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Betulinic Acid Protects against Ischemia/Reperfusion-induced Renal Damage and Inhibits Leukocyte Apoptosis

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The possible protective effect of betulinic acid on renal ischemia/reperfusion (I/R) injury was studied. Wistar Albino rats were unilaterally nephrectomized and subjected to 45 min of renal pedicle occlusion followed by 6 h of reperfusion. Betulinic acid (250 mg/kg, i.p.) or saline was administered at 30 min prior to ischemia and immediately before the reperfusion. Creatinine, blood urea nitrogen (BUN), lactate dehydrogenase (LDH) and TNF-α as well as the oxidative burst of neutrophil and leukocyte apoptosis were assayed in blood samples. Malondialdehyde (MDA), glutathione (GSH) levels, Na^+, K^+-ATPase and myeloperoxidase (MPO) activities were determined in kidney tissue which was also analysed microscopically. I/R caused significant increases in blood creatinine, BUN, LDH and TNF-α. In the kidney samples of the I/R group, MDA levels and MPO activity were increased significantly, however, GSH levels and Na^+, K^+-ATPase activity were decreased. Betulinic acid ameliorated the oxidative burst response to both formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 13-acetate (PMA) stimuli, normalized the apoptotic response and most of the biochemical indices as well as histopathological alterations induced by I/R. In conclusion, these data suggest that betulinic acid attenuates I/R-induced oxidant responses, improved microscopic damage and renal function by regulating the apoptotic function of leukocytes and inhibiting neutrophil infiltration. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: betulinic acid; ischemia/reperfusion; kidney; apoptosis; lipid peroxidation.

INTRODUCTION

Ischemia reperfusion (I/R) injury is a crucial element in the pathogenesis of acute renal failure (ARF) and influences the early functional recovery and even long term survival of a transplanted kidney (Lieberthal and Nigam, 2000; Abernethy and Lieberthal, 2002). Despite advances in medical care, ARF continues to be associated with significant morbidity and mortality (Singri *et al.*, 2003). Ischemia leads to hypoxia, and if it is severe enough, the rate of metabolism is diminished and the generation of high energy compounds subsequently declines (e.g. ATP). Although the return of blood flow to ischemic tissue can result in a recovery of normal functions, the tissue may also be injured paradoxically during the process of reperfusion (Parks and Granger, 1988). Reperfusion leads to the formation and activation of a variety of humoral mediators of injury and inflammation, including oxygen derived free radicals (e.g. superoxide radicals, hydroxyl radicals, hydrogen peroxide) and lipid mediators (e.g. platelet activating factor and leukotriene B4) (Weight *et al.*, 1996; Werns and Lucchesi, 1990). Thus the mechanisms underlying I/R damage to the kidneys are most likely multifactorial, and interdependent involving hypoxia, inflammatory responses and free radical damage.

A large number of synthetic and natural compounds have been demonstrated to induce beneficial effects on human health and disease prevention. Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against the deleterious effects of reactive oxygen species (ROS) and other free radicals induced by I/R (Granger and Korthuis, 1995; Chatterjee, 2007). However, the structure–activity relationship, bioavailability and therapeutic efficacy differ extensively. Betulinic acid is a naturally occurring pentacyclic triterpene. It has several botanical sources, but can also be derived chemically from betulin, a substance found in abundance in the outer bark of white birch trees (*Betula alba*) (Alakurtti *et al.*, 2006). Betulinic acid has been found selectively to kill human melanoma cells while leaving healthy cells alive and also several betulinic acid derivatives are potent and highly selective inhibitors of HIV-1 (Aiken and Chen, 2005). Since triterpenoids have a similarity to steroidal compounds, their effects have often been attributed to a mechanism related to antiinflammatory action.

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In the view of the above findings, this study was designed to determine the possible protective effect of betulinic acid against I/R-induced oxidative kidney injury, using biochemical and histological parameters.

MATERIALS AND METHODS

Animals. Male Wistar Albino rats (200–250 g) were housed in an air-conditioned room with 12 h light and dark cycles, where the temperature (22 ± 2 ºC) and relative humidity (65–70%) were kept constant. All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee. Rats were anesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine, intraperitoneally) during all surgical procedures.

Surgery and experimental protocol. Under anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine, intraperitoneally), right nephrectomy was performed and the left renal pedicle was occluded for 45 min to induce ischemia and then subjected to reperfusion for 6 h (I/R groups). None of the animals died during the I/R period. Another group of rats underwent only laparotomy, where the kidneys were manipulated without nephrectomy or occlusion (sham-operated control group). The rats in the I/R groups were treated with either betulinic acid [(3beta)-3-hydroxylup-20(29)-en-28-oic acid; Sigma-Aldrich, St Louis, MO, USA, 250 mg/kg, i.p.; I/R + BA group) or saline (I/R group) on two occasions, at 30 min prior to ischemia and immediately before the reperfusion period. Each group consisted of eight rats.

