Effects Of Protease Activated Receptor (PAR)2 Blocking Peptide On Endothelin-1 Levels In Kidney Tissues In Endotoxemic Rat Model

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Abstract

Aims: Septic shock, the severe form of sepsis, is associated with development of progressive damage in multiple organs. Kidney can be injured and its functions altered by activation of coagulation, vasoactive-peptide and inflammatory processes in sepsis. Endothelin (ET)-1, a potent vasoconstrictor, is implicated in the pathogenesis of sepsis and its complications. Protease-activated receptors (PARs) are shown to play an important role in the interplay between inflammation and coagulation. We examined the time-dependent alterations of ET-1 and inflammatory cytokine, such as tumor necrosis factor (TNF)-α in kidney tissue in lipopolysaccharide (LPS)-induced septic rat model and the effects of PAR2 blocking peptide on the LPS-induced elevations of renal ET-1 and TNF-α levels.

Main methods: Male Wistar rats at 8 weeks of age were administered with either saline solution or LPS at different time points (1, 3, 6 and 10 h). Additionally, we treated LPS-administered rats with PAR2 blocking peptide for 3 h to assess whether blockade of PAR2 has a regulatory role on the ET-1 level in septic kidney. Key findings: An increase in ET-1 peptide level was observed in kidney tissue after LPS administration time-dependently. Levels of renal TNF-α peaked (around 12-fold) at 1 h of sepsis. Interestingly, PAR2 blocking peptide normalized the LPS-induced elevations of renal ET-1 and TNF-α levels.

Significance: The present study reveals a distinct chronological expression of ET-1 and TNF-α in LPS-administered renal tissues and that blockade of PAR2 may play a crucial role in treating renal injury, via normalization of inflammation, coagulation and vaso-active peptide.

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Introduction

Septicemia and septic shock are still associated with high mortality, and current therapy is mostly supportive and largely ineffective (Mayeux, 1997). Some of the most common and serious complications associated with septicemia include, pulmonary, hepatic, and renal failures (Rackow and Astiz, 1991; Wardle, 1982), and the most common etiological agents are gram-negative bacteria. Lipopolysaccharide (LPS), a component of the gram-negative bacterial cell wall released during septicemia, is a major cause of septic shock in humans (Mayeux, 1997) and has been implicated in the pathogenesis of acute and chronic renal disease. Although hypotension and reduced renal blood flow can contribute to renal failure, animal models show that LPS can cause renal injury even in the absence of significant falls in systemic blood pressure or renal blood flow (Millar and Thiemermann, 1997). Admittedly, not all of the pathophysiological changes associated with sepsis and shock are a direct consequence of bacterial products. Other factors may include inflammatory mediators, such as cytokines tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), platelet-activating factor (PAF), leukotrienes, thromboxane A2, and inducible nitric oxide synthase (iNOS).

Kidney dysfunction in LPS-induced sepsis is thought to be characterized by severe intra-renal vasoconstriction in the face of profound

vasodilation in the systemic circulation, based on the disruption of the overall balance between vasodilation and vasoconstriction in the kidney. Endothelin-1 (ET-1) is the most potent vasoconstrictor peptide known so far (Yanagisawa et al., 1988) and LPS stimulates its synthesis and release in cultured endothelial cells (Marsden and Brenner, 1992; Nakamura et al., 1991) and is also known to significantly elevate its plasma levels in experimental animals (Nakamura et al., 1991; Pernow et al., 1990; Sugiura et al., 1989; Vemulapalli et al., 1991) and patients with septic shock (Pittet et al., 1991; Takakuwa et al., 1994; Voerman et al., 1992; Weitzberg et al., 1991). The possible involvement of the ET-1 system in human septic shock is further supported by a clear correlation between ET-1 plasma levels and morbidity and mortality of septic patients (Brauner et al., 2000; Hartemink et al., 2001). However, it is still controversial whether elevated plasma ET-1 levels are markers of endothelial dysfunction/damage or mediators of the disease. Increased systemic and/or local production of ET-1 has been implicated in blood pressure elevation (Gardiner et al., 1995; Ruetten et al., 1996), mediation of renal vasoconstriction and failure (Pernow et al., 1990), and also in pulmonary and portal hypertension during endotoxic shock (Yamamoto et al., 1997). Clearly, ET-1 system is likely involved in renal complications during sepsis. Accordingly, recently we reported that ET-1 level in kidney tissue is increased time-dependently after LPS administration with a higher plasma ET-1 level (Yamaguchi et al., 2006).

