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Protection through L-Carnitine on Tissue Oxidant Status and Sialic Acid Content in Tilmicosin-Induced Alterations in BALB/c Mice

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Abstract

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The macrolide antibiotic tilmicosin is known to induce cardiotoxic effect when administered at large doses. In this work, the effects of tilmicosin were evaluated with respect to alterations in total sialic acid, malondialdehyde and glutathione content of the heart, liver, kidney and lung tissues after single subcutaneous injection of 75 mg/kg tilmicosin with or without L-carnitine (500 mg/kg for 5 days daily via s.c. route) in BALB/c mice. L-carnitine is a co-factor serving in the mitochondrial β-oxidation of long chain fatty acids, and it was reported to be protective in several types of toxicity cases probably via multi-factorial mechanisms. Twenty eight mice were divided into 4 groups including group 1 (control), group 2 (L-carnitine), group 3 (tilmicosin) and group 4 (tilmicosin plus L-carnitine). Following the administration of treatments, tissue samples were collected, and the samples were assayed for malondialdehyde, glutathione and total sialic acid content. Mice receiving tilmicosin treatment alone had significantly higher malondialdehyde and total sialic acid concentrations (except for MDA of lungs) but lower glutathione concentration in selected tissues compared to those of the control, group 2 (Carnitine only) and group 4 (L-carnitine plus tilmicosin) ($p < 0.05$). However, no significant difference was found associated with the assayed indicators between the control and mice treated with L-carnitine plus tilmicosin. These results suggest that tilmicosin may cause oxidative stress in the heart, liver, lung and kidneys, but the adverse effects could be attenuated by L-carnitine administration.

Total sialic acid, glutathione, malondialdehyde, tilmicosin, L-carnitine

Tilmicosin is a macrolide antibiotic which is used as therapeutic and prophylactic agent against the respiratory disease of cattle known as the bovine respiratory disease (BRD), associated with *Pasteurella* spp. and *Mycoplasma* spp. (Prescott and Baggot 1993). It has been reported that tilmicosin causes cardiotoxicity and hepatotoxicity when used in high doses (Jordan et al. 1993). It is possible for humans to be affected by tilmicosin toxicity due to a small amount of accidental injection (McGuigan 1994; Von Essen et al. 2003). It was reported that a single injection of 25 mg/kg of tilmicosin resulted in decreased superoxide dismutase and glutathione peroxidase in the heart tissue of mice (Yazar et al. 2002), suggesting that tilmicosin may cause oxidative stress by decreasing antioxidant enzymes in cardiac tissue.

L-carnitine is a naturally occurring amino acid-like compound which plays an important role in β-oxidation of long chain fatty acids in mitochondria. L-carnitine is also associated with buffering of excess acyl-Co A which is potentially toxic to the cells (Brass 2000). It was reported that L-carnitine had a protective effect on lipid peroxidation, and it may improve the antioxidant status in rats. Moreover, L-carnitine can increase the scavenging of

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free radicals from the cellular sites (Kalaiselvi and Panneerselvam 1998; Rani and Panneerselvam 2002).

Serum sialic acid levels in various types of diseases have a diagnostic value in that serum sialic acid levels have been found to be increased in cancer patients and several types of inflammatory diseases such as arthritis, Crohn's disease and psoriasis (Silver et al. 1983; Shamberger 1984). In addition, serum sialic acid concentrations are increased in chronic glomerulonephritis, chronic renal failure, chronic liver disease and pneumonia (Sillanaukee et al. 1999). An increase in the level of sialic acid was also correlated with the rate of cardiovascular mortality (Lindberg et al. 1991).

In this study, we studied the alterations in malondialdehyde (MDA), glutathione (GSH) and total sialic acid (TSA) of the heart, liver, lungs and kidneys in BALB/c mice in response to tilmicosin treatment. We also evaluated the protective effect of L-carnitine on the changes induced by tilmicosin.