Biochemical analysis. The animals were decapitated at the end of the reperfusion period (6 h) and trunk blood samples were collected. Serum creatinine (Slot, 1965), urea (Talke and Schubert, 1965) and lactate dehydrogenase (LDH) activity (Martinek, 1972) were determined spectrophotometrically using an automated analyser. Plasma levels of tumor necrosis factor alpha (TNF-α) were quantified according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines (Biosource International, Nivelles, Belgium).

Furthermore in heparinized blood samples the oxidative burst of neutrophil and leukocyte apoptosis were assayed.

In the renal tissue samples stored at –80 ºC malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) and Na^+-K^+-ATPase activities were determined. Additional tissue samples were placed in 10% formaldehyde for histological evaluation.

Evaluation of neutrophil oxidative burst. Heparinized blood samples from the groups mentioned above were processed to determine oxidative burst of neutrophils. Oxidative burst was evaluated using a standard method defined previously (Robinson, 1993). Briefly, white blood cells were isolated by lysing the erythrocytes with a lysate containing 0.1 mmol/L EDTA, 155 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$ followed by washing the cells with phosphate buffered saline (PBS), then a standardized kit (Phagoburst from ORPEGEN Pharma, Heidelberg-Germany) was used according to the manufacturer's instruction. Oxidative burst was measured by flow cytometry (FACS CANTO, Becton Dickinson, Mountain View, CA, USA). Burst responses followed by stimulation of cells with *E. coli,* formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 13-acetate (PMA) were evaluated by using DIVA software.

Evaluation of apoptosis of neutrophils and lymphocytes. White blood cells obtained with the above mentioned method were also used for evaluation of apoptosis in neutrophils and lymphocytes. For this purpose, an annexin V apoptosis assay kit (Biovision, Mountain View, CA) was used according to the manufacturer's instructions. Briefly 1 μL of annexin V was added to the tubes and cells were incubated in the dark for 15 min. Once propidium iodide was added to label late apoptosis, the cells were immediately acquired by flow cytometry. For analysis, lymphocytes and neutrophils were gated separately according to their granularity and size on forward scatter (FSC) versus Side Scatter (SSC) plot. Early apoptosis and late apoptosis were evaluated on fluorescence 1 (FL1 for annexin V) versus fluorescence 3 (FL3 for propidium iodide) plots. The percentage of cells stained with annexin V only was evaluated as early apoptosis, the percentage of cells stained with both annexin V and propidium iodide was evaluated as late apoptosis.

Tissue malondialdehyde and glutathione assays. Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. The MDA levels were assayed for the products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously (Beuge and Aust, 1978). Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and the results were expressed as nmol MDA/g tissue. GSH measurements were performed using a modification of the Ellman procedure (Beutler *et al.*, 1963). Briefly, after centrifugation at 2000 × **g** for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/L $Na_2HPO_4.2H_2O$ solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}. The results were expressed in μmol GSH/g tissue.

Tissue myeloperoxidase activity. Myeloperoxidase is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMN). Tissue MPO activity correlates significantly with the number of PMN determined histochemically in inflamed tissues, and therefore, it is frequently utilized to estimate tissue PMN accumulation. MPO activity was measured in tissues in a procedure similar to that documented by Hillegass *et al.* (1990). Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0), and centrifuged at 41400 × **g** (10 min); the pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles,

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the samples were centrifuged at 41400 × **g** for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 mM PB, o-dianisidine and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Na^+,K^+-ATPase activity. Since the activity of Na^+,K^+-ATPase, a membrane-bound enzyme required for cellular transport, is very sensitive to free radical reactions and lipid peroxidation, reductions in this activity can indicate membrane damage indirectly. Measurement of Na^+,K^+-ATPase activity is based on the measurement of inorganic phosphate released by ATP hydrolysis during incubation of homogenates with an appropriate medium containing 3 mM ATP as a substrate. The total ATPase activity was determined in the presence of 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 30 mM Tris HCl (pH 7.4), while the Mg^{2+}-ATPase activity was determined in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+,K^+-ATPase activity (Reading and Isbir, 1980; Kim *et al.*, 1986). The reaction was initiated with the addition of the homogenate (0.1 mL) and a 5 min preincubation period at 37 ºC was allowed. Following the addition of 3 mM Na_2ATP and a 10 min re-incubation period, the reaction was terminated by the addition of ice-cold 6% perchloric acid. The mixture was then centrifuged at 3500 × **g**, and Pi in the supernatant fraction was determined by the method of Fiske and Subbarow (Fiske and Subbarow, 1925). The specific activity of the enzyme was expressed as nmol Pi/mg protein/h. The protein concentration of the supernatant was measured by the Lowry method (1951).