Protease-activated receptors (PARs), members of a recently described family of G-protein-coupled, seven-transmembrane-domain receptors, are activated through proteolytic cleavage of their N-terminal domain by proteinases, resulting in the generation of a new N-terminal "tethered ligand", which can auto activate the receptor function (Ossovskaya and Bunnett, 2004). Four members of the PAR family have been cloned. PAR1, PAR3, and PAR4 can be activated by thrombin, and PAR2 can be activated by trypsin, mast cell tryptase, neutrophil proteinase 3, tissue factor/factor VIIa/factor Xa, and membrane-tethered serine proteinase-1 (Lourbakos et al., 2001; Vergnolle et al., 2001a). The involvement of PAR2 in inflammation is supported by several studies. Early studies reported that PAR2 expression was upregulated by inflammatory mediators such as TNF-α, IL-1 and LPS (Nystedt et al., 1996). Moreover, a number of studies have demonstrated that activation of PAR2 can lead to blood vessel relaxation, hypotension, increased vascular permeability, granulocyte infiltration, leukocyte adhesion and margination, and pain (Cocks and Moffatt, 2000; Coughlin and Camerer, 2003; Vergnolle et al., 2001b) — all effects that encounter for the cardinal signs of inflammation. Recently, the role of PAR2 in the pathogenesis of sepsis has begun to be appreciated. A combination of thrombin inhibition and PAR2 deficiency reduced inflammation and mortality in a mouse model of endotoxemia (Pawlinski et al., 2004). We have also demonstrated that the blockade of PAR2 ameliorates liver injury via the normalization of the inflammation, coagulation and fibrinolytic pathways (Jesmin et al., 2006).

In the kidney, PAR2 expression has been reported in collecting duct cells, mesangial cells, interstitial fibroblasts, vascular endothelial cells and proximal tubule cells (Coughlin, 2000; Vesey et al., 2007). The present study intended to use a PAR2 blocking peptide to LPS-administered endotoxemic rats to investigate whether PAR2 blockade would have any role on the upregulated ET-1, TNF-α and iNOS levels in kidney tissues.

Materials and methods

Animals and treatments

In the first series of our study, endotoxemia was induced by administering a single (2 ml) intra-peritoneal injection of LPS (in sterile saline solution) derived from *Escherichia coli* 055:B5 (15 mg/kg body weight) to adult male Wistar rats. The animals were killed in groups using pentobarbital (80 mg/kg body weight intraperitoneal [ip]) at different time points after LPS administration (1, 3, 6, and 10 h, n = 30 rats/group). The control group received an equal volume of sterile saline (2 ml/rat) containing no LPS. Whole kidney tissues were carefully harvested, immediately frozen in liquid nitrogen, and stored at −80 °C. For the paraffin section preparations, after the kidney tissues were harvested, they were postfixed in 4% paraformaldehyde overnight and processed routinely for paraffin embedding. All experimental procedures in the present study were performed in accordance with the institutional guidelines of the University of Tsukuba Graduate School of Medicine Animal Care and Use Committee.

In the second part of the study, in order to investigate the specific role of PAR2 in the LPS-induced elevated ET-1 peptide level in the kidney, the PAR2 blocking peptide (BP) was used to treat the rats in addition to LPS. For this purpose, some rats were anesthetized with urethane (35% ethyl carbamate [Wako Pure Chemical Industries, Osaka, Japan] + 4% alpha-chloralose [Wako] saline solution wt./vol., 0.4 to 0.8 g/kg, ip), and the left jugular vein was cannulated for drug administration. All drugs being tested were administered intravenously as a slow bolus injection. LPS (15 mg/kg, intravenous [iv]) was administered through the jugular vein for 3 h (n = 20). Thirty minutes (min) before LPS administration, the PAR2 BP (sc-9278 P, Santa Cruz Biotechnology, Santa Cruz, CA) treated group was administered PAR2 BP intravenously as a slow bolus injection (100 μg/kg, 500 μl/kg PBS) and PAR2 BP was continuously infused (20 μ/kg/h, 100 μl PBS/h) through the jugular vein with a pump for 3 h (n = 20). Non-treated rats were used as a control (n = 20).

