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RESEARCH ARTICLE

Protection against tacrolimus-induced cardiotoxicity in rats by olmesartan and aliskiren

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Abstract

Context: Tacrolimus (TAC), a calcineurin inhibitor, is commonly used as an immunosuppressive agent in organ transplantation, but its clinical use may be limited due to cardiotoxicity. Olmesartan (OLM; angiotensin receptor blocker) and aliskiren (ALK; renin inhibitor) may attenuate cardiotoxicity induced by TAC by inhibition of renin–angiotensin aldosterone system.
Objective: The aim of this study was to evaluate the effect of OLM and ALK on TAC-induced cardiotoxicity.
Materials and methods: Male Wistar albino rats weighing 200–250 g (10–12 weeks old) were used in this study. Animals were divided into four groups. Group 1 received normal saline, group 2 received TAC (2 mg/kg, intraperitoneally for 14 d), group 3 received OLM (2 mg/kg, p.o. for 28 d) + TAC and group 4 received ALK (50 mg/kg, p.o. for 28 d) + TAC. TAC-induced cardiotoxicity was assessed biochemically and histopathologically.
Results: Treatment with OLM or ALK decreased the TAC-induced changes in biochemical markers of cardiotoxicity such as serum aspartate transaminase, creatine kinase and lactate dehydrogenase. OLM or ALK also attenuated the effects of TAC on oxidant–antioxidant parameters such as malondialdehyde, reduced glutathione and catalase. Histopathological and ultrastructural studies showed that OLM or ALK also attenuated TAC-induced cardiotoxicity.
Discussion and conclusion: These results suggest that OLM as well as ALK has protective effects against TAC-induced cardiotoxicity; implying that angiotensin receptor blocker or renin inhibitor, respectively, may counteract cardiotoxicity associated with immunosuppressant use.

Keywords

Angiotensin receptor blocker, aliskiren, cardiotoxicity, olmesartan, renin angiotensin aldosterone system, renin inhibitor, tacrolimus

History

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Introduction

Tacrolimus (TAC), a potent calcineurin inhibitor, is a commonly used immunosuppressive agent in organ transplantation (Ciancio et al., 2004; Naesens et al., 2009). Although cardiotoxicity is less common with TAC, it has been reported in earlier studies with serious consequences (Jarzembowski et al., 2005; Pappas et al., 2000; Turska-Kmiec et al., 2007). For example, arrhythmia and cardiomyopathy have been reported with TAC treatment (Coley et al., 2001; Cox et al., 1997; Hodak et al., 1998; Kobashigawa et al., 2006; Sawabe et al., 1997). Various mechanisms have been proposed to explain the TAC-induced cardiotoxicity, which include arteritis of cardiac arteries, hypertension, renal vasoconstriction and decreased nitric oxide production (Atkison et al., 1995; Jeong et al., 1998; Khanna et al., 2002; Roberts et al., 2002). However, the true underlying mechanism of TAC-related cardiotoxicity remains unclear.

In cardiomyocytes, angiotensin II (AngII) triggers cellular signaling by binding to its receptors: AngII type I receptor (AT1R) and AngII type II receptor. The physiological effect of AngII may be mainly through its binding to AT1R. This is supported by findings which show that AT1R transgenic mice demonstrate no obvious changes in hearts size (Paradis et al., 2000; Sugino et al., 2001). AngII control electrolyte balance and blood pressure by stimulating the heart to release aldosterone (Sachse & Wolf, 2007). AngII is also involved in the development of cardiac hypertrophy, endothelial dysfunction and even organ damage. Inhibition of renin–angiotensin aldosterone system (RAAS) may be an effective way to inhibit the progression of cardiovascular disorders (Flather et al., 2000; Ruggenenti et al., 1999; Turnbull & Blood Pressure Lowering Treatment Trialists, 2003). It has been shown earlier that direct renin inhibition with aliskiren

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(ALK) prevents RAAS activation, leading to mitigation in the development of cardiomyopathy (Rashikh et al., 2011).

