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ORIGINAL ARTICLE

Cyclosporine decreases prostaglandin E2 production in mouse medullary thick ascending limb cultured cells

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cyclooxygenase 2, cyclosporine, medullary thick ascending limb, nitric oxide, prostaglandin E2.

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Summary

Intrarenal vasoconstriction is thought to be the major pathogenesis of cyclosporine (CsA) nephrotoxicity. Nitric oxide (NO) and prostaglandin E2 (PGE2) are two of the major intrarenal vasodilators, which protect kidney from ischemia. CsA inhibited NO production in renal epithelial cells. The interaction between CsA and intrarenal PGE2 and NO production is still unclear. The aim of the study is to evaluate the interaction of CsA with intrarenal PGE2 and NO production in renal epithelial cells. Models of cultured mouse thick ascending limb (TAL) cells are chosen to perform the experiments, as TAL cells are the major site of intrarenal PGE2 production and target of CsA nephrotoxicity. We investigated the PGE2 production by enzyme-linked immunosorbent assay, and cyclooxygenase (COX-1 and COX-2) mRNA expression by RT-PCR in cultured cells treated with or without CsA. TAL cells maintained the main characteristics of their parental cells. TAL cells produce PGE2 mainly by COX-1 in steady state and by COX-2 in stimulated state by lipopolysaccharide (LPS). CsA (100 ng/ml) significantly reduced the PGE2 production up to 43% in TAL cells in LPS stimulated status (control versus CsA: 375.1 ± 15.5 vs. 187.2 ± 12.2 nM/mg protein, $n = 7$, $P < 0.001$). The effects were dose-dependent. The mRNA expression of *COX1* is not affected and *COX-2* is decreased in CsA-treated TAL cells. NO donor could prevent the inhibitory effects of CsA. We concluded that CsA decreased intrarenal PGE2 production in stimulated status mainly by decreasing COX-2 expression. NO might play a role in the CsA effect. The results suggested the role possible of PGE2 in CsA nephrotoxicity.

Introduction

Prostaglandins (PGs) are important mediators of human physiology and disease. The PGs are derived from the metabolism of arachidonic acid by cyclooxygenase (COX) [1]. The renal PGs have important local functions but only little systemic activity, as they are rapidly metabolized in the renal circulation [1]. The renal PGs are involved in three general areas of renal function. They play roles in control of renin secretion, regulation of vascular tone and control of tubular transport function [2]. It is in settings of compromised renal status that PGs can exert these functions to maintain renal blood flow and glomerular filtration rate [3].

Different cell types along nephron can synthesize PGE2 [4]. Glomerular cells including epithelial, endothelial or mesangial cell are capable of generating PGE2 as well as PGF2 and PGI2 [5]. Renal tubule cells, particularly those of renal medulla, are important sites of renal PGs synthesis and PGE2 is the major prostanoid synthesized [4].

COX enzymes, both in human and animals, can be separated into two isoforms, COX-1 and COX-2. COX-1 is a constitutively present in renal vascular, glomerular cells and along most segments of tubules, although with

different concentration [4]. Basal levels of COX-2 are present in the macula densa, thick ascending limbs (TAL) and papillary interstitial cells under normal condition. COX-2 expression is markedly increased in volume-depleted rats and dogs [6]. The subsequent increase of PGE2 mediates the local renal vascular dilatation and prevents the ischemic renal injury [5].

Cyclosporine (CsA) is an immunosuppressive agent widely used in kidney transplantation and glomerulonephritis patients. CsA nephrotoxicity is not an uncommon complication of chronic CsA administration. Intrarenal vasoconstriction has been implicated as an important pathogenic mechanism of CsA nephrotoxicity. Renal medulla is vulnerable to renal ischemic and hypoxic injury induced by CsA. We have previously reported that CsA decreased NO synthesis in mouse medullary TAL cells [7]. CsA decreases renal medullary COX-2 expression as well as PGE2 production *in vivo* [8]. It is still unclear about the interaction between the NO and PGE2 production after the CsA. We hypothesized that CsA can decrease *COX-2* formation with the consequence of decrease in PGE2 formation via the inhibition of NO production in renal TAL cells, which are the main target of CsA tubular effect and play an important role in the regulation of intrarenal hemodynamics.

