 \pm 0.03	0.86 \pm 0.38
MCV fl	48.21 \pm 1.93	51.67 \pm 1.89	48.06 \pm 2.01	52.66 \pm 2.96
MCH pg	14.25 \pm 0.61	14.77 \pm 0.62	14.21 \pm 0.57	14.43 \pm 0.54
MCHC g/dl	29.56 \pm 0.33	28.58 \pm 1.48	29.65 \pm 0.63	27.45 \pm 0.89

* Statistically different from control values ($P < 0.05$). MDA; malondialdehyde, GSH; reduced glutathione, RBC; red blood cell, WBC; white blood cell, PCV; packed cell volume, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration.

Blood cell counts slightly decreased in the experimental groups, but it was not statistically significant ($P > 0.05$). Hepatic MDA and GSH concentrations only increased in Group 4 ($P < 0.05$).

Discussion

Tilmicosin has been used for treatment of respiratory disease in animals such as cattle, swine (Moran et al. 1997), rabbit (Mckay et al. 1996) and rat (Modric et al. 1999). Macrolides are generally hepatotoxic (Barragry 1994). In addition, it is well known that tilmicosin has cardiotoxic effects. After tilmicosin injection, positive chronotropy and negative inotropy are observed (Jordan et al. 1993; McGuigan 1994).

In the present study, cardiac MDA and GSH did not change at any dose we used. In our previous studies, tilmicosin caused an increase in cardiac creatine kinase activity (Yazar et al. 2001) and decreases in cardiac superoxide dismutase and glutathione peroxidase activities in mice at the dose of 25 mg/kg (Yazar et al. 2002). In the present study, cardiac MDA, which accepted as an indicator of lipid peroxidation (Valenzuela 1990; Neilsen et al. 1997), was not affected. It may be stated that tilmicosin only depressed activities and/or synthesizes of superoxide dismutase and glutathione peroxidase.

In this study, tilmicosin caused increased hepatic MDA and GSH levels at the highest dose (75 mg/kg) in Group 4. Macrolides are metabolized by the liver. Therefore tilmicosin might have achieved a high concentration in the liver and cause changes in MDA and GSH concentrations. However, this increase of MDA level was not reflected in the plasma.

In the haematological analysis, tilmicosin caused slightly decreased RBC and WBC counts, but these decreases did not differ ($P > 0.05$). In the previous studies, tilmicosin caused statistically significant decrease in RBC and WBC counts of rabbits (Altunok et al. 2002) and it achieved at high levels in phagocytes of avian, porcine and bovine (Scorneaux and Shryock 1999). Also, it was reported that other macrolides might cause similar effect. Azithromycin and clarithromycin decreased RBC and WBC counts in humans (Fujii et al. 1995; McEvay and Litvak 2001; Ohtsuka et al. 1996; Tajima et al. 1995).

Our results indicate that although tilmicosin caused increases in MDA and GSH levels at the highest dose (75 mg/kg) in the liver, it did not affect cardiac MDA and GSH levels, and haematological parameters. It may be stated that tilmicosin did not cause lipid peroxidation in the heart of Balb/C mice.

Účinek různých dávek tilmicosinu na koncentraci malondialdehydu a glutathionu

Tilmicosin má kardiotoxický účinek a po léčbě tímto preparátem byla pozorována pozitivní chronotropie a negativní ionotropie. Cílem této studie bylo zjistit účinek různých dávek tilmicosinu na koncentraci malondialdehydu, redukováného glutathionu v srdci a játrech a na hematologické parametry. K experimentu bylo použito 40 Balb/c myši samčího pohlaví. Myši byly rozděleny do čtyř skupin ($n = 10$). Myším byly aplikovány s.c. injekce tilmicosinu v následujících dávkách: skupině 1 (kontrolní) byl podán izotonický fyziologický roztok, 2. skupině 25 mg/kg, 3. skupině 50 mg/kg a 4. skupině 75 mg/kg. Po 3 dnech byla pomocí spektrofotometru měřena koncentrace malondialdehydu a redukováného glutathionu v krevní plazmě, v srdci a játrech. Byly též stanoveny počty červených a bílých krvinek, krevních destiček, hemoglobinu, hematokrit a diferenciální krevní obraz.

Na konci experimentu bylo zjištěno, že podání tilmicosinu nevyvolalo žádné statisticky významné změny ($P > 0.05$) sledovaných hematologických ukazatelů. Koncentrace malondialdehydu a redukováného glutathionu v játrech se zvýšila pouze ve čtvrté skupině ($P < 0.05$). Tilmicosin tedy nezpůsobuje lipoperoxidaci v srdeční tkáni.

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