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Ghrelin Alleviates Tilmicosin-Induced Myocardial Oxidative Stress in Rats

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Abstract: The aim of this study was to evaluate the possible antioxidant effect of ghrelin against tilmicosin-induced myocardial damage in rats. Forty male Sprague Dawley rats were equally divided into four groups: control (saline for 5 days), tilmicosin (single dose of 75 mg kg^{-1}, s.c.), ghrelin (10 ng/kg/day for 5 days, s.c.) and ghrelin plus tilmicosin group (pretreatment with ghrelin followed by tilmicosin treatment). The heart were excised for evaluating Malondialdehyde (MDA) content, Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) activity. The results showed that tilmicosin treatment alone significantly ($p<0.05$) elevated the levels of MDA and lowered the activities of SOD, CAT and GPx when compared with the control group. Pretreatment with ghrelin ameliorated the SOD, CAT and GPx activities and inhibited the levels of MDA production in the heart tissue compared to tilmicosin-treated rats. The results of the study provide evidence that the ghrelin pretreatment enhances the antioxidant defense against tilmicosin-induced myocardial oxidative injury in rats and exhibit cardioprotective property.

Key words: Tilmicosin, ghrelin, antioxidant enzyme, myocardial injury, Turkey

INTRODUCTION

Ghrelin, a peptide hormone originally identified as the endogenous ligand of the Growth Hormone Secretagogue Receptor (GSHR) is secreted primarily from the stomach and secondarily from the small intestine and colon (Kojima *et al*., 1999). Ghrelin is also produced in the several tissues such as kidney, pancreas, lung, eye, gonads, thyroid, placenta and heart (Kojima *et al*., 1999; Leite-Moreira and Soares, 2007). Previous reports have indicated that ghrelin has beneficial effects on gastrointestinal, reproductive, immune and coagulation systems (Kojima *et al*., 1999; Eter *et al*., 2007; Iseri *et al*., 2005; Kheradmand *et al*., 2009; Yada *et al*., 2006; Arici and Cetin, 2010). It was also reported that ghrelin have a variety of cardiovascular effects, including increased myocardial contractility, vasodilatation and protection from isoproterenol-induced myocardial injury or septic shock *in vivo* (Chang *et al*., 2001, 2004; Kawczynska-Drozdz *et al*., 2006; Nagaya *et al*., 2001; Tritos and Kokkotou, 2006). In addition, ghrelin has been reported to possess free radical scavenging and antioxidant effect (Iseri *et al*., 2005; Obay *et al*., 2008).

Tilmicosin is a macrolide antibiotic intended for use in the treatment of bacterial infections in livestock (Main *et al*., 1996) and laboratory animals such as rabbit (McKay *et al*., 1996) and rat (Modric *et al*., 1999). Although, macrolide antibiotics are considered to be one of the safest anti-infective drugs, adverse cardiovascular effects of several macrolides have been reported (Freedman *et al*., 1987; Tamargo *et al*., 1982). It has been stated that the heart is the target organ of acute tilmicosin toxicity (Jordan *et al*., 1993). Tilmicosin-induced adverse effects on the heart have been previously shown in dogs (Jordan *et al*., 1993; Main *et al*., 1996), cattle and sheep (Modric *et al*., 1998). It was shown that tilmicosin increased MDA level (Kart *et al*., 2007a, b; Yapar and Karapehlivan, 2006) and decreased superoxide dismutase and glutathione peroxidase level in the heart tissue of mice (Yazar *et al*., 2002).

On the basis of this background, the present study was designed to evaluate the putative protective effect of ghrelin pretreatment on tilmicosin-induced myocardial oxidative injury in rats.

MATERIALS AND METHODS

Animals: Forty male Sprague Dawley rats weighing 260-305 g were used in the study. They were obtained from Experimental and Clinical Research Center of Erciyes University, Kayseri, Turkey. Rats were housed in policarbon cages and were exposed to a 12 h light-dark cycle at a room temperature of 20±2°C and 50-60% relative humidity. Animals had free access to standard pellet chow and drinking water. The study was approved by the Local Ethics Committee.