Material and methods

Culture media (DMEM, HAM's F12) were obtained from Gibco BRL Laboratory (Life Technologies, Taiwan), CsA was from Novatis (Basle, Switzerland); Indomethacin, a nonspecfic *COX* inhibitor [6], and MK966, a specific *COX-2* inhibitor [6], were from MSD (Merck/MSD, Whitehouse Station, NJ, USA). S-nitroso-*N*-acetylpenicillamine (SNAP), a nitric oxide (NO) donor [7], was from RBI (Natick, MA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for PGE2 were from Amersham (Piscataway, NJ, USA).

Cell culture

The TAL segments were isolated from the renal medulla of 1-month-old male B57/6J mice fed a standard diet by microdissection technique and cultured in DMEM:HAM's F12 medium as described [7]. The primary cultures reached confluence after 4 weeks. Cell growth accelerated after the first passage and medium was changed every 4 days. After three passages, cells were routinely subcultured and the medium was changed every 3 days. A line of mouse TAL cultured cells could thus be subcultured for a long time (over 25 passages). All experiments were performed between the 6th and 15th passages on sets of confluent cells grown on Petri dishes.

RNA isolation and RT-PCR

The TAL cells cultured to confluence with DMEM/F12 medium in six well dishes were incubated with or without lipopolysaccharide (LPS) 100 ng/ml for 24 h. Various concentration of CsA was then added to the medium of confluence TAL cultured cells with or without LPS priming for another 24 h. RNAs were extracted from the confluent cells and RNA (100 μg) were reversely transcribed with avian myeloblastosis virus reverse transcriptases (RT AMV; Boehringer Mannheim, Mannheim, Germany) at 42 °C for 60 min. A 150 ng cDNA and non-reverse-transcribed RNA were amplified for 30–42 cycles in 100 μl total volume containing 50 mM KCl, 20 mM Tris-HCl pH 8.4, 10 mM dNTP, 1.5 mM $MgCl_2$, 1 unit Taq polymerase and 10 pM of COX-1 or COX-2 primers. The two primers for COX-, COX-2 and β-actin were as described [1,9]. The amplification cycles were 27 for COX-1 and 32 for COX-2 and β-actin. The thermal cycling program was as follows: 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 3 min. Amplication products were run on 4% agarose gel with ethidium bromide and photographed.

Western blotting for COX-2 protein

The TAL cells cultured in condition as that of RT-PCR were homogenized with lysis buffer (1% Nonidet P40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate in PBS buffer) and centrifuged at $600 \times g$ for 30 min at 4 °C to removed debris. Equal amount (50 μg) of protein was loaded in each lane, separated on a 7.5% SDS-PAGE, and transferred onto a polyvinyldifluoride membrane. Goat anti-mouse COX-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:1000) as primary antibody and rabbit anti-goat as secondary antibody (1:10000) were used for immunoblotting. ECL plus system (Amersham) was used as blot detecting agents.

Quantitation of PCR products: competitive PCR assay

Competitive RT-PCR was performed for the measurement of *COX-2* and β-*actin* mRNA. The test template for all PCR reaction was an aliquot of cDNA collected from TAL cultured cells. To quantitate the tested cDNAs, various amounts of mutant cDNA templates were added to compete with test cDNA on an equimolar basis, as previously described [10]. For *COX-2* and β-*actin*, deletion cDNA mutant templates were developed to create 89 and 104 bp deletions in the middle of the molecules, resulting in mutant cDNAs of 322 and 357 bp respectively. The two primers sequence of mutant *COX-2* competitor were as follows: sense strand 5′-TTACAGCTCAGTTGAACG

CC-3′ and antisense strand 5′-GGAGAGAAGGAAATGGC TGC-3′. Following 4% agarose gel electrophoresis, amplification bands stained by ethidium bromide were quantitated from the film negative by scanning densitometry. The ratio of mutant to wild type band density was calculated for each lane and plotted as a function of the amount of initial mutant template added to the reaction. The amount of cDNA was derived from linear regression analysis with duplicate or triplicate assays. The mean values for assays were expressed as a percentage change to the control.