Histological analysis. For the light microscopic investigations, kidney samples were fixed with 10% formaldehyde, dehydrated in graded alcohol series, cleared in toluene and embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined under an Olympus BX51 photomicroscope (Tokyo, Japan). All tissue sections were examined microscopically for the characterization of histopathological changes by an experienced histologist who was unaware of the treatment conditions.

Statistics. Statistical analysis was carried out using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). Each group consisted of eight animals. All data were expressed as mean ± SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were regarded as significant.

RESULTS

As shown in Table 1, the BUN and creatinine levels in the I/R group were found to be significantly higher than those in the control rats ($p < 0.001$). When betulinic acid was administered before ischemia and the subsequent reperfusion period, these elevations in BUN and serum creatinine levels were depressed significantly ($p < 0.05$–0.001). Similarly, lactate dehydrogenase activity, as a marker of generalized tissue damage, showed a significant increase in the I/R animals ($p < 0.001$), while betulinic acid administration reversed this effect ($p < 0.001$). When compared with the sham-operated control group, in the I/R group, the proinflammatory cytokine, TNF-α, was increased significantly ($p < 0.001$). On the other hand this I/R-induced increase was also attenuated ($p < 0.05$) with betulinic acid treatment (Table 1).

In the evaluation of neutrophil oxidative burst, betulinic acid increased and normalized burst function following stimulations by both fMLP and PMA in comparison with the I/R group, whereas no difference was found following *E. coli* stimulation (Fig. 1).

Early apoptotic response in neutrophils measured with annexin V staining was increased in both I/R and I/R + BA groups in comparison with controls has been found ($p < 0.05$ and $p < 0.001$ respectively) (Fig. 2a). The late apoptotic response in neutrophils was significantly increased in the I/R group, whereas no differ-

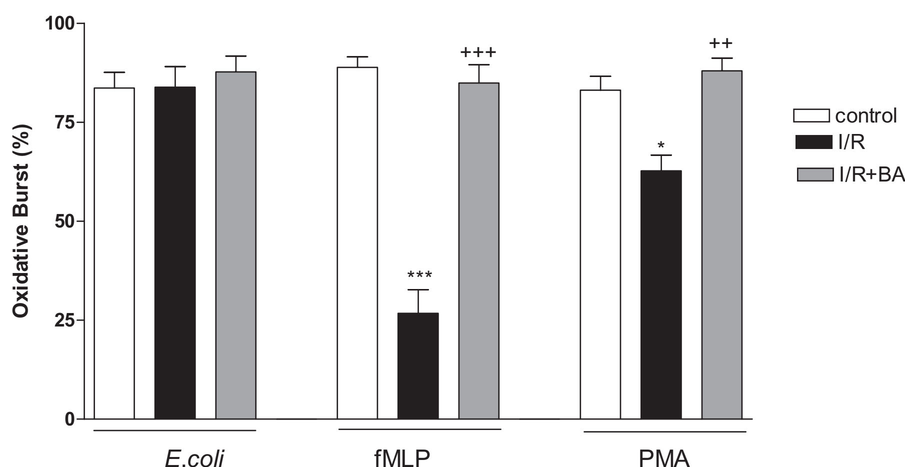

Figure 1. Oxidative burst in neutrophils in control, saline-treated I/R (I/R) and betulinic acid-treated I/R (I/R + BA) groups. * $p < 0.05$, *** $p < 0.001$ compared with the control group; $^{++}p < 0.01$, $^{+++}p < 0.001$ compared with the I/R group. Each group consists of eight animals.