Measurements of hemodynamic and biochemical parameters

On the day of the experiment, the rats were anesthetized with pentobarbital sodium (40 mg/kg body weight ip) and a microtip pressure transducer catheter (SPC-320, Millar Instruments, Houston, TX, USA) was inserted into the left carotid artery. The arterial blood pressure and heart rate were monitored with a pressure transducer (model SCK-590, Gould, OH, USA) and recorded using a polygraph system (amplifier, AP-601G; Tachometer, AT-601G; thermal-pen recorder, WT-687G; Nihon Kohden, Tokyo, Japan). Thereafter, plasma and serum samples were prepared. In preparing the plasma samples, the proportion of blood to the sodium citrate dehydrate anticoagulant volume was 9:1. Serum creatinine levels and blood urea nitrogen (BUN) were measured using kits from Wako Pure Chemical Industries, LTD (Osaka, Japan).

Histopathological examination

For the histopathological analysis, the tissue specimens were fixed in a 4% buffered formalin solution, dehydrated through an ethanol series, embedded in paraffin, and sliced into 5-μm-thick sections. After deparaffinization, the sections were stained with hematoxylin and eosin (HE) using the standard histological method.

TNF-α and iNOS immunoassays

TNF-α levels in the serum and kidney tissue specimens were detected using a rat TNF-α enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems, Minneapolis, MN). This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat TNF-α has been pre-coated onto a microplate. Standards, controls, and samples are pipetted into the wells and any rat TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of rat TNF-α bound in the initial step.

The sample values are then read off the standard curve. For iNOS, we used a Human iNOS Immunoassay kit (R and D Systems, Minneapolis, MN).

Enzyme immunoassay for plasma and renal ET-1 levels

Concentration of ET-1 in plasma and renal tissue extracts was determined using an ET-1 Enzyme Immuno Assay Kit (Immuno-Biological Laboratories, Fujioka, Japan). This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetraethyl Benzidine (TMB) is used as a coloring agent (Chromogen), the strength of which is in proportion to the quantities of ET-1. In plasma and kidney tissue extracts, an equal volume of 0.1% trifluoroacetic acid (TFA) in water was added. Then, the sample was centrifuged at 3000 ×*g* for 15 min at 4 °C to clarify and the supernatant was saved. After a 200 mg C 18 Sep-Pak column (Waters Inc. Part No. 235D1) was washed with 4 × 1 ml of 60% acetonitrile in 0.1% TFA followed by 4 × 5 ml of 0.1% TFA in water, the supernatant was applied to the column and washed with 4 × 5 ml of 0.1% TFA in water. The sample was eluted slowly by applying 3 × 1 ml of 60% acetonitrile in 0.1% TFA in water and the eluant was collected in a plastic tube. The eluant was then evaporated to dryness using a centrifugal concentrator under vacuum. The sample was reconstituted with assay buffer and measured immediately according to the manufacturer's instructions.

Statistical analysis

Results are expressed as mean ± S.E., and the sample number equals the number of animals in each group. Means were compared by one factor analysis of variance, followed by the Scheffé's test for multiple comparisons. Differences were considered significant at $p < 0.05$.