Enzyme-linked immunosorbent assay

Concentrations of PGE2 were determined by using competitive binding ELISA kits (Amersham). Cells were incubated in the same condition as that of RT-PCR experiments and supernatants were collected for PGE2 assay following kits manual. Cellular proteins were extracted after cell homogenization and lysis. Protein concentrations were measured with Bradford method. PGE2 production was calibrated with cellular protein.

Cell viability

To examine the potential nonspecific cytotoxicity of CsA on cultured TAL cells, the cell viability was determined by the MTT [(3–4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye assay. Cells were seeded and grown for 5 days on 96-well trays (Corning, New York, NY, USA) and then incubated for the next 24 h in 100 μl of defined medium, with or without increasing concentrations of CsA (5–600 ng/ml). The cell viability found for each concentration of CsA tested was compared with that of the untreated cells, and the results were expressed as a percentage of viable cells. All measurements were performed in duplicate.

Statistical analysis

All the numeric results were represented as mean ± SEM. Student's *t*-test or one-way ANOVA were used to determine whether there was a significant difference between different groups ($P < 0.05$ or less). StatView software (SAS Institute, Cary, NC, USA) was used as a statistical tool in this study.

Results

COX expression in TALs and TAL cultured cells

COX-1 and *COX-2* mRNAs were found in TAL segments, microdissected from B57/6J mice, and subcultured TAL cells in RT-PCR experiments (Fig. 1). The presence of both isoforms of the *COX* suggested the TAL cultured cell was an appropriate model to test the effects of the CsA on the *COX* expression *in vitro*.

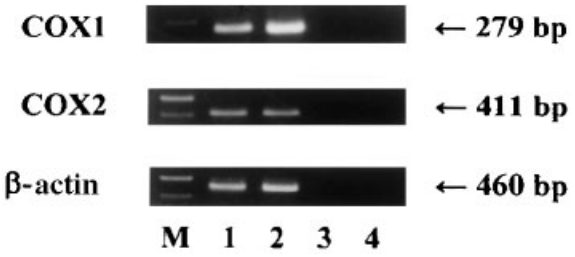

Figure 1 *COX-1* and *COX-2* mRNA expression. Ethidium bromide stained gel of *COX-1*, *COX-2* and β-*actin* (used as control) showed amplified products of the expected size (*COX-1*: 279 bp, *COX-2*: 411 bp, β-*actin*: 460 bp long) obtained by RT-PCR from microdissected TALs (lane 1) and TAL cultured cells (lane 2). No band was detected when nonreverse-transcribed RNA from cultured cells was used (lane 3) or when the cDNA was omitted (lane 4). Molecular weight standards (M) were the 1 kb ladder from Gibco-BRL.

PGE2 production in TAL cultured cells

To determine the best timing of the PGE2 production in LPS-stimulated TAL cells, we stimulated confluent TAL cultured cells with LPS 100 ng/ml and measured the PGE2 production of TAL cells for 1, 2, 6, 12 or 48 h. The PGE2 production in TAL cells was significantly increased after 6 h of LPS *incubation* (−LPS versus +LPS: 101.9 ± 12.8 vs. 179.2 ± 14.7 nmol/mg protein, $n = 4$, $P < 0.001$). The enhancement reached its maximal effect after 24 h of incubation (−LPS versus +LPS: 225.2 ± 21.4 vs. 386.2 ± 27.8 nmol/mg protein, $n = 4$, $P < 0.001$) (Fig. 2). Twenty-four hour incubation was then taken for the subsequent experiments to study the CsA or COX inhibitor effect on the LPS-stimulated TAL cells. Indomethacin (10^{-5} M), a nonspecific COX inhibitor, significantly decreased PGE2 production (control versus indomethacin: 242.7 ± 16.7 vs. 165.9 ± 11.9 nmol/mg protein, $n = 5$, $P < 0.001$), but MK966 (10^{-5} M), a specific COX-2 inhibitor, did not affect basal PGE2 production (control versus MK966: 242.7 ± 16.7 vs. 228.1 ± 12.7 nmol/mg protein, $n = 5$, NS) without the presence of LPS (100 ng/ml) (Fig. 3). LPS increased PGE2 production and the increase was inhibited by both indomethacin (10^{-5} M) and MK966 (10^{-5} M) (control versus indomethacin and MK966: 375.1 ± 15.5 vs. 200.2 ± 6.3 nmol/mg protein, $n = 5$, $P < 0.001$; control versus MK966: 203.9 ± 13.7 nM/mg protein, $n = 5$, $P < 0.001$) (Fig. 3). The results implicated that PGE2 formation was mainly through COX-1 activity in basal state, but LPS stimulated PGE2 formation was largely by COX-2 activity. The results paralleled the RT-PCR studies for *COX* isoforms expression. LPS enhanced *COX-2*, but not *COX-1* mRNA