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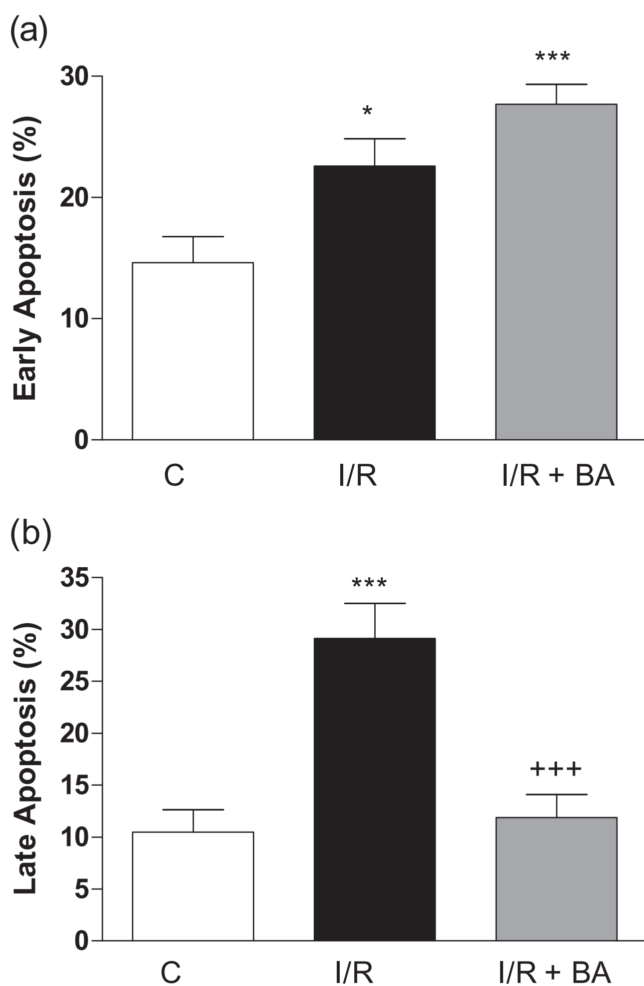

Figure 2. (a) Early apoptosis and (b) late apoptosis in neutrophils in control (C), saline-treated I/R (I/R) and betulinic acid-treated I/R (I/R + BA) groups. Apoptosis ratios were calculated by dividing the values of after-stimulation by the values obtained prior to phorbol myristate acetate stimulation. *$p < 0.05$, ***$p < 0.001$ compared with the control group; $^{+++}p < 0.001$ compared with the I/R group. Each group consists of eight animals.

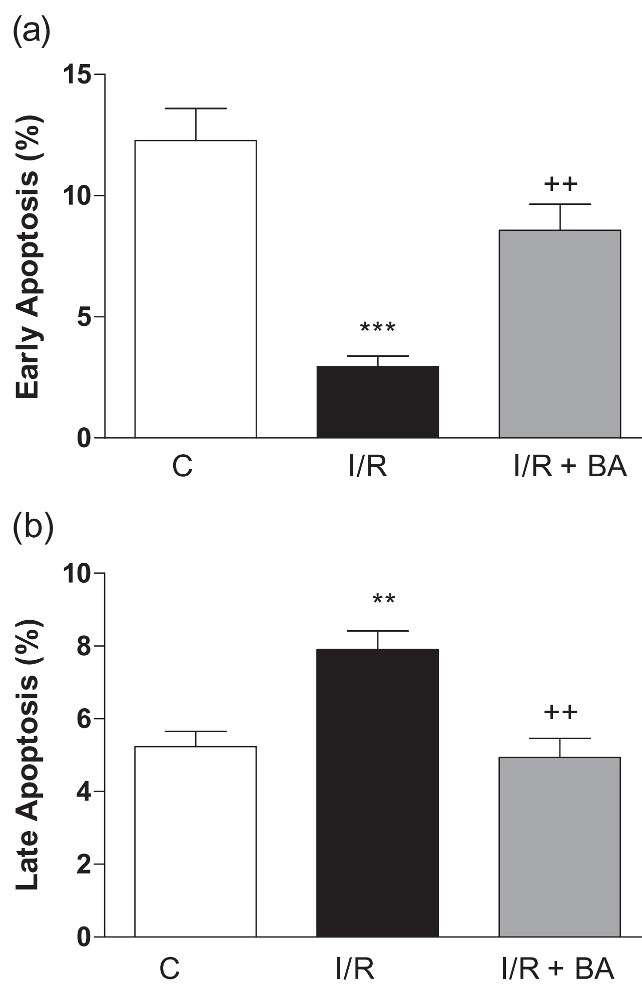

Figure 3. (a) Early apoptosis and (b) late apoptosis in lymphocytes in control (C), saline-treated I/R (I/R) and betulinic acid-treated I/R (I/R + BA) groups. Apoptosis ratios were calculated by dividing the values of after-stimulation by the values obtained prior to phorbol myristate acetate stimulation. **$p < 0.05$, ***$p < 0.001$ compared with the control group; $^{++}p < 0.01$ compared with I/R group. Each group consists of eight animals.

ences were defined in the I/R + BA group ($p < 0.001$) (Fig. 2b).

In lymphoctytes, no increased early apoptosis has been observed as a result of a rapid movement through the late apoptotic process (Fig. 3a). I/R provoked late apoptosis as seen in neutrophils ($p < 0.01$) (Fig. 3b). No difference was observed in late and early apoptosis between the control and I/R + BA groups. Betulinic acid ameliorated the late apoptotic response of lymphocytes ($p < 0.01$).