Experimental design: Ghrelin (Sigma Chemical, St. Louis, MO) was dissolved in saline. The rats were randomly divided into four groups containing ten rats each. The

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first group served as the control group and was given 1 mL subcutaneous injections of normal saline solution for 5 dats. Group 2 was treated with 75 mg kg^{-1} of tilmicosin (Micotil 300; Lilly Elanco, Istanbul, Turkey) by a single subcutaneous injection (Kart *et al*., 2007b). Group 3 received daily subcutaneous injections of 10 ng kg^{-1} body weight of ghrelin (Iseri *et al*., 2005) for 5 days. Group 4 was pre-treated everyday subcutaneously with ghrelin (10 ng kg^{-1}) for 5 days before treatment with a single dose of tilmicosin (75 mg kg^{-1}, b.w. subcutaneously).

Animals were sacrificed 24 h after the last injection. Under the sodium thiopentone anaesthesia (20 mg kg^{-1}, intraperitoneally), the chest cavities of the rats were opened and heart was removed immediately and tissues rinsed with 0.9% NaCl. The tissues were homogenized and the centrifuged homogenates were stored at -25°C until they were analyzed.

Biochemical evaluation: Concentration of myocardial Malondialdehyde (MDA, an index of lipid peroxidation) was analyzed according to the method by Yoshioka *et al*. (1979). Briefly, 2.5 mL of trichloroacetic acid solution (20%) and 0.5 mL of 2-thiobarbituric acid (0.67%) solution added to 0.5 mL supernatant in centrifuge tubes were placed in a boiling water bath for 30 min. Tubes were then immediately cooled in ice-cold water and 4 mL of n-botanol added. After centrifugation at 3, 000 rpm for 10 min, absorbance intensity of the upper (n-butanol) phase was measured by a spectrophotometer (Shimadzu UV-1700, Japan) at 535 nm. Absorbance values were compared with a series of standard solutions (1, 1, 3, 3-tetramethoxypropane). Data were expressed as nmol mg^{-1} protein. Total protein content in the homogenates were determined by the method of Lowry *et al*. (1951).

Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities in the heart homogenates were spectrophotometrically determined using commercially available assay kits (Cayman, Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. All enzyme activities were expressed as U mg^{-1} protein.

Statistical analysis: Statistical analysis was carried out using the SPSS for Windows software, Version 12.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to check for normality. Groups of data were compared by ANOVA followed by Tukey's multiple comparison tests. All data are expressed as means±standard error. Values of $p<0.05$ were regarded as significant.

RESULTS

Myocardial MDA level was found to be significantly ($p<0.05$) higher in the tilmicosin-treated group as compared to the control group. Pre-treatment with ghrelin prior to the administration of tilmicosin led to a significant decrease ($p<0.05$) in MDA levels when compared to the tilmosin-treated group (Fig. 1).

Results revealed that treatment with tilmicosin alone caused a significant decrease ($p<0.05$) in the myocardial SOD, CAT and GPx activities as compared the control group. The reduction in SOD, CAT and GPx activities by tilmicosin alone was increased by pre-administration of ghrelin as compared to the tilmicosin-treated group (Fig. 1-4).

The present studies demonstrate that administration of ghrelin alone does not cause any significant alteration on myocardial MDA level and SOD, CAT and GPx activities.

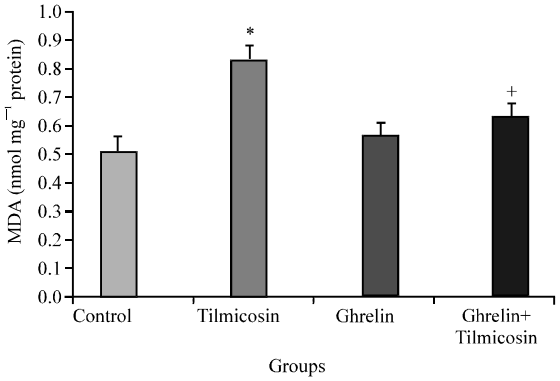

Fig. 1: The myocardial MDA levels in the control and treatment groups. *$p<0.05$ compared with the control group; $^{+}p<0.05$ compared with tilmicosin-treated group