ROS contribute to all aspects of cellular function including gene expression, proliferation, migration and cell death (Ushio-Fukai & Alexander, 2004). In the vasculature, there is accumulating evidence that implicates ROS has a role in the development of hypertension, endothelial dysfunction, and hypertrophy (Cai et al., 2003; Landmesser et al., 2003). Previous literature suggests that TAC produces ROS *via* NADPH oxidase activation and causes disturbance in anti-oxidant defense, which may be responsible for cardiotoxicity (Brandes & Kreuzer, 2005). Therefore, TAC-induced cardiotoxicity could be through oxidative stress where RAAS may play an important role.

Olmesartan (OLM) is AngII receptor (type A1) antagonist where as ALK is a renin inhibitor and selectively inhibits renin without affecting other systems (Wood et al., 2003). Since activation of RAAS leads to ROS generation, it is reasonable to propose that cardioprotective effects of OLM or ALK may be mediated through inhibition of oxidative stress. Based on these evidences, our study investigated the effect of OLM and ALK on TAC-induced cardiotoxicity in rats using biochemical markers of oxidative stress, cardiac function and histopathological measures of cellular damage.

Materials and methods

Animals

In this study, male Wistar albino rats weighing 200–250 g (10–12 weeks old) were used. The animals were obtained from Experimental Animal Care Center, College of Pharmacy at King Saud University. They were housed under ideal laboratory conditions (12-h light/darkness cycle, 45–55% relative humidity and temperature 23–25°C), maintained on standard pellet diet and water *ad libitum* throughout the experimental period. All experiments were carried out according to the guidelines of the animal care and use committee at King Saud University.

Drugs and chemicals

TAC purchased from Sigma Aldrich (Saint Louis, MO), OLM from Ranbaxy Research Laboratory (Gurgaon, India) and ALK from Novartis Ltd. (Hyderabad, India) were used in the study. Biochemical parameters were done using kits (Dimension®, Siemens, Malvern, PA). All the other chemicals used were of analytical grade.

Experimental protocol

Rats were randomly divided into four groups: Group 1, control group, received normal saline for 28 d. Group 2, toxic group, received TAC [2 mg/kg, intraperitoneally (i.p.)] for 14 d (Mitamura et al., 1994). Group 3, treatment group, received TAC (2 mg/kg, i.p.) for 14 d with the same schedule as group-2, and also OLM [2 mg/kg, dissolved in distilled water administered *per os* (p.o.)] for 28 d; and group 4, treatment group, received TAC (2 mg/kg, i.p.) for 14 d with the same schedule as group-2, and also ALK (50 mg/kg, dissolved in distilled water administered p.o.) for 28 d (Rashikh et al., 2011).

TAC administration (in groups 2 and 3) started on day 15 and was continued till the end of the study. All the rats were sacrificed at the end of the study by decapitation under ether anesthesia, as per the protocol. Blood samples were collected followed by serum separation at 3000 *g* for 10 min. Samples were then kept at −20 °C until analysis of cardiac function parameters. The rat's heart were isolated, washed in ice-cold physiological saline and used for assessment of oxidative stress, histopathology and ultrastructural changes.

Biochemical estimation

Biochemical estimations were done by autoanalyzer (Dimension® RXL MAX™, Siemens).

Determination of lipid peroxides, measured as malondialdehyde

Level of malondialdehyde (MDA), a product of membrane lipids peroxidation, was estimated in cardiac tissue by the method of Ohkawa et al. (1979), using the standard calibration curve prepared with tetraethoxy propane. MDA was expressed as nmoles of MDA per milligram of protein. Protein was estimated by the method of Lowry et al. (1951).

Determination of reduced glutathione

Glutathione (GSH) content was estimated in cardiac tissue by the method of Sedlak & Lindsay (1968). The absorbance of reaction mixture was read within 5 min of addition of dithiobis-2-nitrobenzoic acid at 412 nm using UV-spectrophotometer, against a reagent blank.