Materials and Methods

Thirty two BALB/c mice weighing 20 - 30 g were divided into 4 groups. Clinically healthy conventional BALB/c mice were obtained from the University of Kafkas Animal Research Farm, Kars, Turkey. The mice were fed a standard pelleted diet; feed and water were provided *ad libitum*. The animals were treated according to the Animal Care and Use Regulation (European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purpose 1996). Mice in group 1 (control) were injected with a single dose of subcutaneous saline solution (2.5 ml/kg b. m.). Group 2 (L-carnitine) was applied 500 mg/kg of L-carnitine daily (CARNITENE®, injectable solution in 5 ml sterile ampoule containing 200 mg/ml L-carnitine, Santa Farma Ilac Sanayii A.S., Istanbul, Turkey) with the injected volume of 50 - 75 µl for 20 - 30 g b.m. mice using Hamilton microinjector applied for 5 days. Group 3 was treated with 75 mg/kg of tilmicosin (Micotil 300® Lilly Elanco, Istanbul, Turkey) by single subcutaneous injection. One ml of tilmicosin solution (containing 300 mg/ml tilmicosin) was diluted with 9 ml of saline solution, and diluted tilmicosin solution was given to the mice with the volume of 50 - 75 µl for 20 - 30 g body mass (75 mg/kg b.m.), respectively. Group 4 was daily injected with subcutaneous 500 mg/kg of L-carnitine for 5 days which is followed by single subcutaneous injection of 75 mg/kg of tilmicosin.

Liver, heart, kidneys and lungs were collected, and the tissues were rinsed with 0.9% NaCl. The 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 samples were stored at -25 °C until they were analyzed. Analyses were carried out by the method of Beutler et al. (1963) and Yoshoiko et al. (1979) for GSH and MDA concentrations, respectively. Total sialic acid concentrations were measured colorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) by the method of Sydow (1985).

Statistical differences between the groups were tested by analysis of variance (ANOVA) and Tukey's test using SPSS for Windows version 10.0. Data were presented as mean ± standard errors, and p values less than 0.05 were considered significant.

Results

Table 1 shows TSA, MDA and GSH concentrations in selected tissues according to mice treatments. While MDA and TSA concentrations in the heart, liver, kidneys and lungs (except for MDA of lung) were lower in group 4 (tilmicosin plus L-carnitine) than those of group 3 (tilmicosin alone), the MDA and TSA concentrations in selected organs were not significantly different between the control and group 4 (tilmicosin plus L-carnitine). Glutathione concentrations of the heart, liver, kidneys and lungs were decreased in group 3 (tilmicosin alone) compared to group 4 (tilmicosin plus L-carnitine) and group 1 (control). Moreover, no difference was found in GSH concentration between group 4 (tilmicosin plus L-carnitine) and group 1 (controls) in the heart, liver, kidney and lung tissues.

Discussion

Administration of tilmicosin in high doses is known to induce cardiotoxicity and hepatotoxicity (Jordan 1993; McGuigan 1994; Yazar et al. 2004). Increased free radical generation is reported in various types of diseases including ischemia-reperfusion, cancer,

Table 1. Heart, liver, kidney and lung tissue malondialdehyde (MDA), glutathione (GSH) and total sialic acid (TSA) concentrations in the treatment groups

