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RESEARCH ARTICLE

Protective Effect of S-Methyl Cysteine against Tilmicosin-Induced Cardiotoxicity in Rats

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The present study was carried out to investigate whether S-methyl cysteine (SMC) would ameliorate the acute cardiotoxic effect of tilmicosin antibiotic in treated Wister rats. Thirty-two male rats were equally divided into four groups: control, SMC (100 mg/kg orally for five consecutive days), tilmicosin (a single dose, 75 mg/kg BW, S/C on the sixth day) and SMC+Tilmicosin (pretreated with SMC and co-injected with 75 mg/kg of tilmicosin at the sixth day). The biochemical results demonstrated marked increase in serum aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) activities and cardiac troponin T (cTnT) concentrations in tilmicosin-treated rats indicating severe cardiotoxicity. On the other hand, pretreatment of rats with SMC revealed marked decrease in cardiac biochemical parameters toward the normal limits. Histopathological findings of the heart sections revealed multifocal myocarditis in tilmicosin-treated rats meanwhile, (SMC+Tilmicosin) treated group showed slight vacuolation of myocardial fiber. Furthermore, the ultrastructure findings revealed myolysis and necrosis in tilmicosin-treated rats compared with intact myocardial fiber in (SMC+Tilmicosin) group.

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INTRODUCTION

Tilmicosin is a potent semisynthetic macrolide antibiotic, has an antimicrobial activity against *Pasteurella* spp., *Mycoplasma* spp., and a variety of Gram-positive organisms (Barragry, 1994). Although, macrolide antibiotics are considered to be one of the safe anti-infective drugs, cardiotoxic effects of tilmicosin such as positive chronotropy, negative inotropy, acute heart failure and alteration in electrocardiogram have been reported in sheep, cattle, horses, goats and dogs (Main *et al.*, 1996; Modric *et al.*, 1998; Abou-El-Hassan *et al.*, 2003). Tilmicosin-induced cardiotoxicity is through increasing free radical production and decreasing antioxidant enzymes in heart (Yazar *et al.*, 2002; Yapar *et al.*, 2006).

S-Methyl cysteine (SMC), a water-soluble organosulfur compound (OSC), exists in various plants, including Allium plants such as garlic and onion (Amagase, 2006). Garlic represents a known cardioprotective agent (Ginter and Simko, 2012; Khatua *et al*., 2012), its efficacy could be attributed to various active organosulfur compounds (Yun *et al*., 2014). Hydrophilic sulfur compounds include SMC, N-Acetyl cysteine (NAC), S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) which exhibited marked antioxidant protection (Siveski-Iliskovic *et al.*, 1994). S-Methyl cysteine is found at a concentration of ~40 mg/g of fresh garlic (Fukushima *et al*., 2001).

S-Methyl cysteine has an antioxidative, anti-inflammatory and anti-fibrogenic effects, its supplementation might be helpful for prevention or treatment of different diseases (Chen *et al*., 2007; Yin *et al*., 2007). Furthermore, SMC can serve as chemo-preventive agent for hepatocarcinogenesis, reducing cellular proliferation and oxidative stress (Nishikawa-Ogawa *et al*., 2006). However, less is known about its protective effect against cardiac toxicity. Therefore, this study was designed to investigate the possible cardioprotective effect of SMC against tilmicosin-induced cardiac toxicity in rats.

MATERIALS AND METHODS

Animals: Thirty-two male Wister albino rats weighing (150-200 g) were obtained from animal research center, El Zagazig University, Egypt. Rats were housed in plastic cages, and acclimatized for one week to laboratory

conditions. The animals were provided commercial balanced diet and water, *ad libitum* throughout the experiment. All animal related procedures were carried out in accordance with the ethical committee of Kafrelshiekh University.

Experimental design: Thirty two rats were randomly divided into four equal groups, i.e., control, SMC, tilmicosin and SMC+Tilmicosin groups. Control rats received saline (oral and S/C injections) and SMC group received orally SMC (S-methyle cysteine, Sigma-Aldrich, USA; 100 mg/kg BW) for 5 days and injected with saline (S/C) on the 6th day. Tilmicosin group received orally saline for 5 days and then 24 hours later, the animals administered a single injection of tilmicosin (Micotil 300, Lilly Elanco, Istanbul, Turkey; 75 mg/kg BW S/C) whereas SMC+Tilmicosin group received SMC (100 mg/kg BW orally) for 5 days followed by single S/C injection of tilmicosin (75 mg/kg BW) at the sixth day. All animals were sacrificed on the 7th day.