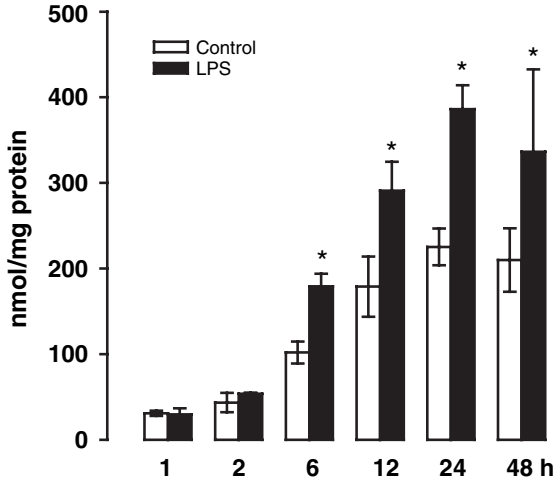

Figure 2 Time course of LPS effect on PGE2 production in TAL cultured cells. LPS (100 ng/ml) significantly increased PGE2 production after 6-h incubation (–LPS versus +LPS: 101.9 ± 12.8 vs. 179.2 ± 14.7 nmol/mg protein, $n = 4$, $P < 0.001$) and the maximal effect appeared 24 h (–LPS versus +LPS: 225.2 ± 21.4 vs. 386.2 ± 27.8 nmol/mg protein, $n = 4$, $P < 0.001$) after LPS incubation (*$P < 0.001$).

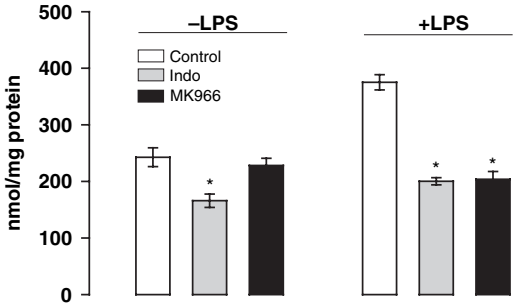

Figure 3 Effects of COX inhibitors on PGE2 production in TAL cultured cells. Indomethacin (Indo), nonspecific COX inhibitor, significantly decreased PGE2 production of TAL cultured cells without LPS stimulation. MK966, a specific COX-2 inhibitor, did not affect the PGE2 production under no LPS. In TAL cells treated with LPS, both Indomethacin and MK966 significantly decreased PGE2 production (*$P < 0.001$, $n = 5$, compared with control). These results indicated that PGE2 production in TAL cells cultured in basal condition was mainly by COX-1. LPS stimulated PGE2 production is mainly via COX-2.

expression (Fig. 4a). These results explicated that LPS increased PGE2 formation mainly via the increase in COX-2 activity.

CsA decreased COX-2 expression

Cyclosporine reduced *COX-2* expression in LPS stimulated TAL cultured cells, but not in basal state in RT-PCR studies. Taking –LPS/–CsA as control, LPS 100 ng/ml