Tissue	Indicators	Control	L-carnitine	Tilmicosin	Tilmicosin+ L-carnitine
Liver	MDA (μmol/g)	0.278 ± 0.01^{b}	0.285 ± 0.01^{b}	0.397 ± 0.01^{a}	0.292 ± 0.01^{b}
	GSH (mg/g)	0.357 ± 0.06^{b}	0.478 ± 0.01^{a}	0.214 ± 0.01^{c}	0.336 ± 0.02^{b}
	TSA (mg/g)	0.604 ± 0.03^{b}	0.706 ± 0.04^{b}	1.130 ± 0.09^{a}	0.751 ± 0.05^{b}
Kidneys	MDA (μmol/g)	0.174 ± 0.02^{b}	0.168 ± 0.01^{b}	0.242 ± 0.01^{a}	0.196 ± 0.01^{b}
	GSH (mg/g)	0.223 ± 0.06^{ab}	0.265 ± 0.02^{a}	0.128 ± 0.01^{c}	0.196 ± 0.01^{b}
	TSA (mg/g)	1.376 ± 0.16^{b}	1.293 ± 0.08^{b}	2.082 ± 0.13^{a}	1.462 ± 0.07^{b}
Heart	MDA (μmol/g)	0.142 ± 0.02^{b}	0.105 ± 0.01^{b}	0.185 ± 0.02^{a}	0.127 ± 0.01^{b}
	GSH (mg/g)	0.140 ± 0.02^{a}	0.130 ± 0.01^{a}	0.099 ± 0.01^{b}	0.134 ± 0.01^{a}
	TSA (mg/g)	0.184 ± 0.02^{b}	0.123 ± 0.01^{c}	0.272 ± 0.03^{a}	0.192 ± 0.01^{b}
Lungs	MDA (μmol/g)	0.107 ± 0.03^{ab}	0.093 ± 0.05^{b}	0.131 ± 0.01^{a}	0.100 ± 0.01^{b}
	GSH (mg/g)	0.115 ± 0.05^{a}	0.119 ± 0.01^{a}	0.078 ± 0.01^{b}	0.111 ± 0.02^{a}
	TSA (mg/g)	0.294 ± 0.02^{b}	0.233 ± 0.01^{b}	0.370 ± 0.03^{a}	0.262 ± 0.02^{b}

Results with different superscripts within the same row are significantly different ($p < 0.05$).

nephritis, hepatitis and drug/toxin induced alterations (Dröge 2002). Lipid peroxidation in cellular membranes generates malondialdehyde (MDA) as a by-product, and it has been accepted as a marker of lipid peroxidation in membranous structures (Neilsen et al. 1997; De Zwart 1999). In the present study, MDA concentrations in selected tissues (except for lung) in group 3 (tilmicosin) were higher compared with those of other groups (groups 1, 2 and 4). In addition, GSH concentration of mice in group 3 was decreased in the selected organs compared to those of the control, group 2 (L-carnitine) and group 4 (L-carnitine plus tilmicosin). These results suggest that tilmicosin results in increased oxidative stress, and L-carnitine might have a protective effect on lipid peroxidation induced by tilmicosin. Indeed, L-carnitine has been shown to have a protective effect on lipid peroxidation and free radical damage (Arduini 1992; Kalaiselvi and Panneerselvam 1998; Sener et al. 2004). L- carnitine is a co-factor essential for the beta oxidation of long chain fatty acids by providing the translocation of fatty acids into the mitochondrial matrix and also a buffer for the potentially toxic acyl-Co A (Kelly 1998; Brass 2000). This function of L-carnitine is especially important in the cardiac tissue since the energy requirement of cardiac tissue is heavily dependent on the beta-oxidation of fatty acids for the energy production (Kelly 1998). L-carnitine has been shown to ameliorate several toxicity cases. Doxorubicin-induced apoptosis in cardiac myocytes is prevented by L-carnitine *in vitro* (Andrieu-Abadie et al. 1999). Methamphetamine neurotoxicity, mediated by peroxynitrite radicals, was protected by L-carnitine (Ashraf et al. 2002). Acetyl-L-carnitine, an esterified form of carnitine, reduced the lipid peroxidation and decreased the oxidative stress in aged rats (Hagen et al. 1998; Kaur et al. 2001). Another way of protection by L-carnitine might be related to increasing antioxidant level in tissues since L-carnitine has been shown to increase GSH levels (Di Giacomo et al. 1993). Reduced GSH concentration of mice treated with tilmicosin alone in the organs could increase the burden on the cellular oxidant state since GSH is an important part of antioxidant defense system which plays an important role in preventing harmful effects of free radicals by scavenging hydroxyradicals and singlet oxygen (Wu et al. 2004). Similar to our results, Yazar et al. (2002) reported that tilmicosin decreased superoxide dismutase and glutathione peroxidase levels in the heart tissue of mice treated with tilmicosin. Another study reported that while administration of tilmicosin at 75 mg/kg dose had no effect on cardiac MDA and GSH, it increased MDA and GSH concentration in the liver tissue (Yazar et al. 2004) .