Serum biochemical analysis: The serum levels of AST, LDH, and CK were determined using commercially available standard diagnostic kits (Stanbio Laboratory Boerne, USA). The serum cTnT concentration was determined using ELISA kit (Roche Diagnostics, Mannheim Germany).

Histopathological and ultra-structural examination: The heart tissue sections were directly fixed in 10% formalin, dehydrated, cleared and embedded in paraffin wax. Then blocks were sectioned in 4 µm thickness and stained by H&E. Heart specimens were also processed for electron microscopy as described by (Nawrot *et al.*, 2013). The samples were then observed under a JEM, 100CXII electron microscope.

Statistical analysis: Statistical analysis was carried out by using the SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). Groups data were compared by one-way analysis of variance (ANOVA), followed by LSD test. The statistical significance was accepted at ($P<0.05$).

RESULTS

This study was carried out to investigate the protective effect of SMC against timicosin-induced cardiotoxicity by measuring the cardiac enzyme markers, cTnT, and cardiac histopathology. Firstly, Serum enzyme biomarkers were investigated; as presented in Table 1, Animals treated with tilmicosin showed significant increase in the serum levels of AST, LDH, and CK compared to normal control group and SMC-only treated group ($P<0.05$). Pretreatment with SMC in the SMC+Tilmicosin group significantly reduced the serum levels of AST, LDH, and CK as compared to tilmicosin treated group ($P<0.05$).

Also, cardiac troponin T was measured; animals treated with tilmicosin showed significant increase in the serum concentration of cTnT compared to normal control group and SMC-only treated group ($P<0.05$) (Table 1). SMC+Tilmicosin treated group showed significantly lower serum levels of cTnT as compared to tilmicosin treated group ($P<0.05$). No significant difference was observed in the rats treated with SMC alone compared with normal control rats.

Histopathological findings of heart sections from normal and SMC treated animals showed normal morphological appearances (Fig. 1, A-C), whereas tilmicosin-treated group revealed myocardial necrosis associated with lymphocytic infiltration and multifocal vacuolation of myocardial fibers (Fig. 1, E). The rats pretreated with SMC and co-injected with tilmicosin demonstrated slight vacuolation of the myocardial fibers (Fig. 1, G).

Transmission electron microscopic images, from the ventricular wall of both control and SMC groups, showed normal myocardial fibrils (Fig. 1, B-D). Tilmicosin demonstrated severe fragmentation and loss of myofibrils, swelling of mitochondria and loss of their cristae (Fig. 1, F). SMC-Tilmicosin treated rats showed only minimal myocytolysis (Fig. 1, H).

DISCUSSION

Cardiotoxicity represents one of the most serious side effects associated with new drug development. Tilmicosin has been prepared by chemical modification of desmycosin and used for the treatment of respiratory tract infections. The heart is the target organ of acute tilmicosin toxicity (McGuigan, 1994). Tilmicosin increased the free radical production and also decreased the antioxidant enzymes as SOD, CAT and GPx activities in cardiac tissue (Yazar *et al*., 2002; Cetin *et al.*, 2011). It is well known that the heart is more susceptible to free radical-induced damage, because it has relatively low levels of these antioxidant enzymes (Abou-El-Hassan *et al.*, 2003).

