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Original Article: Laboratory Investigation

Attenuation of ureteral obstruction-induced renal injury by polyenylphosphatidylcholine

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Background: The cytoprotective, antioxidant and antifibrotic effects of polyenylphosphatidylcholine (lecithin, PPC) have been demonstrated both experimentally and clinically. The present study investigated whether PPC treatment has any beneficial effect on renal injury in unilateral partial ureteral obstruction (UUO) in rats.

Methods: Forty Wistar–Albino rats were split into three groups (sham-operated controls, untreated and treated rats). Rats of the untreated and treated groups (n = 15) underwent UUO with two-thirds of the left ureter embedded in the psoas muscle. In group 3, PPC was given orally at a dose of 100 mg/day for 30 days. At the end of the 30th day of the experimental period, obstructed kidneys and blood samples were harvested. To investigate the therapeutic efficacy of PPC treatment in UUO kidneys, oxidant and antioxidant enzyme levels, lipid peroxidation, proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor alpha), transforming growth factor beta-1 (TGFβ-1), alpha smooth muscle actin (α-SMA) and nuclear factor kappa beta (NF-κβ) expression, leukocyte infiltration (ED1, ED2, CD4 and CD8 immunohistochemistry), and tubulointerstitial damage in the obstructed kidneys were studied.

Results: Oxidative stress, neutrophil infiltration, release of cytotoxic mediators, TGFβ-1 levels, tubulointerstitial damage, alpha-SMA and NF-KB expressions in kidney tissue were significantly increased in the UUO rats. PPC treatment attenuated oxidative stress, leukocyte infiltration, cytotoxic mediator, and TGFβ-1 levels and also decreased expressions of α-SMA and NF-κβ. It was associated with decreased tubulointerstitial damage, compared with UUO alone.

Conclusions: These results indicate that PPC treatment protects against UUO-induced renal injury in rats possibly through its antioxidant, anti-inflammatory and antifibrotic actions.

Key words: alpha smooth muscle actin, nuclear factor kappa beta, polyenylphosphatidylcholine, transforming growth factor-beta1, unilateral ureteral obstruction.

Introduction

Obstructive uropathy represents an important cause of end-stage renal disease in infants and children. Interstitial fibrosis is one of the major characteristics in obstructive nephropathy and correlates with a loss of renal function.[1,2] Hallmarks of the development of fibrosis are inflammatory processes leading to a strong elevation of multiple chemoattractants, cytokines and growth factors.[3–5] Free radical release is a recently recognized mechanism in the pathogenesis of the rat and mice unilateral ureteral obstruction (UUO) model.[6–8] It has been shown that oxidative stress in UUO contributes to the development of glomerular and tubulointerstitial lesions.

The polyenylphosphatidylcholine (PPC) study medication contained a soybean lecithin extract, 'essential' phospholipids (EPL), consisting of 72–76% phosphatidylcholine of which the two major species are dilinoleoylphosphatidylcholine (DLPC; 32–42%) and palmitoyllinoleoylphosphatidylcholine (18–19%).[9] It has been previously shown that PPC exhibits remarkable antioxidant, cytoprotective, antifibrotic and anti-inflammatory properties in different pathological events in both experimental and clinical settings.[10–14] Therefore, this study investigated the effect of PPC treatment on ureteral obstruction-induced renal injury using an *in vitro* model of partial ureteral obstruction in rats.

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Materials and methods

Experimental protocol

Female Wistar–Albino rats, weighing 180–210 g, were used in this study. Incomplete ureteral obstruction was performed with two-thirds of the left ureter embedded in the psoas muscle through a midline abdominal incision as described previously (group 2 and 3 rats, $n = 15$).[15] Sham-operated rats had their ureters manipulated but not embedded; kidneys from these animals served as controls (group 1, $n = 10$). Rats were killed on the 30th day of UUO. Having collected blood by cardiac puncture, blood samples were stored at −70°C until assayed. Kidney samples were quickly removed and divided transversally into discoid parts. One part was washed in cold saline and stored at −70°C and the other fixed in 10% phosphate-buffered formalin for histopathological examination. All tissue homogenates were prepared on the same day for the estimation of biochemical markers. Polyenylphosphatidylcholine was given orally in group 3 rats at a dose of 100 mg/day for 30 days during the experiment.