Results

Effects of LPS on hemodynamic and biochemical parameters (Table 1)

Both the systolic and diastolic blood pressures significantly decreased at different time points after LPS administration compared to control rats (Table 1). Levels of serum iNOS (a crucial inflammatory mediator) increased significantly after LPS administration, peaking at 1 h (more than 3-fold increase compared to control group) and maintained at significantly higher levels during all the time points following LPS administration (Table 1). The serum levels of TNF-α (another potential inflammatory cytokine), as determined by ELISA, were elevated after administration of LPS (Table 1), reaching their peak levels at 1 h (1200-fold from control), but decreasing thereafter. Thus, from the present study, it is obvious that serum levels of potential inflammatory mediators were much more elevated at circulatory level during the early hours of endotoxemic induction. Furthermore, immunoreactive ET-1 levels in plasma were significantly higher following LPS administration for all time points, reaching peak levels at 3 h after LPS administration. Further, serum creatinine levels increased during all the time points after LPS administration. In contrast, levels of the serum BUN began to increase significantly only 3 h after LPS administration. Both levels of serum creatinine and BUN (important parameters of kidney function) exhibited a time-dependent-like increasing trends after LPS administration.

Histopathology after LPS administration

Evaluation of renal histology of H&E-stained tissue sections showed no evidence of intra-glomerular thrombosis and/or disseminated intravascular coagulation in any of the treated animals (Fig. 1). Besides, these sections showed only minimal tubular degenerative changes; that is, focal tubular dilatation with mild swelling or thinning of the epithelial cells at 3 h after LPS administration (Fig. 1). These findings are consistent with a septic kidney.

Effects of PAR2 blocking on serum creatinine and BUN levels

The elevated serum levels of both creatinine (1.42 ± 0.11 mg/dl) and BUN (34.70 ± 2.80 mg/dl) in LPS-treated rats at 3 h were almost unchanged (creatinine, 1.37 ± 0.13 mg/dl; BUN, 37.50 ± 3.20 mg/dl) after the administration of PAR2 BP to the LPS-treated rats (Fig. 2A and B), respectively. These results suggest that PAR2 BP failed to reverse the important parameters of kidney function in LPS-induced endotoxemic rats.

Effects of blocking PAR2 on serum and renal levels of TNF-α

The elevated levels of serum TNF-α (710.00 ± 55.00 pg/ml) in LPS-administered rats were significantly normalized at 3 h of endotoxemia after blocking PAR2 (290.00 ± 38.00 pg/ml) (Fig. 2C). At 1 h, the amount of TNF-α in renal tissue, as determined by ELISA (Fig. 3A), was significantly elevated compared to control group in the LPS-treated animals (about 17-fold increase) and thereafter declined. Thus, levels of renal TNF-α increased significantly in the early hours following LPS administration (Fig. 3A) and were remarkably normalized (more than 50% reversal) by PAR2 blockade for 3 h (Fig. 3B).

Effects of PAR2 blocking on renal levels of iNOS

iNOS protein expression was induced during all the time-points after LPS administration compared to control rats in renal tissues, as determined by ELISA (Fig. 3C), peaking at 3 h after LPS injection. A 60% increase in renal iNOS protein expression was noted at 3 h after LPS administration (Fig. 3C) compared to control tissues. Treatment with PAR2 BP for 3 h greatly normalized the elevated levels of iNOS in renal tissues of LPS-administered rats {Control (0.97 ± 0.11 U/mg): LPS (1.65 ± 0.17 U/mg): LPS + PAR2 BP (1.27 ± 0.12 U/mg)}, respectively.

Table 1
Effects of LPS on hemodynamic and biochemical parameters.

	LPS				
	Control	1 h	3 h	6 h	10 h
Systolic BP (mm Hg)	132 ± 5	121 ± 4*	111 ± 5**	102 ± 5**	114 ± 7**
Diastolic BP (mm Hg)	87 ± 4	75 ± 3*	73 ± 4*	72 ± 5*	76 ± 4*
iNOS (serum, U/ml)	7.8 ± 1.0	25.1 ± 3.6**	17.3 ± 3.1*	11.4 ± 2.1**	11.5 ± 0.5**
ET-1 (plasma, pg/ml)	0.8 ± 0.4	8.2 ± 2.0**	23.4 ± 9.9**	9.4 ± 6.0*	4.8 ± 1.9*
TNF-α (serum, pg/ml)	23 ± 2	2340 ± 340**	512 ± 32*	125 ± 11*	81 ± 7*
BUN (serum, mg/dl)	20.2 ± 1.6	22.1 ± 1.3	36.7 ± 3.3*	46.4 ± 7.2*	72.5 ± 9.3*
Creatinine (serum, mg/dl)	0.8 ± 0.1	1.3 ± 0.1*	1.4 ± 0.1*	1.6 ± 0.1*	1.5 ± 0.1*

LPS, lipopolysaccharide; BP, blood pressure; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; BUN, blood urea nitrogen; ET-1, endothelin-1.
* $p < 0.05$ vs control.
** $p < 0.01$ vs control.