The renal tissue MDA content in the control group (28.8 ± 2.2 nmol/g) was elevated by I/R injury (39.3 ± 2.8 nmol/g, $p < 0.05$); however, betulinic acid treatment decreased significantly the I/R-induced elevation in renal MDA level (27.3 ± 1.9 nmol/g, $p < 0.05$; Fig. 4a). In accordance with this, I/R caused a significant decrease in renal GSH level (0.73 ± 0.04 μmol/g; $p < 0.001$) when compared with the control group (1.96 ± 0.11 μmol/g), while in the I/R + BA group, the renal GSH content was found to be partially preserved (1.21 ± 0.14 μmol/g; $p < 0.05$) (Fig. 4b).

Myeloperoxidase activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the saline-treated I/R group (11.5 ± 1.1 U/g, $p < 0.01$) than in the control group (6.5 ± 0.9 U/g). On the other hand, betulinic acid treatment in the I/R group significantly decreased the renal tissue MPO level (7.8 ± 0.6, $p < 0.01$), which was found to be not different than that of the control group; Fig. 5a).

Na^+,K^+-ATPase activity, was reduced in the saline-treated I/R group (6.1 ± 0.4 μmol/mg protein/h), indicating impaired transport function (Fig. 5b). However, in the betulinic acid-treated I/R rats (8.9 ± 0.6 μmol/mg protein/h), the measured Na^+,K^+-ATPase activity in the renal tissue was not different from those of the control rats (9.91 ± 0.6 μmol/mg protein/h).

In the control group, regular kidney morphology with Bowman's spaces, glomeruli and tubular epithelium (Fig. 6a) was observed. In the IR group (Fig. 6b), severe vascular congestion, degeneration of the tubular epithelium, glomerular and Bowman's space structures were

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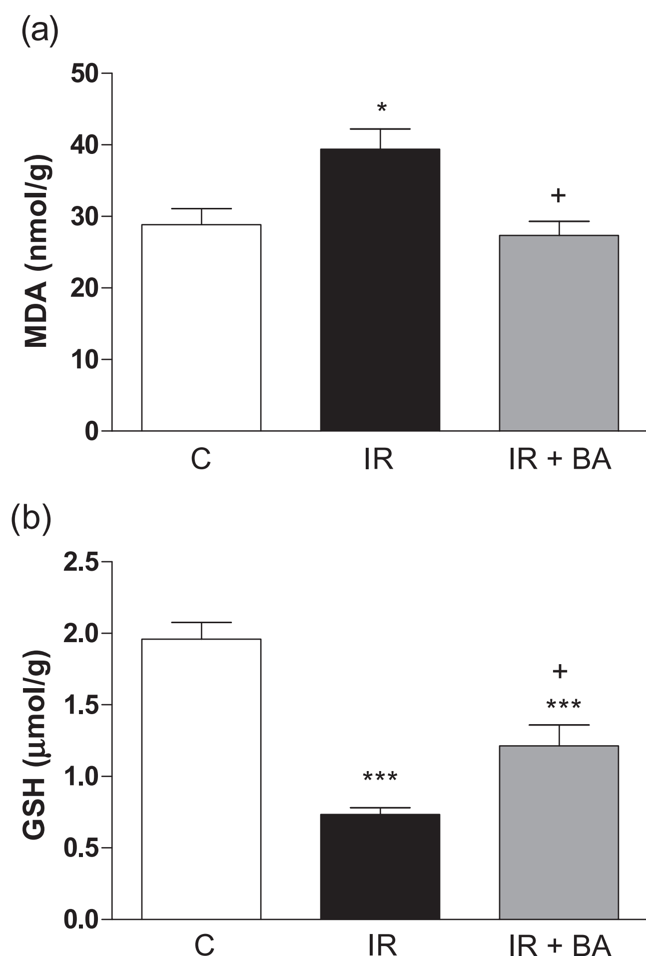

Figure 4. (a) Malondialdehyde (MDA) and (b) glutathione (GSH) levels in the kidney tissue of control (C), saline-treated I/R (I/R) and betulinic acid-treated I/R (I/R + BA) groups. $^{*}p < 0.05$, $^{***}p < 0.001$; compared with the control group. $^{+}p < 0.05$; compared with the I/R group. Each group consists of eight animals.