Determination of catalase

Cardiac catalase (CAT) activity was estimated in post mitochondrial supernatant (PMS) using the method of Clairborne (1985). The reaction mixture consisted of 1.95 ml of phosphate buffer (0.1 M, pH 7.4), 1.0 ml of hydrogen peroxide (0.019 M) and 0.05 ml of PMS in a final volume of 3 ml. Changes in absorbance were recorded at 240 nm every minute for 5 min. The enzyme activity was calculated as nmoles of H_2O_2 consumed/min/mg protein.

Histopathological studies

Heart were harvested from the rats and fixed in 10% buffer formosaline. Paraffin sections of thickness 3–4 μm were prepared and stained with hematoxylin and eosin for histopathological examination under light microscopy (Iqbal et al., 2008).

Ultrastructural studies

Immediately after removal of heart from the dissected rats, tissues were sliced into small size (1 mm^3) and fixed in 3% buffered glutaraldehyde. Tissue specimens were then post fixed in 1% osmium tetroxide (OsO_4) for 90 min. Dehydration of the fixed tissue was performed using ascending grades of ethanol followed by transfer of tissue to epoxy resin via propylene oxide. After impregnation with the pure resin (SPI Resin), tissue specimens were embedded in the same resin mixture. Ultra-thin sections of silver shades (60–70 nm) were

cut using an ultra-microtome (Leica, UCT, Tokyo, Japan) with a diamond knife; sections were then placed on copper grids and stained with uranyl acetate (20 min) and lead citrate (5 min). Stained sections were observed under transmission electron microscopy (JEOL JEM-1011, Tokyo, Japan) operating at 80 kV (Reynolds, 1963; Singal et al., 1985; Tong et al., 1991).

Statistical analysis

All results are expressed as mean ± SEM. Comparisons among different groups were analyzed by analysis of variance, followed by Tukey–Kramer multiple comparisons test to identify significance among groups. Values were considered statistically significant when $p < 0.05$. Statistical analysis was carried out using GraphPad Prism 3.0 (La Jolla, CA).

Results

Effects of ALK and OLM on TAC-induced changes on parameters of cardiac function in serum

In this study, administration of TAC for two weeks treatment resulted in cardiac damage to rats as evidenced by a significant ($p < 0.05$) increase in serum aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) as compared to control group. Treatment with ALK or OLM significantly ($p < 0.05$) reversed TAC-induced increase in AST, LDH and CK levels (Table 1). A significant ($p < 0.05$) decrease in serum high density lipoprotein (HDL), cholesterol and triglycerides (TG) was seen in toxic group, which was reversed by treatment with OLM or ALK (Table 2).

Table 1. Effects of ALK or OLM on TAC-induced changes in parameters of cardiac function in serum.

	AST (U/L)	CK (U/L)	LDH (U/L)
Control	49.00 ± 8.61	249.78 ± 4.62	195.67 ± 14.59
TAC	128.64 ± 10.25[a]	755.37 ± 31.42[a]	709.08 ± 11.25[a]
TAC + OLM	67.82 ± 7.83[b]	349.53 ± 14.40[b]	354.33 ± 42.11[b]
TAC + ALK	54.62 ± 5.04[b]	294.63 ± 20.33[b]	303.77 ± 44.35[b]

AST, aspartate transaminase; CK, creatine kinase; LDH, lactate dehydrogenase; U/L, unit per liter; TAC, tacrolimus; OLM, olmesartan; ALK, aliskiren; and SEM, standard error of mean. The data are expressed as mean ± SEM ($n = 6$). ANOVA followed by Tukey–Kramer multiple comparison test.
[a]$p < 0.05$ versus control; [b]$p < 0.05$ versus tacrolimus.

Table 2. Effects of ALK or OLM on TAC-induced changes in serum lipid profile.