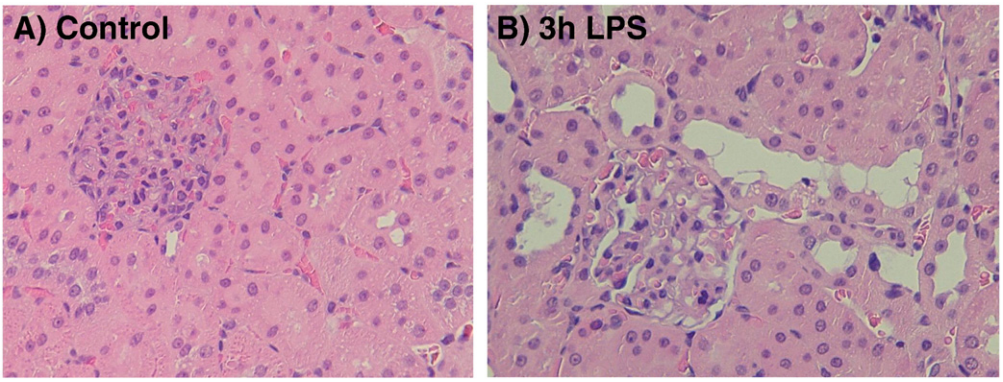

Fig. 1. Morphological findings by hematoxylin and eosin staining in the kidney tissues in control rats (A) and 3 h after lipopolysaccharide (LPS) administration (B). Magnification, 100×.

Effects of blocking PAR2 on levels of plasma and renal tissue ET-1

A time-dependent increase in levels of renal ET-1 peptide was noted in LPS-administered compared to the control group (Fig. 4A). Further, after the 3 h time point, ET-1 levels were significantly higher in LPS administered rats compared to the corresponding control group. Ten hours (10 h) after LPS administration, a 4.5-fold increase in ET-1 level was observed (Fig. 4A) in LPS administered renal tissues compared to the control group. Treatment with PAR2 BP for 3 h significantly normalized the upregulated ET-1 level in renal tissues of LPS-administered rats {Con (0.90 ± 0.10 pg/mg): LPS (3.50 ± 0.30 pg/mg): LPS + PAR2 BP (1.80 ± 0.20 pg/mg)}, respectively.

Discussion

The present study demonstrates that the levels of ET-1 peptide increased in kidney tissues after LPS administration time-dependently with a rise in plasma ET-1 concentration compared to control rats. These increases in renal and plasma ET-1 levels were associated with a rise in TNF-α and iNOS. Both the systolic and diastolic blood pressures significantly decreased in LPS-administered rats. The present study is the first time to demonstrate that blockade of PAR2 could reduce elevation of renal ET-1 as well as TNF-α and iNOS in LPS-induced endotoxemic rats without affecting the circulatory levels of renal function parameters, such as creatinine and BUN.

We show here significant increases in levels of serum creatinine and BUN after LPS administration, indicating impairment of kidney function in the endotoxemic models used in the present study. Regarding the histological injury in the renal tissues, minimal injury was seen at 3 h after LPS administration, consistent with our previous study (Yamaguchi et al., 2006). Further, we also show here, using Wistar rats, that blood pressure continued to decrease even at 10 h after LPS administration, although the extent of decrease was not abrupt and remarkable. It is possible that it may be the reason why there were no remarkable histological changes in renal tissues after LPS administration. Thus, collectively, the endotoxemic models used in the present study had impairment of renal function parameters, minimal morphological injury in kidney as well as decrease of systemic blood pressure.