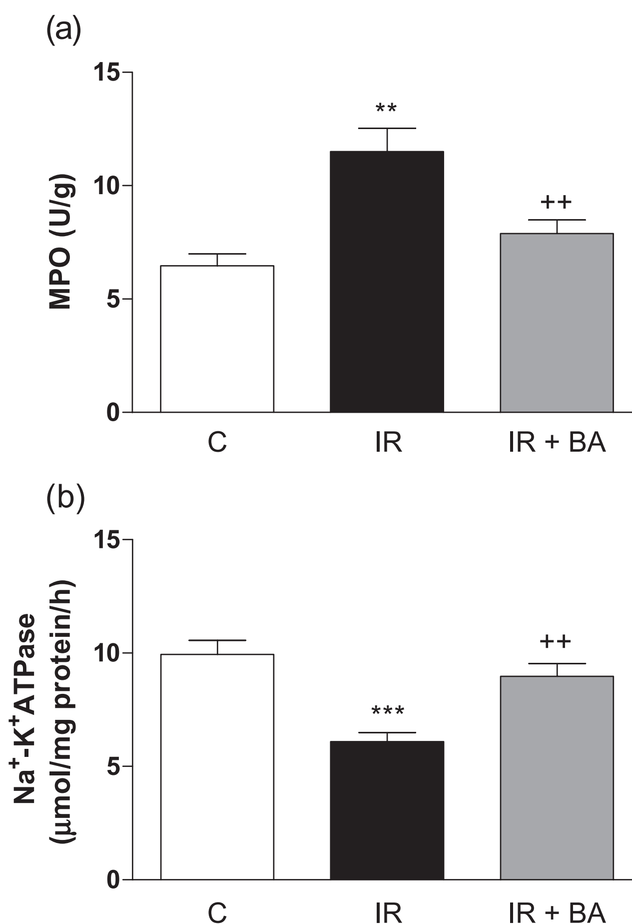

Figure 5. (a) Myeloperoxidase and (b) Na^{+}-K^{+}ATPase activities in the kidney tissue of control (C), saline-treated I/R (I/R) and betulinic acid-treated I/R (I/R + BA) groups. $^{**}p < 0.01$, $^{***}p < 0.001$; compared with the control group. $^{+}p < 0.05$; compared with the I/R group. Each group consists of eight animals.

observed. On the other hand, morphologically demonstrated degenerated kidney (Fig. 6c) tissues of the rats were clearly improved when the animals were treated by betulinic acid.

DISCUSSION

The intracellular and molecular mechanisms involved in the development of renal I/R injury are complex and not yet fully understood. Many pharmacological interventions have been investigated for their ability to reduce renal I/R injury and ischemic ARF. Although many have been evaluated clinically with a view to reducing renal I/R injury and improving renal function, none has been able to reduce mortality significantly. I/R induced organ damage is multifactorial and interdependent, involving hypoxia, inflammatory responses and free radical damage (Weight *et al.*, 1996; Chatterjee, 2007; Boros and Bromberg, 2006). Thus, agents with antioxidant and antiinflammatory effects are thought to be useful in the clinical setting of I/R damage.

Under normal conditions, ROS play a physiological role in intracellular and cell-to-cell communications (Nose, 2000). However, during renal I-R, excessive ROS generation occurs as demonstrated in many biochemical and immunohistochemical studies (Paller, 1994; Chatterjee *et al.*, 2000; Güz *et al.*, 2007; Şehirli *et al.*, 2008). It has been demonstrated that in accordance with the increases in toxic oxygen metabolites, the renal MDA level was increased significantly, indicating the presence of enhanced lipid peroxidation due to I/R injury (Chen *et al.*, 2003; Şener *et al.*, 2006a, 2006b). Similarly, in the current study MDA levels of the kidney tissues in the I/R group was increased while the levels of tissue GSH were declined, demonstrating the depletion of the antioxidant pool. It is well known that lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Stark, 2005). On the other hand, GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stress. However reduced GSH as the main component of endogenous non-protein sulfhydryl pool, is known to be a major low molecular weight scavenger of free radicals in the cytoplasm. Because of their exposed sulfhydryl groups, non-protein sulphydryls bind a variety of electrophilic radicals and metabolites that may be damaging to cells (Ross, 1988). The results of the present study further support the notion that depletion of tissue