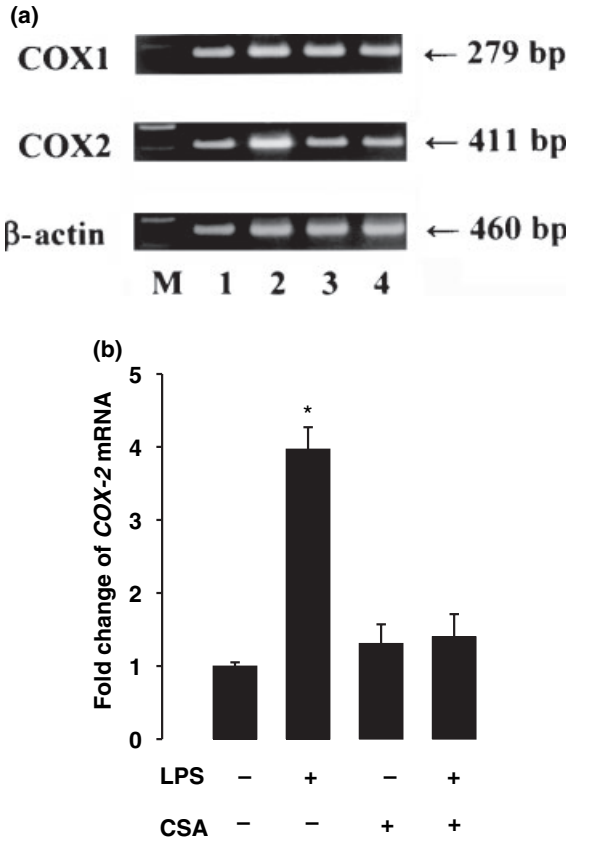

Figure 4 The effects of CsA on *COX-1* and *COX-2* expression in TAL cultured cells. (a) Ethidium bromide-stained 4% agarose gel of RT-PCR showing the amounts of amplified products of *COX-1*, and *COX-2* compared with that of β-*actin*, used as standard, in TAL cultured cells incubated without (lane 1), with 100 ng/ml LPS only (lane 2), with 100 ng/ml CsA only (lane 3) or with LPS plus CsA (lane 4). Molecular weight standards (M) were the 1 kb ladder from Gibco-BRL. (b) The lower panel showed the change of *COX-2* mRNA after scanning densitometry analysis. Taking –LPS/–CsA as control, LPS 100 ng/ml (+LPS/–CsA) alone increased *COX-2* mRNA by 3.97 ± 0.30-fold (control versus +LPS/–CsA: 1.00 ± 0.05 vs. 3.97 ± 0.30) (*$P < 0.001$, $n = 4$). LPS-induced *COX-2* mRNA can be blocked by CsA (+LPS/–CsA versus –LPS/+CsA: 3.97 ± 0.30 vs. 1.40 ± 0.36) (*$P < 0.001$, $n = 4$).

(+LPS/–CsA) alone increased *COX-2* mRNA by 3.97 ± 0.30-fold (control versus +LPS/–CsA: 1.00 ± 0.05 vs. 3.97 ± 0.30) (*$P < 0.001$, $n = 4$). LPS induced *COX-2* mRNA can be blocked by CsA (+LPS/–CsA versus –LPS/+CsA: 3.97 ± 0.30 vs. 1.40 ± 0.36) (*$P < 0.001$, $n = 4$) (Fig. 4b). *COX-1* expression was not affected by CsA in neither basal nor LPS stimulated condition (Fig. 4a). Similar results could be seen in competitive RT-PCR studies, LPS increased *COX-2* expression by around eightfold (7.92 ± 1.92, $n = 4$, $P < 0.001$) (Fig. 5a and b). The stimulated *COX-2* expression was inhibited by CsA in TAL

cultured cells (Fig. 5a and b). Both the results of the RT-PCR and competitive RT-PCR studies suggested that CsA might decrease *COX-2* expression in LPS-stimulated status in our TAL cultured cells model. To further confirm this conclusion, we did Western blotting for cells cultured in similar condition as in that of RT-PCR. LPS can enhance COX-2 protein expression and CsA will reverse this enhancement ($n = 3$, $P < 0.05$) (Fig. 5c).

CsA effect on PGE2 production

Parallel to the *COX-2* RNA expression change, LPS induced a significant increase in PGE2 production (Fig. 6a). CsA (100 ng/ml) significantly reduced PGE2 production up to 50.1% in LPS stimulated TAL cells (−CsA versus +CsA: 375.1 ± 15.5 vs. 187.2 ± 12.3 nmol/mg protein, $n = 7$, $P < 0.001$). CsA did not alter the basal PGE2 production (−CsA versus +CsA: 219.3 ± 13.4 vs. 256.9 ± 18.2 nmol/mg protein, $n = 7$, NS) (Fig. 6a). The effect of CsA in significantly preventing LPS induced PGE2 increase was dose-dependent from 10 ng/ml up to the concentration of 600 ng/ml ($n = 5$, $P < 0.001$) (Fig. 6b).