Serum AST, LDH and CK enzymes activities have been used as markers of myocardial oxidative stress, usually associated with ischemic or toxic myocardial injury, and reflect the extent of damage in its musculature (Zhou *et al.*, 2008; Jahan *et al.*, 2012). Intriguingly, the significant alterations of these enzymes could be also indicating hepatic and cardiac intoxication. Tilmicosin administration markedly elevated serum activities of AST, LDH, and CK. Interestingly, pre-administration of SMC in tilmicosin treated group markedly reduced the activities of the cardiac enzymes to the normal levels. Tilmicosin treatment demonstrated also significant elevation in the serum concentration of cTnT which nearly reversed towards control group levels by SMC pretreatment. CTnT is a contractile protein that released from myocardium in proportion to the degree of tissue injury and disruption of myocyte membranes. CTnT is one of the most preferred biomarkers for myocardial infarction as proposed by the American College of Cardiology and the European Society of Cardiology (Babuin and Jaffe, 2005; Korff *et al*., 2006). Serum levels of cTnT have been shown to increase in myocardial infarction (Kaur *et al*., 2013). Several dozen published studies have directly confirmed its effectiveness in laboratory animals for assessment of cardiotoxicity (O'Brien, 2008). AST, LDH and CK measurement has long been used to detect cardiac and skeletal muscle injury, but these three biomarkers lack sensitivity and specificity (Chan and Ng, 2010; Kaur *et al*., 2013).