Estimation of oxidative stress (total nitrate + nitrite), lipid peroxidation (malondialdehyde) and antioxidant enzyme activities

The rat tissue were homogenized in 1.15% KCl buffer (1:9, w/v) using a manual glass homogenizer (Tempest Virtishear, Model 278069; The Virtis Company, Gardiner, NY, USA) for approximately 5 min. The supernatant was used for analysis of the malondialdehyde (MDA) content of homogenates and was determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances.[16] Three millimeters of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution were added to 0.5 mL of plasma pipetted into a tube. The mixture was heated in boiling water for 45 min. After cooling, the color was extracted into 4 mL of n-butanol The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, Cambridge, UK) with 532 nm. The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and given as nmol/g tissue.

Tris hydrochloride (Tris-HCl; pH 7.4) was used for homogenization of tissue nitrite (NO_2^-) and nitrate (NO_3^-) levels and glutathione (GSH) activity. First, a supernatant phase was used for nitration plus nitrate determination as a NO product. This product can be used to estimate NO production. Quantitation of NO_2 and NO_3 was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO_2 with a mixture of naphthylethylenediamine and sulfanilamide.[17] Results are expressed as umol/g wet tissue. GSH was determined by the spectrophotometric method which was based on the use of Elman's reagent.[18] Results are expressed as nmol/mg tissue. Total (Cu–Zn and Mn) superoxide dismutase (SOD) activity was determined according to the method of Sun *et al*.[19]

Estimation of cytokines and TGFβ-1

Tissue homogenates were prepared using a homogenizer IKA Ultra-Turnax (2 cycles of 45 sec at 0°C) in 0.5 mol Tris/1.5 mol NaCl/ 50 mmol CaCl2/2 mmol sodium azide buffer at pH 7. The homogenates were then centrifuged at 10.000 *g* for 15 min at 4°C and the supernatants were used for enzyme-linked immunosorbent assay (ELISA). Rat interleukin (IL)-1α, rat IL-6 (rIL-6) and rat tumor necrosis factor-alpha (rTNF-α) and rat transforming growth factor-beta 1 (rTGF-β1) (BioSource Immunoassay kit, Camarillo, CA, USA) levels were measured using a sandwich ELISA protocol supplied by the manufacturer of the antibodies and resultant optical density determined using a microplate reader at 450 nm. Results were expressed as pg or ng/gram tissue.

Histopathological examination

The sections were fixed in 10% phosphate-buffered formaldehyde and stained with hematoxylin–eosin and Masson's trichrome. For the evaluation of leukocyte infiltration, the widening of interstitial space with focal leukocyte infiltration was assessed from each histology sample. We collected five non-overlapping microscopic fields (×400) and the number of leukocytes per 0.28 mm^2 was calculated from these.

To estimate the grade of interstitial fibrosis, the green-stained interstitial area with Masson-trichrome was evaluated as a percentage of area of the total examined area, using the image analyzer (Leica, Leica Micros Imaging Solutions, Cambridge, UK). Using 3–5 high-power fields (HPF; ×400) in each slide, the widening of interstitial space with focal leukocyte infiltration and interstitial fibrosis were assessed to quantitate the results.

Descriptive statistics are given as mean ± standard error of the mean (SEM). Analysis of variance (one-way ANOVA) was done with Bonferroni's *t*-test. $P < 0.05$ was considered significant.

Immunohistochemistry for ED1, ED2, CD4 and CD8

Paraffin block sections (5μ) were placed on poly-L-lysine-coated slides. After drying in an oven for 1 h at 60°C, sections were dewaxed in xylene for 20 min and dehydrated in 96% alcohol for 10 min. Then, sections were incubated for 7 min in 3% hydrogen peroxide to block endogenous peroxidase. After washing with phosphate-buffered saline (PBS), each section was covered with two drops of trypsin and incubated at 37°C for 13 min. After washing in PBS, the sections were incubated for 5 min at ultra V block. This was followed by incubation with primary monoclonal antibodies (Chemicon, Temecula, CA, USA) for identification of $CD4^+$ cells (W3/25) and $CD8^+$ cells (OX-8), and for identification of rat ED1 and ED2 (resident macrophage) cells (Serotec, Oxford , UK) specific for a monocyte/macrophage cytoplasmatic antigens for 80 min. A second layer of biotinylated goat antimouse IgG (Chemicon and Serotec) was used as the second antibody for 15 min. After the sections were washed with PBS three times, they were placed with the third layer of mouse streptavidin labeled with peroxidase for 15 min. Finally, the preparations were developed in AEC chromogen, counterstained in Mayer's hematoxylin and mounted. Immunohistochemical staining for ED1, ED2, CD4 and CD8 were assessed quantitatively with a Leica image analyzer (Leica Micros Imaging Solutions, Cambridge, UK) using specialized computer software (Leica Q Win), a color video camera (Leica DFC 280) and a Compaq computer. Because it was difficult to count ED1-, ED2-, CD4- and CD8-positive cells, the positive areas stained with the antibodies were evaluated as a percentage of the total examined area. These data can be read directly on the display of the analyzer and are reported in Figure 5. Descriptive statistics are given as mean ± SEM. Analysis of variance (one-way ANOVA) was done with Bonferroni's *t*-test. $P < 0.05$ was considered significant.