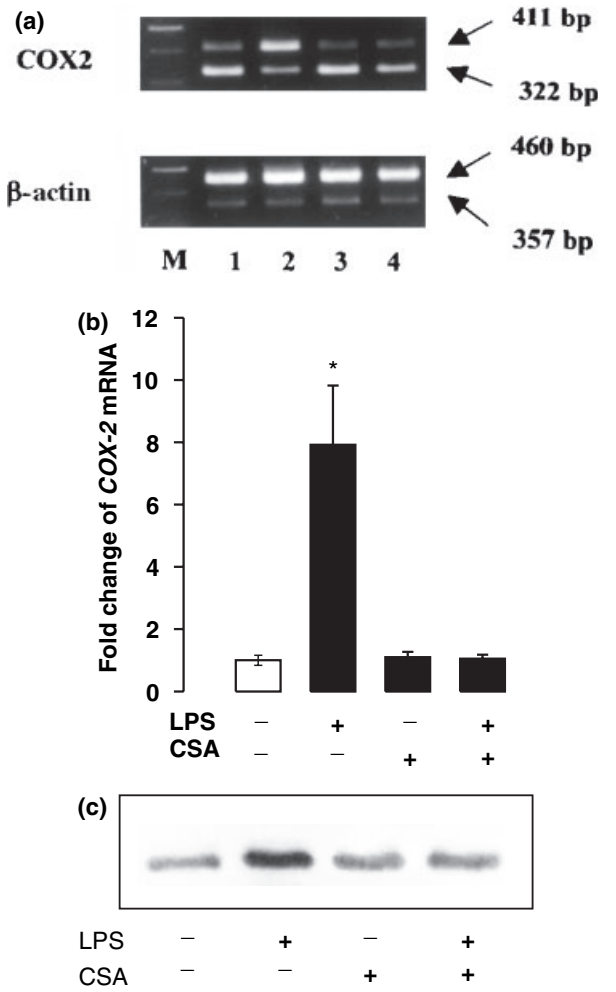

Figure 5 (a) Representative RT-competitive PCR gel and the results of *COX-2* mRNA by CsA (M represented molecular weight standards and the other numbers represented the same manipulation as in Fig. 4). (b) Bar chart showed that the LPS (100 ng/ml) induced an eighfold (7.92 ± 1.92) (*$P < 0.001$, $n = 4$) increase of *COX-2* expression. CsA prevented the stimulatory effect of LPS. (c) Representative Western blotting of COX-2 protein showed that LPS can enhance COX-2 protein expression and this enhancement can be blocked by CsA.

Nitric oxide reversed the CsA-related PGE2 suppression

Nitric oxide plays a role in the regulation of PGE2 production by regulating COX-2 activity [2]. It is interesting