	LDL (mmol/L)	HDL (mmol/L)	CHOL (mmol/L)	TG (mmol/L)
Control	0.91 ± 0.04	1.12 ± 0.08	2.33 ± 0.08	0.50 ± 0.10
TAC	1.42 ± 0.19[a]	0.98 ± 0.04	3.26 ± 0.16[a]	2.86 ± 0.17[a]
TAC + OLM	0.29 ± 0.04[b]	1.08 ± 0.12[b]	2.10 ± 0.10[b]	1.58 ± 0.08[b]
TAC + ALK	0.62 ± 0.17[b]	1.17 ± 0.11[b]	2.40 ± 0.18[b]	1.33 ± 0.06[b]

LDL, low density lipoprotein; HDL, high density lipoprotein; CHOL, cholesterol; TG, triglycerides; TAC, tacrolimus; OLM, olmesartan; ALK, aliskiren; and SEM, standard error of mean. The data are expressed as mean ± SEM ($n = 6$). ANOVA followed by Tukey–Kramer multiple comparison test.
[a]$p < 0.05$ versus control; [b]$p < 0.05$ versus tacrolimus.

Effects of ALK and OLM on TAC-induced changes on parameters of oxidative stress in heart

The results are summarized in Figures 1–3. Administration of TAC for two weeks resulted in a significant ($p < 0.05$) increase in heart MDA level compared to the control group. Treatment with ALK or OLM showed a significant ($p < 0.05$) reversal in TAC-induced increase in cardiac MDA levels (Figure 1). Consequently, significant ($p < 0.05$) decrease in cardiac GSH level was found in TAC-treated rats as compared to control group, which was reversed by ALK or OLM treatment (Figure 1). Treatment with ALK and OLM also showed a significant ($p < 0.05$) reversal in TAC-induced increase in CAT activity (Figure 2).

Effects of ALK and OLM on TAC-induced histopathological changes in heart

Normal morphological structures of cardiac tissue were observed in the control group (Figure 3a). However, administration of TAC for two weeks showed myocardial degeneration and broken myocardial fibers with cytoplasmic vacuoles. Clusters of hypochromatic cells with pyknotic nuclei and inflammatory cells infiltration were the most

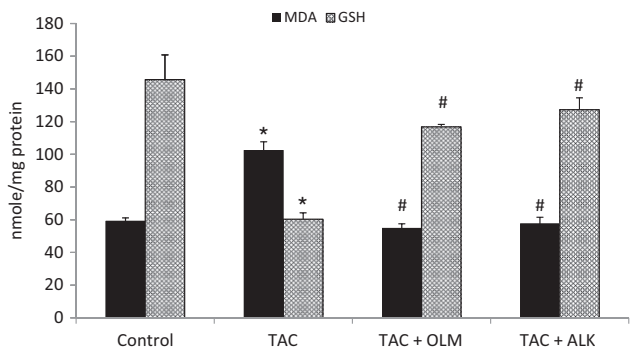

Figure 1. Effect of OLM or ALK on TAC-induced changes in cardiac lipid peroxidation and GSH levels of different experimental groups. The data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$, versus control group; #$p < 0.05$, versus toxic group. ANOVA followed by Tukey–Kramer multiple comparison tests.

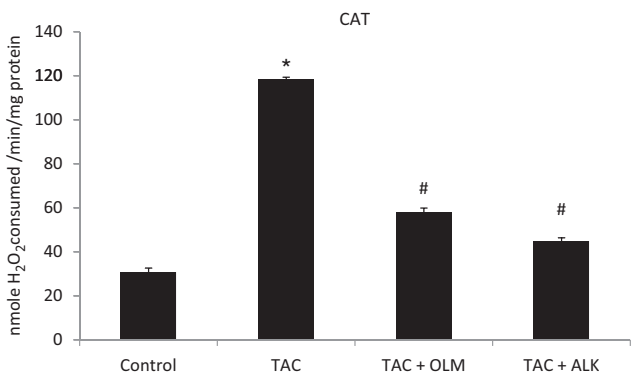

Figure 2. Effect of OLM or ALK on TAC-induced changes in cardiac catalase activity of different experimental groups. The data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$, versus control group; #$p < 0.05$, versus toxic group. ANOVA followed by Tukey–Kramer multiple comparison tests.