The models used in the present study showed increase in levels of inflammatory cytokines such as TNF-α and iNOS. Further, consistent with our previous studies (Jesmin et al., 2004, 2006, 2007) and earlier reports from other labs (Cohen, 2002; Norman et al., 1995; Putensen and Wrigge, 2000), increase in levels of plasma TNF-α was noted in the earlier hours of endotoxemia. The pathogenic role of TNF-α in endotoxemic renal failure has been suggested. For example, an intravenous infusion of TNF-α in rabbits or perfusion of isolated rat kidney with TNF-α led to a decrease in glomerular filtration rate (GFR) (Bertani et al., 1989; Van der Veen et al., 1999). In rabbits, an intravenous infusion of TNF-α induced glomerular fibrin deposition and renal failure (Bertani et al., 1989). In the present study, we found elevated levels of TNF-α after LPS administration in kidney tissues, meaning that the elevated levels of TNF-α would possibly be involved in the pathogenesis of LPS-induced renal dysfunction. Similarly, consistent to our previous study (Yamaguchi et al., 2006), we also show here elevated levels of circulatory and renal iNOS after LPS administration. Various inflammatory stimuli, such as LPS and proinflammatory cytokines, stimulate the endogenous NO production by activation of iNOS (Feihl et al., 2001). A local induction of iNOS, which is constitutively expressed in the kidney, in particular in the medulla and proximal tubules, may be the cause of peroxynitrite-related tubular injury as a result of local formation of reactive oxygen and nitrogen species during systemic inflammation (Ortiz and Garvin, 2003).

Consistent with our previous study (Yamaguchi et al., 2006) in the current investigation also we found a time-dependent increase in ET-1

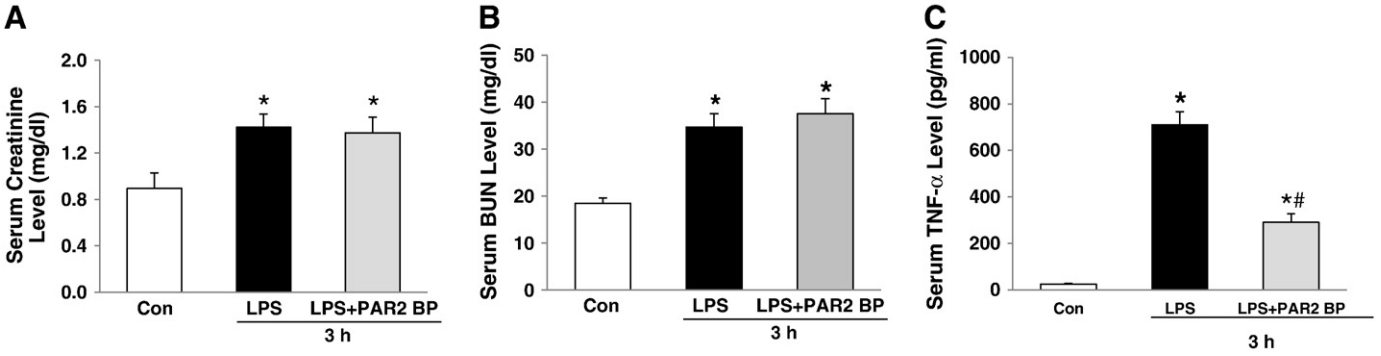

Fig. 2. Effects of PAR2 BP on serum (A) creatinine, (B) BUN and (C) TNF-α levels from the control and LPS-treated rats. Values are mean ± S.E. (n = 22). *p < 0.01 vs control, #p < 0.01 vs LPS 3 h.