Assaying sialic acid may be of value for diagnostic purposes since sialic acid content has been reported to be altered in cancer patients, cardiovascular disease, some inflammatory diseases as well as in chronic glomeruloneprítis and renal failure (Silver et al. 1983; Shamberger 1984; Lindberg et al. 1991). In the present study, we found that while administration of tilmicosin alone resulted in elevated tissue TSA concentrations in the heart, liver kidneys and lungs, mice receiving L-carnitine plus tilmicosin showed no difference in TSA concentration when compared to the control. An increased level of TSA in tilmicosin-treated mice could be associated with the effect of tilmicosin on the cellular membranes due to generation of free radicals leading to lipid peroxidation and a release of sialic acid residues from cellular membranes. Sialic acids are derivatives of neuraminic acid located on the terminal portions of oligosaccharide chains of glycolipids and glycoproteins in membranes (Sillanaukee et al. 1999; Wang and Brand-Miller 2003). Events leading to disintegration in the membranous structures as a result of lipid peroxidation could result in the release of the sialic acid content, thus increasing the TSA concentrations in the tissues. Indeed, the observed similar values between group 1 (control) and group 4 (L-carnitine plus tilmicosin) may reflect a protection by L-carnitine in tilmicosin induced oxidative stress.

In conclusion, we may suggest that a single dose of tilmicosin at 75 mg/kg induces oxidative stress in the heart, liver, kidney and lung tissues. However, L-carnitine may prevent these adverse effects when applied together with tilmicosin.

Ochranný vliv L-karnitinu na obsah oxidantů v tkáních a na obsah kyseliny sialové v rámci Tilmicosinem vyvolaných poškození u BALB/c myší

Je známo, že makrolidové antibiotikum tilmikosin při aplikaci ve vysokých dávkách působí kardiotoxicky. V této studii byly u BALB/c myší zkoumány účinky tilmikosinu vzhledem k ovlivnění celkového množství kyseliny sialové, malondialdehydu a glutathionu v srdci, ledvinách, jaterní a plicní tkáni po jeho jednorázové aplikaci 75 $mg \cdot kg^{-1}$ s nebo bez přídavku L- karnitinu (500 $mg \cdot kg^{-1}$ s.c. po dobu 5 dní). L-karnitin je kofaktorem a-oxidace dlouhých řetězců mastných kyselin v mitochondriích. Byl popisován jeho protektivní účinek u různých typů toxicity, pravděpodobně v rámci multifaktoriálních mechanismů. Celkem 28 myší bylo pak rozděleno do 4 skupin: skupina 1 (kontrola), skupina 2 (L- karnitin), skupina 3 (tilmikosin) a skupina 4 (L-karnitin a tilmikosin). Po podání léčiv byly následně odebrány vzorky tkání, a v nich pak byly analyzovány koncentrace malondialdehydu, glutathionu a celkového množství kyseliny sialové. Myši ošetřené pouze tilmikosinem měly, ve srovnání s kontrolou, skupinou 2 (pouze karnitin) a skupinou 4 (karnitin a tilmikosin) ve vybraných tkáních výrazně vyšší koncentraci malondialdehydu a celkový obsah kyseliny sialové (kromě MDA v plicích) ale zároveň nižší koncentraci glutathionu ($p < 0,05$). Nebyly zjištěny žádné signifikantní rozdíly v měřených ukazatelích u kontrol a myší ošetřených L-karnitinem i tilmikosinem. Tyto výsledky naznačují, že tilmikosin může způsobovat oxidativní stres v srdci, játrech, plicích a ledvinách, ale vedlejší účinky mohou být redukovány podáním L- karnitinu.

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