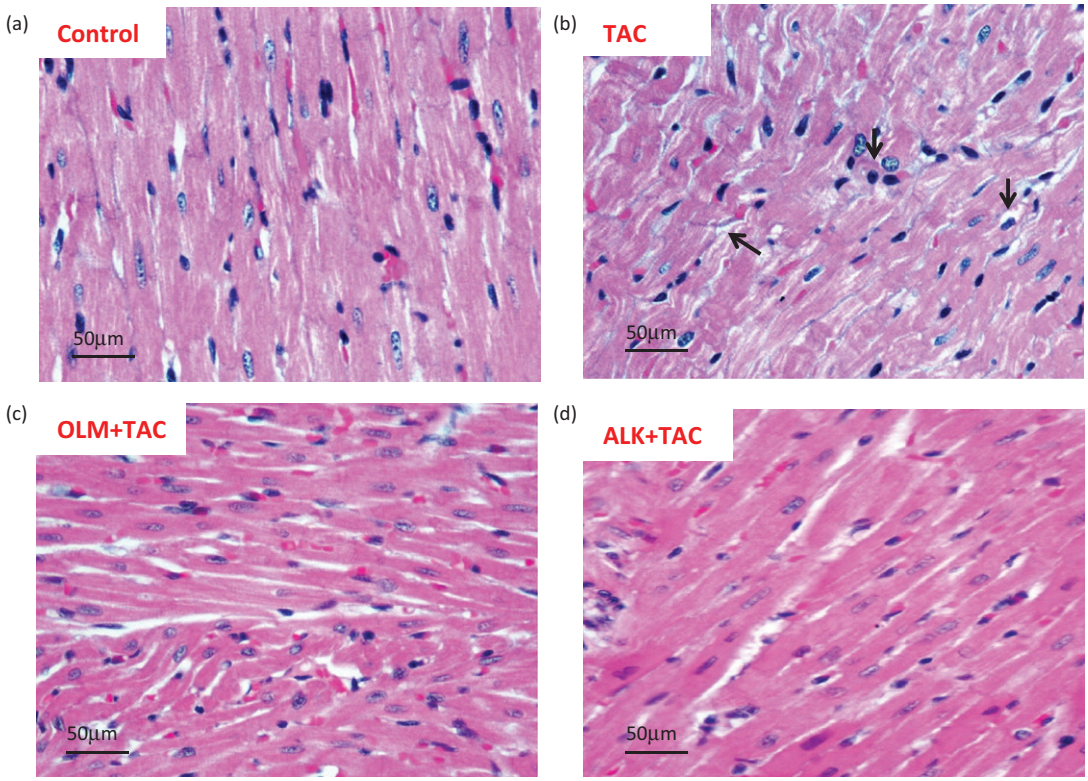

Figure 3. Effect of OLM or ALK on TAC-induced changes in cardiac histopathology of different experimental groups. (a) Control; (b) TAC; (c) OLM + TAC and (d) ALK + TAC ($n = 6$ per group; magnification = 20×).

significant change all over the heart (Figure 3b). Treatment with OLM or ALK reversed TAC-induced cardiac damage as evidenced by normalization of shape and size of cardiac muscle fibers (Figure 3c and d).

Effects of ALK and OLM on TAC-induced ultrastructural changes in heart

Ultrastructural changes in cellular structures were further visualized by TEM studies. At ultra-structural level, normal structures of heart were observed in control group (Figure 4a). By contrast, the ultrastructural changes in TAC-treated hearts showed cellular damage, i.e. calcification, extensive fibrotic tissue proliferation throughout cardiac tissue, disruption in branching structure with loss of striations, mitochondrial swelling, presence of inflammatory cells and multi vesicle bodies with scarring of myofibrils and cell debris (Figure 4b). However, OLM or ALK treatment demonstrated a massive and sustained regenerative cell proliferation, seen as normalization of myofibrils striation, increase in mitochondria and number of myofibrils (Figure 4c and d).