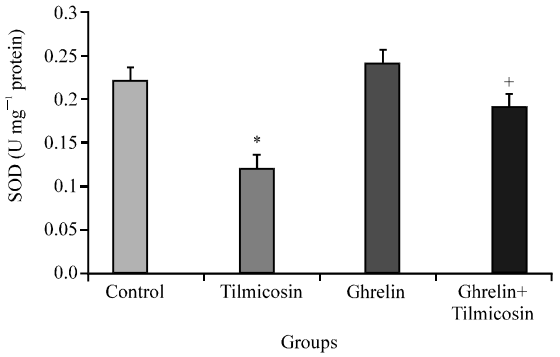

Fig. 2: The myocardial SOD activities in the control and treatment groups. *$p<0.05$ compared with the control group; $^{+}p<0.05$ compared with tilmicosin-treated group

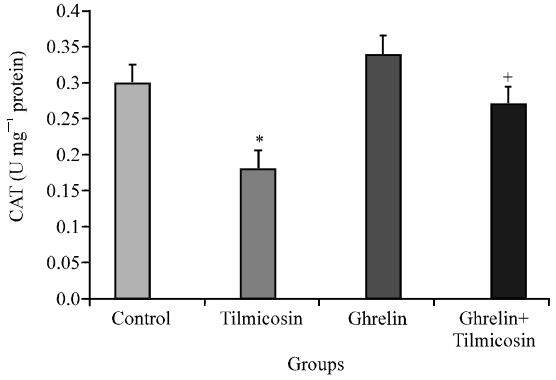

Fig. 3: The myocardial CAT activities in the control and treatment groups. *p<0.05 compared with the control group; $^{+}$p<0.05 compared with tilmicosin-treated group

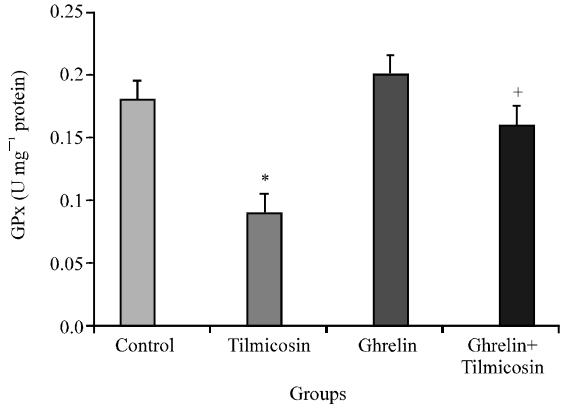

Fig. 4: The myocardial GPx activities in the control and treatment groups. *p<0.05 compared with the control group. $^{+}$p<0.05 compared with tilmicosin-treated group

DISCUSSION

Previous studies showed that tilmicosin can cause cardiotoxic effects such as negative inotropy, positive chronotropy, acute heart failure and alteration in electrocardiogram (Jordan *et al*., 1993; Main *et al*., 1996; Modric *et al*., 1998). It was also demonstrated that tilmicosin could cause oxidative stress by increasing free radical production and decreasing antioxidant enzymes in heart (Yazar *et al*., 2002).

The toxic effects of tilmicosin depend on animal species and route of administration (Jordan *et al*., 1993). Several experimental studies have reported that increased oxidative stress and depressed antioxidant status have deleterious effects on both cardiac structure and function (Kaul *et al*., 1993; Singal *et al*., 1998).

Free radicals can cause membrane and macromolecule injury, both of which lead to the damage of heart. It is well known that heart tissue is highly susceptible to oxidative stress. Evaluation of lipid peroxidation, SOD, CAT, GPx and other antioxidant enzyme activities in myocardia have been used as marker of oxidative stress or tissue injury (Doroshow *et al*., 1980; Gustafson *et al*., 1993). In the study, the development of myocardial injury induced by tilmicosin was established by a significant increase in MDA content and a significant decrease in SOD, CAT and GPx activities.