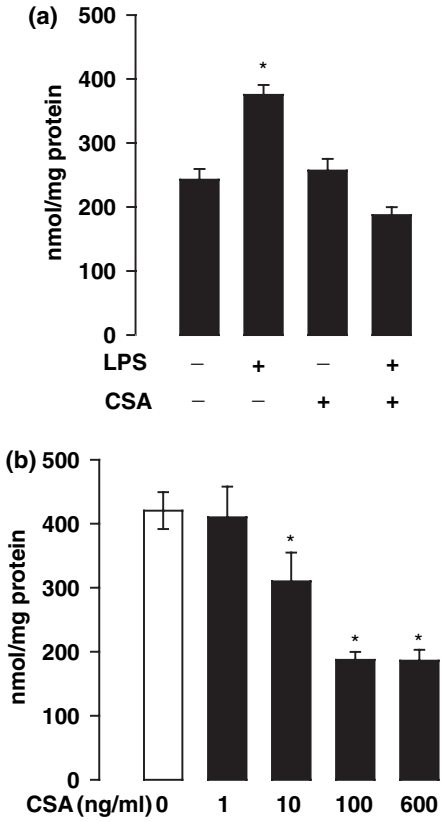

Figure 6 (a) CsA decreased PGE2 production in TAL cultured cells. (a) LPS significantly enhanced PGE2 production in TAL cultured cells (+LPS/−CsA) and the stimulatory effect could be decreased by CsA (+LPS/+CsA). Cells incubated with CsA but without LPS (−LPS/+CsA) did not change PGE2 production when compared with cells in basal condition (−LPS/−CsA) (*$P < 0.001$, $n = 5$, compared with basal condition). (b) The decrease of PGE2 production in LPS stimulated cells by CsA was dose dependent. White bar represented PGE2 production in basal status (control) and black bars stand for cells incubated with LPS and different concentration of CsA (*$P < 0.001$, $n = 5$, compared with control).

to know if CsA associated PGE2 decrease is associated with NO. We co-incubated LPS stimulated TAL cultured cells with CsA and SNAP (10^{-5} M) (an NO donor) [7]. PGE2 production was enhanced in SNAP-treated TAL cells with or without LPS (control versus +SNAP/−LPS: 211.7 ± 77.1 vs. 774.2 ± 98.4 nmol/mg protein, $n = 4$, $P < 0.001$) (control versus +SNAP/+LPS: 211.7 ± 77.1 vs. 1021.4 ± 103.7 nmol/mg protein, $n = 4$, $P < 0.001$) (Fig. 7). CsA suppressed PGE2 production in LPS stimulated TAL cultured cells was reversed by SNAP (−CsA/−SNAP versus +CsA/−SNAP: 420.5 ± 28.8 vs. 187.4 ± 12.3 nmol/mg protein, $n = 4$, $P < 0.001$) (−CsA/−SNAP versus +CsA/+SNAP: 420.5 ± 28.8 vs. 475.3 ± 18.7 nmol/mg protein, $n = 4$, NS). PGE2 production in +CsA/+LPS/+SNAP cells was 678.8 ± 48.6 nmol/mg protein (Fig. 7). CsA also decreased the NO production in LPS-stimulated TAL cells. CsA (6 h, 100 ng/ml) reduced NO production by 33.2% (control versus CsA: 472.2 ± 5.8 vs. 315.4 ± 23.6 nmol/10^6 cells, $P < 0.01$, $n = 5$). The 2results indicated that NO might play an important role in the CsA-mediated PGE2 decrease in TAL cultured cells.

Effects of cyclosporine and LPS on cell viability

To ensure that the change induced by CsA or LPS were not caused by cell damage, cell viability by MTT test used as an index of cell injury, was measured in TAL cultured cells. The percentages of viable cells remained unchanged after treatment of CsA and LPS. The observed decrease in *COX-2* expression or PGE2 production by CsA in LPS stimulated TAL cultured cell was not the result of cell damage.

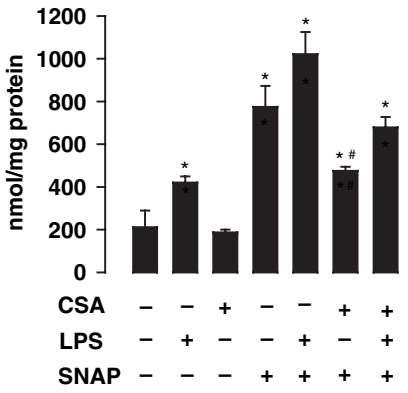

Figure 7 Nitric oxide reversed CsA effect on TAL cultured cells. CsA significantly decreased PGE2 activity in cells cultured with LPS. SNAP, a nitric oxide donor, prevented the CsA effect on PGE2 production [+CsA/−LPS/−SNAP versus +CsA/−LPS/+SNAP 187.4 ± 12.3 nmol/mg protein vs. 475.3 ± 18.8 nmol/mg protein (#$P < 0.001$, $n = 4$, compared with +CsA/−LPS/−SNAP)]. These results implicated that CsA-associated PGE2 suppression was related to nitric oxide production compared with control (−CsA/−LPS/−SNAP) (*$P < 0.001$, $n = 4$, compared with control).