Immunohistochemistry for alpha smooth muscle actin and nuclear factor kappa beta

Formalin-fixed sections were used for alpha smooth muscle actin (α-SMA) and nuclear factor kappa beta (NF-κβ) immunohistochemistry. The antibodies used were mouse monoclonal antiserum to α-SMA (Labvision, Fremont, CA, USA), at 1:150; against the actin, smooth muscle Ab-1; and rabbit polyclonal antiserum to NF-κβ (Labvision), against the NF-κ B/p65 (Rel A) Ab-1.

Cells positive for α-SMA in the renal cortex and medulla were semiquantitated in 20 randomly chosen HPF (×400) as follows: (0) no immunostaining detectable; (1) trace immunostaining; (2) spot-like immunostaining (usually at strongly fibrotic areas); and (3) strong diffuse immunostaining. In each section of kidney, the intensity of immunostaining for NF-κβ was scored separately by two observer as: (0) no specific staining (no cytoplasmic and nuclear staining); (1) weakly positive (mild cytoplasmic staining and no nuclear staining); (2) moderately positive (moderate cytoplasmic staining and present or absent nuclear staining); and (3) strongly positive (severe cytoplasmic and nuclear staining).

Statistical analyses

All analyses (except for histological analyses and immunohistochemistry results) were made using the SPSS statistical software package 13.0 for Windows (SPSS, Chicago, IL, USA). The results were

Table 1 Effect of polyenylphosphatidylcholine (PPC) treatment on oxidant and antioxidant enzyme levels, and lipid peroxidation in ureteral obstruction (UUO)

Groups	Oxidant levels	Antioxidant enzyme activities		Lipid peroxidation
	Total $NO_2 + NO_3$ (μmol/L)	SOD (U/g protein)	GSH (mmol/g wet tissue)	MDA (mmol/mg wet ttissue)
Control (group 1)	316.25 ± 27.08	183.63 ± 30.23	8.43 ± 1.64	102.68 ± 14.28
UUO (Group 2)	548.36 ± 72.43*	69.14 ± 16.87*	3.13 ± 0.98*	309.89 ± 39.02*
UUO + PPC (group 3)	222.7 ± 46.49**	245.96 ± 22.1**	5.44 ± 0.77**	135.80 ± 41.6**

Values are given as mean ± SEM for rats. *Significant difference when compared with group 1 ($P < 0.001$). **Significant difference when compared with group 2 ($P < 0.001$). GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 2 *In vitro* measurements of renal cytokines and transforming growth factor (TGF)-β1 in control, unilaterally obstructed and unilaterally obstructed + PPC treated rats

Groups	IL-1 (pg/g wet tissue)	IL-6 (pg/g wet tissue)	TNF-α (pg/g wet tissue)	TGF-β1 (ng/g wet tissue)
Control (group 1)	1325.95 ± 150.7	18 569.61 ± 1292.84	1 6741.8 ± 1363.4	112.3 ± 26.7
UUO (group 2)	2664.46 ± 585.9*	2 6253.1 ± 3365.2*	27 090.17 ± 1757.42*	190.26 ± 17.59*
UUO + PPC (group 3)	1475.84 ± 307.85*	20 959.45 ± 838.2*	18 547.09 ± 1145.48*	146.58 ± 23.4*

Values are given as mean ± SEM for rats. *Significant difference when compared with group 1 ($P < 0.01$). IL, interleukin; TNF-α, tumor necrosis factor alpha.

Table 3 Effect of PPC treatment on leukocyte infiltration, fibrosis, smooth muscle actin (SMA) and nuclear factor kappa beta (NF-κβ) expression in UUO

Groups	Leukocyte infiltration	Fibrosis	SMA	NF-κβ
Control (group 1)	2.32 ± 0.44	4.37 ± 0.34	–	0.30 ± 0.48
UUO (group 2)	75.01 ± 3.85*	11.78 ± 3.8*	1.86 ± 0.35*	2.26 ± 0.45*
UUO + PPC (group 3)	40.09 ± 1.84**	6.91 ± 3.23**	1.4 ± 0.5**	1.66 ± 0.5**

Values are given as mean ± SEM for rats. *Significant difference when compared with group 1 ($P < 0.0001$). **Significant difference when compared with group 1 ($P < 0.001$).

expressed as mean ± SEM. The Kruskal–Wallis test was used to compare the three groups. In two-group comparisons, the Mann–Whitney *U*-test was used. *P*-values below 0.01 were considered to be statistically significant.