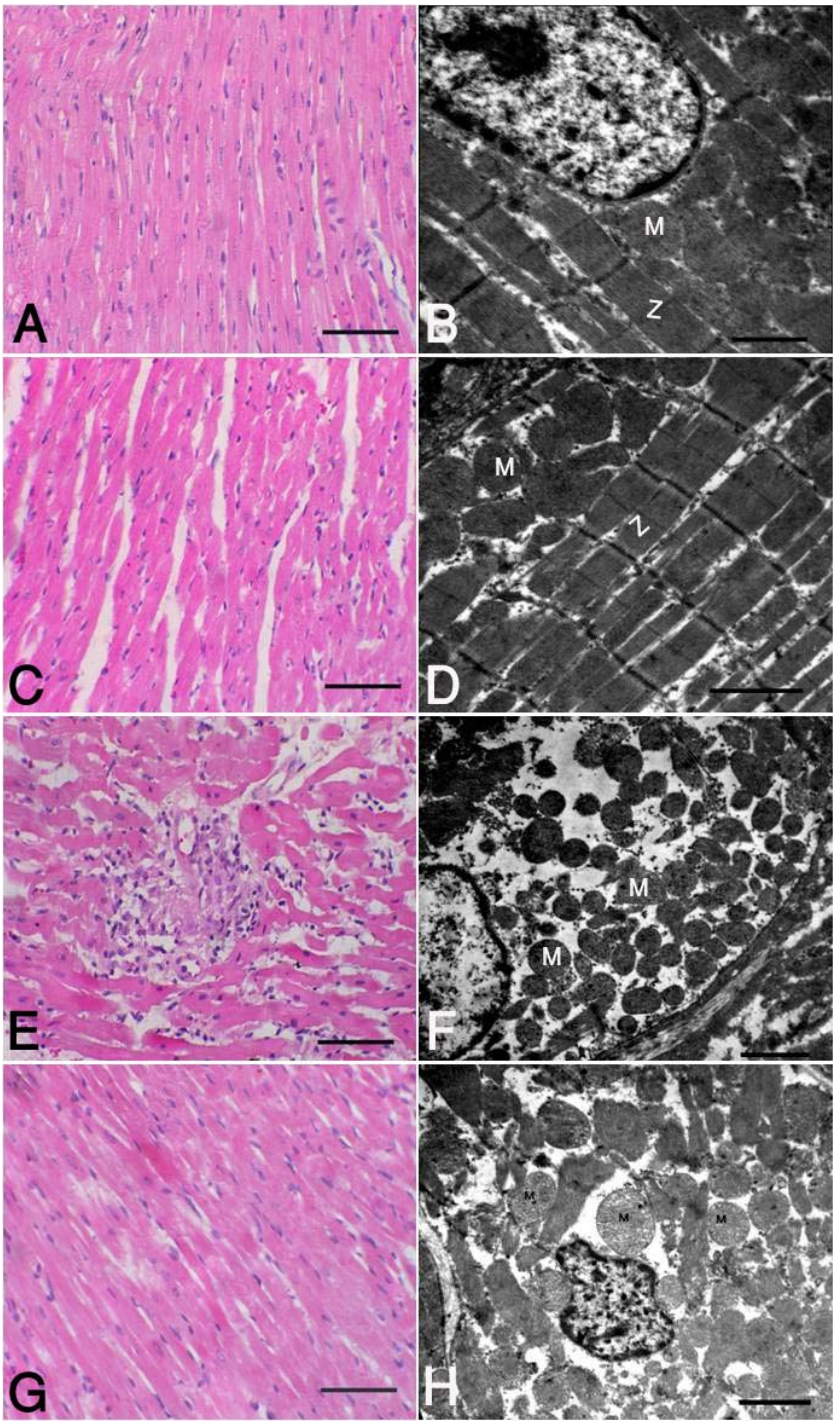

Fig. 1: Histopathological and ultra-structural findings of different tilmicosin treated groups with or without pretreatment with SMC. **A, B)** heart sections of normal non treated rats showing normal myocardial fibers; **C, D)** heart sections from SMC treated group also revealed normal myocardial fibers; **E, F)** heart sections of tilmicosin-treated rats revealing marked degeneration of myocardial fibers and mononuclear inflammatory cells infiltration, also severe loss and fragmentation of myofibrils; **G, H)** heart sections of SMC+Tilmicosin treated rats revealing slight vacuolation of myocardial fibers and fragmentation of the myofibrils. **M** referred to mitochondria, **Z** referred to Z line. A, C, E and G: H&E satin, Scale bar: 100µm. B: E/M, Scale bar: 1µm; D, F and G: E/M, Scale bar: 2µm).

Table 1: Effect of SMC on cardiac enzyme activities and cTnT in tilmicosin-treated rats

Treatment	AST (U/L)	LDH (U/L)	CK (U/L)	cTnT (µg/L)
Control	65.00±5.52	1403.67±30.99	359.50±24.54	15.80±1.10
SMC	74.75±1.08	1268.33±44.68	356.33±11.05	13.76±2.15
Tilmicosin	138.25±15.45[a]	1668.50±23.38[a]	510.50±29.16[a]	40.67±9.23[a]
SMC+Tilmicosin	85.00±6.35	1432.33±65.13	318.67±16.19	15.40±1.04

Data are expressed as mean±S.E. Superscripts within the same column indicate significant difference between the treatments (P<0.05). AST: serum aspartate transaminase, LDH: lactate dehydrogenase, CK: creatine kinase, cTnT: cardiac troponin T. SMC: animals received orally S-methyl cysteine, Tilmicosin: animals administered a single S/C injection of tilmicosin.

Measurement of cTnT along with these biomarkers could supply information that is useful for detection and differentiation between cardiac and skeletal muscle toxicity (Tonomura *et al.*, 2009).

It is noteworthy that myocardial dissolution, necrosis and monocytes infiltrations as well as myofibrils and mitochondrial alterations were correlated well with down regulation of the endogenous SO2/GOT pathway involved in myocardial oxidative stress associated with isoproterenol cardiotoxicity (Liang *et al.*, 2011). Tilmicosin treatment caused marked degeneration and necrosis of cardiac muscle fibers (Xie *et al.*, 2011). Moreover the ultrastructural finding revealed significant cardiomyopathy on the level of both myofibrils and mitochondria in tilmicosin-treated rats whereas SMC-tilmicosin treated group, demonstrated minimal degree of myofibrils loss.

Antioxidants demonstrated beneficial effects against drugs-induced cardiotoxicity in mice and rats (Naidu *et al.*, 2002; Liang *et al.*, 2011; Jahan *et al.*, 2012). Supplementation of OSC such as SAC, SMC, SEC and SPC could increase the levels of reduced glutathione in plasma and organs, elevating the glutathione peroxidase and superoxide dismutase activity as well (Siveski-Iliskovic *et al.*, 1994; Chen *et al.*, 2007). Therefore, pretreatment of SMC might be alleviate the tilmicosin cardiotoxic effect through enhancing the antioxidant protection.

Conclusion: Tilmicosin induced myocardial damage indicated by increase of AST, LDH, CK and cTnT biomarkers as well as myocardial tissue necrosis and myocytolysis. SMC exhibited significant protective effects toward tilmicosin-induced cardiotoxicity in rats. Therefore, SMC could be a promising candidate for further field applications to ameliorate tilmicosin cardiotoxic effect.

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