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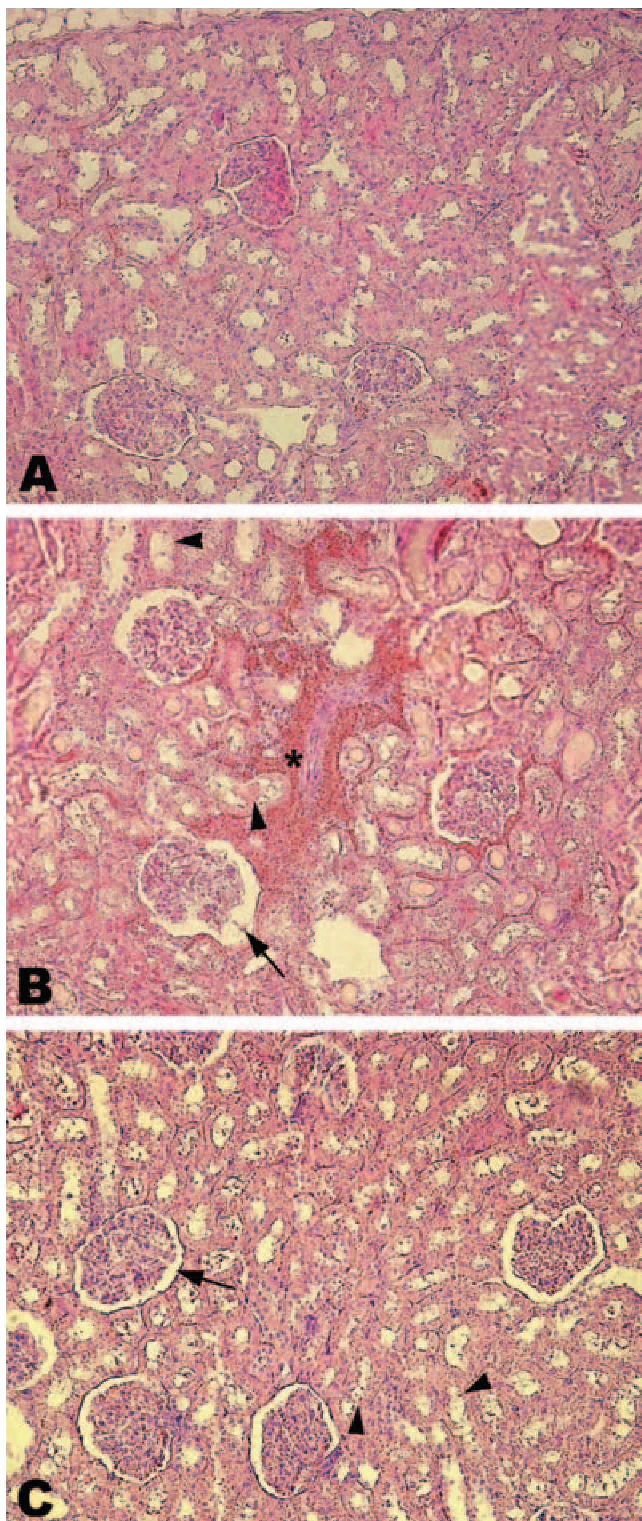

Figure 6. Regular morphology of kidney (A) was observed in the control group. Degenerated Bowman's space and glomerular structure (arrow), severe vascular congestion (asterisk) and degenerated tubular epithelium (arrow head) were observed in the IR groups (B). Quite regular Bowman's space and glomerular structure (arrow), mild degenerated tubular epithelium (arrow head) were observed in betulinic acid-treated IR group (C). H & E staining, original magnifications 200×.

GSH is one of the major factors that permit lipid peroxidation and subsequent tissue damage. Furthermore administration of betulinic acid maintained the GSH levels, reduced lipid peroxidation and thus protected tissue against oxidative stress.

Table 1. Effects of betulinic acid (BA; 250 mg/kg, i.p.) treatment on blood urea nitrogen (BUN), creatinine, lactate dehydrogenase (LDH) and tumor necrosis factor-alpha (TNF-α) in the serum of experimental groups

	BUN (U/L)	Creatinine (U/L)	LDH (U/L)	TNF-α (pg/mL)
Control	23.3 ± 1.8	0.63 ± 0.07	2037 ± 179	4.4 ± 0.5
I/R	40.0 ± 2.3^{c}	1.91 ± 0.11^{c}	5704 ± 357^{c}	15.4 ± 1.4^{c}
I/R + BA	31.6 ± 2.3^{a,d}	0.91 ± 0.10^{e}	2624 ± 321^{e}	10.6 ± 1.2^{b,d}

I/R, ischemia/reperfusion. Each group consists of eight animals.
$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; compared with the control group.
$^{d}p < 0.05$, $^{e}p < 0.001$; compared with the saline treated-I/R group.