In the present study, cardiac MDA level was determined to be statistically increased in the animals treated with tilmicosin alone as compared with the control. The increase determined in the content of MDA in the heart tissue demonstrates the free radicals generated by tilmicosin lead to oxidative injury in the heart tissue. The increase in MDA level is in agreement with the findings of Kart *et al*. (2007a, b) who found that a single injection of 75 mg kg^{-1} of tilmicosin increased the level of MDA in cardiac tissue of mice. Similarly, Yapar and Karapehlivan (2006) reported that doses of 50 and 70 mg kg^{-1} of tilmicosin increased the level of MDA in cardiac tissue of mice. This study showed that ghrelin pretreatment for 5 days prior to the injection of tilmicosin significantly decreased MDA level in the heart tissue as compared to the rats treated with tilmicosin alone. Malonyldialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen derived free radicals is considered a reliable marker of myocardial cell damage (Rao *et al*., 1983). Therefore, it can be concluded that ghrelin pretreatment protects the heart tissue against lipid peroxidation by tilmicosin. Similarly, Obay *et al*. (2008) demonstrated that pretreatment of rats with different doses of ghrelin prevented pentylenetetrazole-induced elevation in lipid peroxidation. It has been recently reported that ghrelin may be antioxidant agent. For example, Zwirska-Korczala *et al*. (2007) showed that ghrelin significantly decreases the level of MDA in preadipocyte cell culture. Iseri *et al*. (2005) showed that ghrelin administration significantly decreased MDA level in the alendronate-induced gastric tissue injury in rats. Likewise, Kheradmand *et al*. (2009) reported that ghrelin administration for 10 days decreased MDA level in the rat testis. The above reports support the findings.

In the present study, tilmicosin caused oxidative stress in animals as evidenced by the decrease in myocardial SOD, CAT and GPx activities in rat's heart. The findings are similar to the data reported by Yazar *et al*. (2002) who showed that tilmicosin treatment decreased cardiac SOD and Gpx activities. Also, Yapar and Karapehlivan (2006) and Kart *et al*. (2007b)

observed that tilmicosin decreased glutathione level in cardiac tissue of mice. In this study, ghrelin pretreatment caused a significant increase in SOD, CAT and GPx activities compared to the rats treated with tilmicosin alone. These results show that ghrelin has significant cardioprotective effect and maintains myocardial tissue integrity. This protective effect of ghrelin may be due to its free radicals scavenging capability.

This observation has been supported by the findings of Zwirska-Korczala *et al.* (2007) who demonstrated that ghrelin significantly increased SOD, CAT and GPx activities in preadipocyte cell culture. Similarly, Obay *et al.* (2008) showed that ghrelin pretreatment prevented reduction of antioxidant enzyme activities against pentylenetetrazole-induced oxidative stress in the erythrocytes, brain and liver of rats. In a previous study, it was reported that GPx activity in rat testis was increased and SOD and CAT activities were unchanged by administration of ghrelin at dose of 1 nmol kg^{-1} for 10 days and suggested that higher dose of ghrelin is needed to induce greater activity of SOD. The mechanism of action behind the ghrelin-induced beneficial effect is not exactly understood. Evans *et al.* (2000) showed that Growth Hormone (GH) replacement therapy significantly improved oxidative stress in patients with growth hormone deficiency. Ghrelin strongly and dose dependently stimulates GH release (Takaya *et al.*, 2000). The antioxidant effect of ghrelin against tilmicosin induced oxidative stress may be depended on an increase in GH release.

CONCLUSION

In this study, the findings show that tilmicosin might cause myocardial oxidative stress in rats but ghrelin pretreatment protects rats' hearts against tilmicosin induced oxidative injury. It is suggested that ghrelin treatment may contribute to developing novel strategies in the prevention of the cardiotoxic effects of macrolide antibiotics.

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