Discussion

Prostaglandin E2 plays an important role in the regulation of intrarenal hemodynamics [2]. Renal PGE2 is primarily a vasodilator. The production of the renal PGE2 is mediated by COX enzymes [11]. In our experiments, indomethacin, a nonspecific COX inhibitor, inhibited PGE2 production, but MK966, a specific COX-2 inhibitor did not (Fig. 3). The results suggested that PGE2 production in TAL cultured cells was largely via COX-1 activity in basal status. On the contrary, LPS-induced PGE2 production was mainly through COX-2 activity, as both the indomethacin and MK966 significantly prevented the LPS-induced change (Fig. 3). The results kept with the notion that COX-1 is a housekeeping enzyme and COX-2 is induced by various inflammatory or infectious stimulations [12]. LPS, acting as a mediator of inflammation, up-regulated *COX-2* expression in many cell types, including macrophage, microglia or alveolar epithelial cells [13]. LPS activates tyrosine kinase, which then activates protein kinase C (PKC). Activated PKC subsequently activates p44/42 or p38 mitogen-activated protein kinase pathways. The subsequent downstream activation of nuclear factor-kappa B (NF-κB) specific DNA-binding protein formation increases the *COX-2* expression and PGE2 production [14]. Reduced renal PGE2 production is harmful to renal hemodynamics [2]. CsA decreased the expression of *COX-2* expression in TAL cells under LPS stimulation. The reduced *COX-2* expression led to the diminishing of PGE2 production in TAL cells. The decrease of *COX-2* expression and PGE2 formation cannot be seen in TAL cultured cells without LPS stimulation. These results implicated that CsA inhibited PGE2 production only in the state of LPS stimulation, a situation commonly seen in infection or inflammation. However, the exact molecular mechanisms by which CsA decreased *COX-2* expression and PGE2 production were not completely understood.

Both the PGE2 and NO are known to play an important role in the regulation of intrarenal hemodynamics [2]. Recent evidence has suggested that the generation of each may affect the other via a feedback loop [15]. The interaction between PGE2 and NO remained unclear. PGE2 might increase NO formation in renal TAL cultured cell and rat gastric mucosa [15]. On the contrary, increasing NO production stimulated PGE2 synthesis in pig ileal mucosa cells [16]. It is likely that the interaction between NO and PGE2 depends on different cell types and stimulation. We found that NO

up-regulated PGE2 production in TAL cultured cells (Fig. 7). COX-2 promoter contained binding site for nuclear factor of activated T cells (NFAT). Activation of NFAT can stimulate COX-2 expression [17]. NO may involve in NFAT activation in mouse [18]. Our study also suggested NO could regulate COX-2 production in TAL cells. Increasing NO by SNAP prevented the CsA related PGE2 inhibition in TAL cultured cells. It is likely that the decrease of the PGE2 production is secondary to the decrease of the NO change from our experiments. The NO alteration appeared 1 h after the incubation of CsA and reached plateau 6 h after incubation [9]. The alteration of PGE2 production started 6 h after the incubation and reached the maximal effect at 24 h. The time lag suggested that NO production alteration might proceed of PGE2 change. In addition, NO donor, SNAP, reversed the CsA effect on the PGE2 production. The results indirectly indicated that CsA altered the PGE2 production through the NO change. Our experiments suggested that CsA might affect COX-2 activity directly or indirectly via NO decrease.

Prolonged CsA is associated with vasoconstriction and sodium retention. TAL is the one of the major sites of sodium reabsorption along the nephron [11]. Our previous studies also suggested the stimulating effect of CsA on Na^+–K^+–Cl^- co-transporter activity in TAL cells [9]. The dual effects of vasoconstriction and sodium retention might lead to renal ischemia and fluid retention in CsA nephrotoxicity.

Cyclosporine is a common immunosuppressive agent widely used in renal transplant and glomerulonephritis patients. Our study suggested that CsA could decrease important PGE2 production in stimulated renal epithelial cells in culture. It is still uncertain if the PGE2 production decreases significantly in patients taking CsA with occult or obvious inflammation. Further clinical investigation is necessary to clarify the effect of CsA in renal PG production. In conclusion, CsA decreased PGE2 production via suppressing *COX-2* expression in a model of TAL cultured cells. The observed results might play an important role in the CsA renal effect.

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