Discussion

Our study shows for the first time that TAC-associated cardiotoxicity is reversed by treatment with ALK or OLM as evidenced by improvement of cardiac function parameters, oxidative stress, histopathological and ultrastructural changes. Protective effects of angiotensin-converting enzyme inhibitor, angiotensin receptor blocker and renin inhibitor against doxorubicin-induced cardiotoxicity have been reported in several previous studies (Ibrahim et al., 2009; Iqbal et al., 2008; Rashikh et al., 2011; Yagmurca et al., 2003). One study has also reported the effects of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker on TAC-induced cardiotoxicity, but its focus was only on histological and immunohistochemical findings (Agirbasli et al., 2007).

Serum AST, LDH and CK enzyme activities are important parameters for assessment of cardiotoxicity (Al-Shabanah et al., 1998; el-Missiry et al., 2001). In this study, serum AST, LDH and CK enzyme activities were significantly increased in TAC-treated rats compared to control group. However, treatment with ALK or OLM caused a significant reversal in serum AST, LDH and CK enzyme activities caused by TAC. These results are in agreement with earlier reports (Iqbal et al., 2008; Rashikh et al., 2011; Yagmurca et al., 2003). There was a dysregulation of serum lipid profile in TAC group, i.e. increase in LDL, TC and TG and decrease in HDL. Treatment with ALK or OLM also caused a significant improvement in serum lipid profile. Therefore, these data suggest that ALK or OLM improves cardiac function by counteracting TAC-induced cardiotoxicity.

We measured lipid peroxidation in cardiac tissue as a measure of oxidative stress. In our study, we observed a significant increase in the concentration of MDA level in cardiac tissue after TAC administration, which was reversed by OLM or ALK treatment. The elevated level of MDA may be due to enhanced production of ROS (superoxide radicals, hydrogen peroxide and hydroxyl radicals). An earlier study has shown increased ROS generation after TAC administration in the cardiac tissue (Ibrahim et al., 2009). Zhou et al. (2004) reported that TAC leading to cell death *via* enhanced production of ROS. Treatment with OLM or ALK