Renal I/R injury is the major cause of ARF in both native and transplanted organs. Reperfusion, although essential for the survival of ischemic renal tissue, causes additional damage. The neutrophils seem to act as an amplifier of the initial reperfusion reaction and are considered as an important factor in the damaging cascade that takes place upon reperfusion. It is well documented that I/R elicits an acute inflammatory response characterized by activation of neutrophils (Willerson, 1997; Heinzelmann *et al.*, 1999). Activated neutrophils induce tissue injury through the production and release of reactive oxygen metabolites and cytotoxic proteins (e.g. proteases, MPO, lactoferrin) into the extracellular fluid (Kettle and Winterbourne, 1997). In the present study, elevated levels of MPO activity, which is an index of tissue neutrophil infiltration, was inhibited by betulinic acid treatment, indicating that the protective effect of betulinic acid is neutrophil dependent.

Sodium–potassium ATPase is a membrane-dependent enzyme that enables the transport of sodium and potassium to cross the membrane against a concentration gradient by hydrolysis of ATP and maintenance of intracellular electrolyte homeostasis (Aperia, 2007). Like many membrane dependent enzymes, Na^{+},K^{+}-ATPase requires phospholipids for its activity. However, in ischemia the depletion of energy stores and also the initiation of lipid peroxidation due to increased free radicals following reperfusion causes inhibition of membrane Na^{+},K^{+}-ATPase activity (Matsuzaki *et al.*, 2007; Şehirli *et al.*, 2008). Thus I/R-induced ARF, characterized by impaired renal function, reduced glomerular filtration and tubular sodium reabsorption have been related to the functional disorders of Na^{+},K^{+}-ATPase (Chatterjee, 2007). In agreement with these investigations, in our study there was a significant decrease in the activity of Na^{+},K^{+}-ATPase in the saline treated I/R group, while BA treatment restored the enzyme activity possibly protecting the membrane structure by its antioxidant effect.

In the present study as shown in the results regarding the neutrophil oxidative burst, following stimulation by both fMLP and PMA, betulinic acid treatment increased and normalized burst function in comparison with the I/R group, whereas no difference was found following *E. coli* stimulation. It is concluded that I/R has no effect on the phagocytosis of *E. coli* that is a gram-negative bacteria. Since bacteria were engulfed successfully, no sequential signalling event was affected by I/R. However,

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different signalling pathways used by fMLP and PMA stimuli were interrupted in the I/R group and administration of betulinic acid immediately restored the running of these pathways in the cells. An increased early apoptotic response inconsecutive to the increased late apoptosis in the I/R + BA group suggests that BA has an activating effect on neutrophils resulting in phosphatidylserine being turned inside out of the membrane and being labelled by Annexin V FITC. Nevertheless, betulinic acid succeeded in overcoming the increased apoptosis seen in the I/R group. On the other hand, betulinic acid normalized both early and late apoptotic responses in leukocytes.

Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of diseases, despite relatively little knowledge of their modes of actions. Triterpenoids are almost ubiquitous in plants and have long been considered to be the antiinflammatory principles of several drugs, particularly in the form of triterpene glycosides. The reported biological effects of triterpenoids include cytotoxic, antitumor, antiinflammatory and antiviral activities (Recio *et al.*, 1995; Fulda and Debatin, 2000; Ryu *et al.*, 2000). In 1995, betulinic acid was reported to be a selective inhibitor of human melanoma (Pisha *et al.*, 1995). It was demonstrated that betulinic acid induces apoptosis in human melanoma *in vitro* and *in vivo* model systems (Schmidt *et al.*, 1997). The antiinflammatory activity of triterpenoids is connected with their interaction with several cellular and extracellular proteins. Betulin and betulinic acid have been shown as potent phospholipase A2 inhibitors (Bernard *et al.*, 2001). Considering their similarity to steroidal compounds, they have often been attributed a mechanism related to that of these antiinflammatories. Bureeva *et al.* (2009) have studied betulin disulfate and betulinic acid sulfate on the classical pathway of complement activation induced by ischemia, and showed that triterpenoids have inhibitory effects on complement activation. Furthermore Nguemfo *et al.* (2009) using a free radical scavenging method, showed weak antioxidant activity for betulinic acid while they demonstrated the antiinflammatory properties of betulinic acid in the carrageenan-induced inflammation model. However, more experiments are necessary to explain the structure/function relationship of triterpenoids.

There is no specific therapy for ARF except for supportive care. ARF is a complex syndrome involving renal vasoconstriction, extensive tubular damage, tubular cell necrosis, glomerular filtration failure and glomerular injury. Different experimental models have shown that treatment with antioxidant and antiinflammatory agents can protect against oxidative damage induced by I/R. With the data in hand, it can be concluded that betulinic acid through its antiinflammatory and antioxidant properties protects the kidney from I/R induced injury. However, further studies are required to show the beneficial effects of betulinic acid for the development of new therapeutic approaches for treating I/R related tissue injury and organ dysfunction.

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