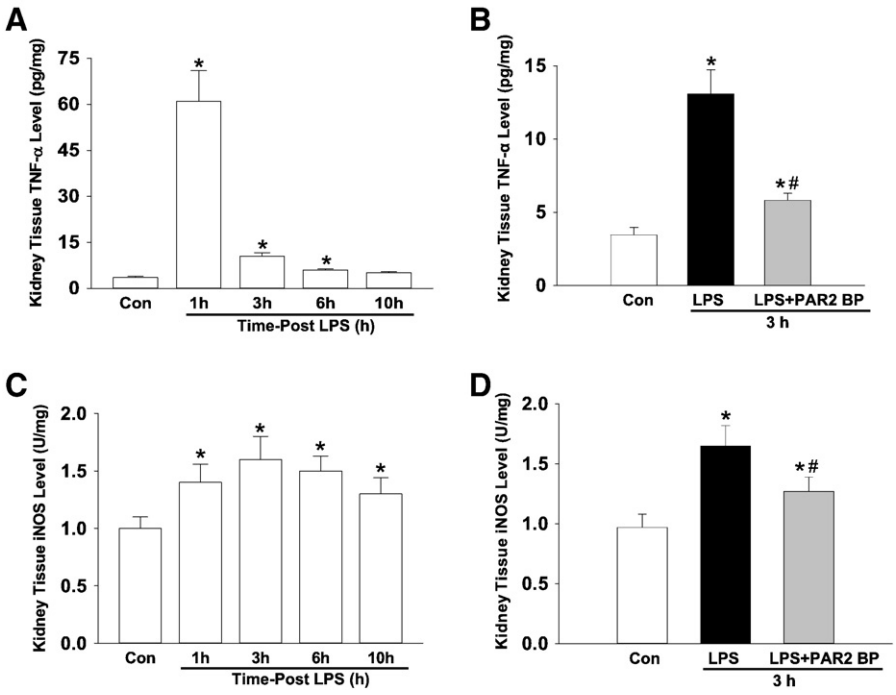

Fig. 3. (A, C) Renal levels of TNF-α and iNOS from the control and LPS-treated rats. TNF-α and iNOS were measured by ELISA. Values are mean ± S.E. (n = 22). *p < 0.01 with respect to the control. (B, D) Effects of PAR2 BP on renal TNF-α and iNOS levels at 3 h after LPS administration. Values are mean ± S.E. (n = 22). *p < 0.01 vs control, #p < 0.01 vs LPS 3 h.

peptide level after LPS administration in renal tissues. In fact, the role of ET-1 in renal complications in sepsis is recently being investigated. ET-1 receptor blockade is useful in preventing albumin escape during endotoxin shock in conscious, chronically catheterized rats (Filep et al., 1995). Moreover, others have shown that ET-1 receptor antagonist prevented LPS-induced metabolic acidosis and hypoxemia, and improved LPS-induced decreases in urine volume, renal blood flow, creatinine clearance, and urine osmolality (Mitaka et al., 1999). The fact that LPS induces expression of both renal ET type A (ET_A) and ET type B (ET_B) receptors, as shown in our previous study (Yamaguchi et al., 2006) indicates that dual ET-1 antagonism could prove useful in preventing renal dysfunction in septic model. Although the precise mechanisms underlying the expression pattern of these molecules are presently unclear, based on the findings of the present and previous studies, we speculate that increased levels of ET-1 are triggered by compensatory mechanisms aimed at counteracting the undesirable effects generated by abnormally high levels of NO/iNOS. In fact, the early renal dysfunction of endotoxemia results from renal hypoperfusion caused mainly by an imbalance in renal vasoconstrictors and vasodilators (Knotek et al., 2001).

The most crucial findings of the present study are the demonstration of effects of PAR2 BP on various circulatory parameters from inflammatory cytokines to renal functions. Although PAR2 BP significantly altered levels of plasma TNF-α, PAR2 BP had no observable effects on serum creatinine and BUN levels. In IgA nephropathy, a positive association between PAR2 positive cells in kidney and serum creatinine level has been previously reported (Liu et al., 2010). This is in contrast with the current findings where PAR2 BP was found to be ineffective to reverse the upregulated levels of important kidney function parameters, such as creatinine and BUN in endotoxemic rat. It is important to state here that the morphological injury at 3 h endotoxemic rat was minimal and we assume that PAR2 BP has the potential in normalizing the alteration of inflammatory cytokine such as TNF-α without altering the renal morphology as well as circulatory parameters of renal function. This conclusion is consistent with the fact that the regulatory effects of PAR2 on TNF-α expression have been documented in many studies. For instance, PAR2 directly regulates TNF-α expression in coronary microcirculation in type 2 diabetes (Park et al., 2011). Kim et al. (2002) reported that PAR2 agonist induces TNF-α secretion through the activation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated

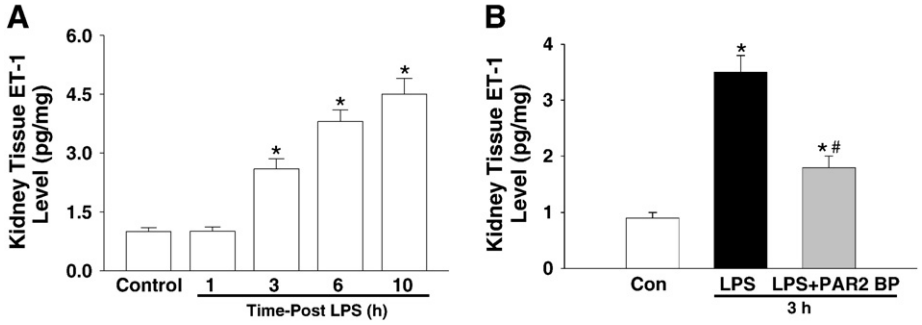

Fig. 4. (A) Renal level of ET-1 from the control and LPS-treated rats. ET-1 was measured by ELISA. Values are mean ± S.E. (n = 20). *p < 0.01 with respect to the control. (B) Effects of PAR2 BP on renal ET-1 level at 3 h after LPS administration. Values are mean ± S.E. (n = 22). *p < 0.01 vs control, #p < 0.01 vs LPS 3 h.

protein kinases (MAP kinase) in atrocytoma cells (Kim et al., 2002; Kleinbongard et al., 2010). Future studies should focus on the effects of PAR2 BP on above signal transduction in the current experimental setting.

PAR2 may contribute to LPS-mediated organ dysfunction not only through the inflammatory cytokine, but also through vasoconstrictive peptides like ET-1. In the present study, we found that blockade of PAR2 significantly suppressed the renal ET-1 level. This suppression of ET-1 by PAR2 blockade may involve TNF and NO pathways. Studies have demonstrated that TNF-α can induce ET-1 expression. TNF-α increases the transcription of ET-1 gene (Miyauchi and Masaki, 1999). NF-kappa B functions as one of main regulatory factors in TNF-α-induced ET-1 gene transcription. In cultured endothelial cells, TNF-α exposure resulted in an increased secretion of ET-1 (Corder et al., 1995) which was accompanied by a corresponding increase in prepro-ET-1 mRNA transcript levels (Marsden and Brenner, 1992). We speculate that, ET-1 might be an important mediator of TNF-α-induced renal dysfunction after LPS administration although the detailed mechanistic insights remain to be explored. And PAR2 may one of the key molecules regulating TNF-α production in sepsis. Accordingly, in the present study we found that blockade of PAR2 lowers both the plasma and renal TNF-α levels. More recently, we showed that blockade of PAR2 significantly improves LPS-induced acute liver injury through the suppression of TNF-α and coagulation system (Jesmin et al., 2006). Another possible mechanism of the suppression of ET-1 level in renal tissue by PAR2 blockade may involve NO/iNOS pathway. Systemic administration of PAR2 agonist in vivo in rats and mice causes hypotension (Cicala et al., 2001; Emilsson et al., 1997). Interestingly, also in humans in vivo PAR2 agonist causes vasodilatation that is dependent on NO release and prostanoids (Robin et al., 2003). Thus, we speculate that PAR2 may regulate ET-1 through modulating NO/iNOS pathway in sepsis. In our future studies we intend to investigate in more depth the mechanisms underlying the reversal effects of PAR2 BP on the elevated levels of renal ET-1 in endotoxemia.

Conclusion

The present study demonstrates that ET-1, TNF-α and iNOS expressions are increased at various time points in renal tissues after LPS administration in rats and reports for the first time that blockade of PAR2 significantly normalizes the elevated renal expressions of TNF-α, iNOS and ET-1.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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