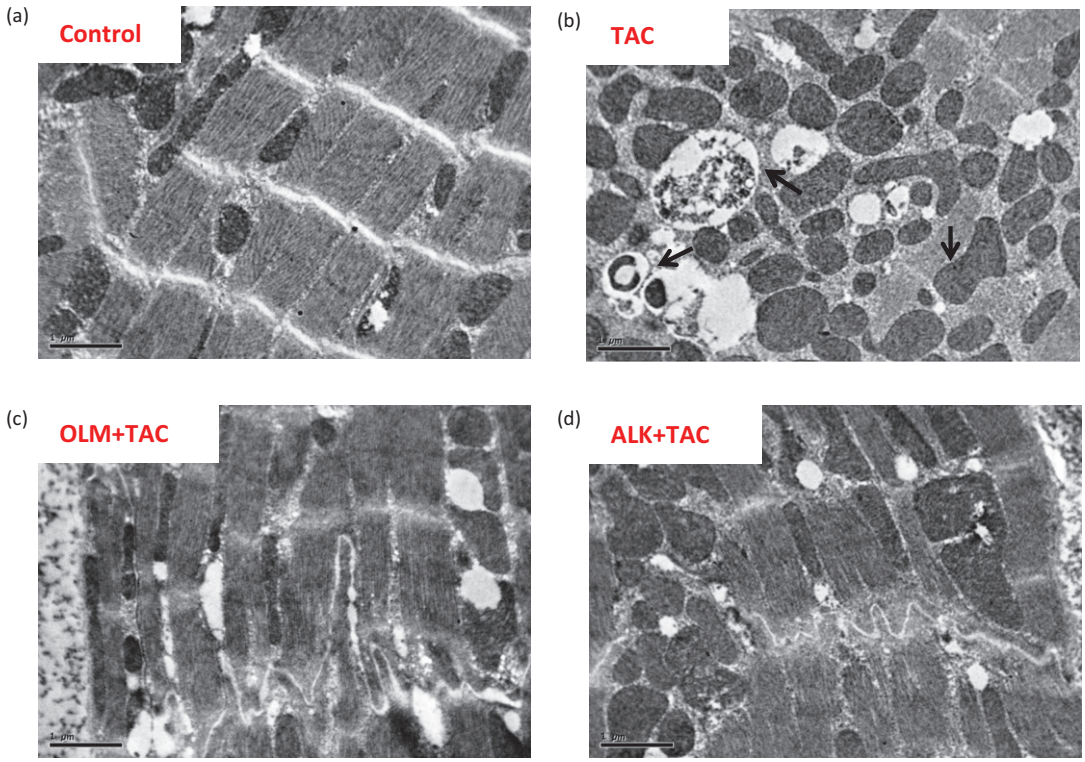

Figure 4. Effect of OLM or ALK on TAC-induced ultrastructural changes in heart of different experimental groups. (a) Control; (b) TAC; (c) OLM + TAC and (d) ALK + TAC ($n = 6$ per group; magnification = 10 000×).

significantly decreased MDA level, which suggests its role in combating oxidative injury through inhibition of RAAS system.

Administration of TAC led to a decrease in GSH level and an increase in CAT activity. The increased CAT activity in cardiac tissue may be due to excess production of hydrogen peroxide, which has potential to promote lipid peroxides formation. Decreased GSH content may be due to scavenging of ROS as GSH in a major intracellular redox buffer and has ability to detoxify various ROS through direct interaction. Treatment with OLM or ALK normalized GSH levels and CAT activity, which could be a direct consequence of ROS inhibition through RAAS pathway inhibition. RAAS activation been reported to lead to ROS generation in earlier studies (Agirbasli et al., 2007; Landmesser et al., 2003; Ruggenenti et al., 1999; Sawabe et al., 1997). OLM or ALK have also been shown to decrease oxidative stress in earlier studies (Lee et al., 2013; Whaley-Connell et al., 2010).

Histopathological and ultrastructural examination of cardiac tissue showed myocardial degeneration, broken myocardial fibers with cytoplasmic vacuoles in toxic group. Clusters of hypochromatic cells with pyknotic nuclei and inflammatory cell infiltrate were the most significant change all over the heart. Similar histopathological and ultrastructural changes have been reported earlier in doxorubicin related cardiotoxicity (Ibrahim et al., 2009; Iqbal et al., 2008; Rashikh et al., 2011). TAC-induced histopathological and ultrastructural changes were reversed by treatment with OLM or ALK. There was restoration of myofibril architecture after treatment with OLM or ALK. Inflammatory cell infiltration induced by TAC was also inhibited by treatment with OLM or ALK. Earlier studies have also shown that RAAS blockade leads to preservation of myocardial integrity (Ibrahim et al., 2009; Iqbal et al., 2008; Rashikh et al., 2011). Our study confirms earlier reports and further shows that biochemical and histopathological parameters parallel each other.

Our study showed no significant difference in the protection by ALK over OLM or vice versa against TAC-induced cardiotoxicity. This was supported by data that showed similar degree of inhibition/attenuation in most of the biochemical/histological parameters. These observations suggest that both pathways probably converge on ROS inhibition for manifestation of cardioprotection *via* RAAS blockade.

Treatment with OLM or ALK ameliorated TAC-induced cardiotoxicity implying that angiotensin receptor blockade or renin inhibition, respectively, leads to improvement in cardiac function. The protective effect of OLM or ALK were evidenced by a significant attenuation of oxidative stress parameters in heart (MDA, GSH and CAT), improvement in cardiac function biochemistry in serum as well as restoration of cardiac structures as assessed by histopathological and ultrastructural findings. In conclusion, this study suggests that both OLM and ALK treatment may be equally beneficial against cardiomyopathy associated with immunosuppressant use.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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