Results

Effects of PPC on oxidative stress (total nitrate + nitrite) and lipid peroxidation (MDA)

Tissue total nitrate plus nitrite levels were increased in the UUO group compared with the other groups, but were significantly reduced by PPC treatment (Table 1). Polyenylphosphatidylcholine treatment reduced the level of lipid peroxidation in group 3 rats compared with the group 2 rats (Table 1).

Effects of PPC on cytokine and TGFβ-1 release in UUO

As shown in Table 2, UUO increased the levels of proinflammatory cytokines in group 2 rats compared with the sham. PPC treatment reduced the levels of proinflammatory cytokines and TGFβ-1 in the obstructed kidneys (Table 2).

Effects of PPC on leukocyte infiltration and interstitial fibrosis

After 30 days following UUO, severe leukocyte infiltration was observed in the periglomerular and peritubular interstitium of the obstructed kidneys in non-treated rats (Fig. 1A). Quantitative analysis of the focal leukocyte infiltration area in the interstitium showed that leukocyte infiltration was significantly reduced in the PPC-treated group (Fig. 1B, Table 3). Polyenylphosphatidylcholine at a dose of 100 mg/day reduced the development of interstitial fibrosis in UUO rats (Fig. 2A,B). The percentage area of interstitial fibrosis in UUO kidneys was significantly greater than that in PPC-treated kidneys (Table 3).

Effects of PPC treatment on immunohistology

An inflammatory reaction involving ED1+ (Fig. 3A) and ED2+ macrophages (Fig. 3C), and CD4+ macrophages (Fig. 4A) and T-helper

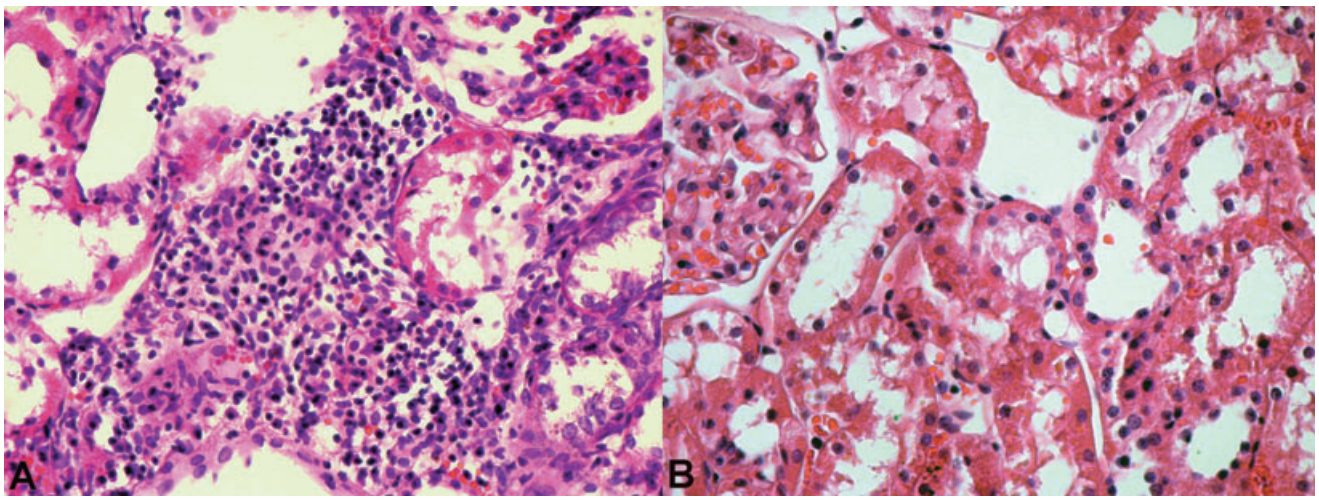

Fig. 1 (A) Severe leukocyte (mainly monocytes) infiltration was observed in the periglomerular and peritubular interstitium of the obstructed kidneys from non-treated rats. (B) Leukocyte infiltration was significantly reduced in the polyenylphosphatidylcholine (PPC)-treated group (HE stain, reduced from original magnification ×400).

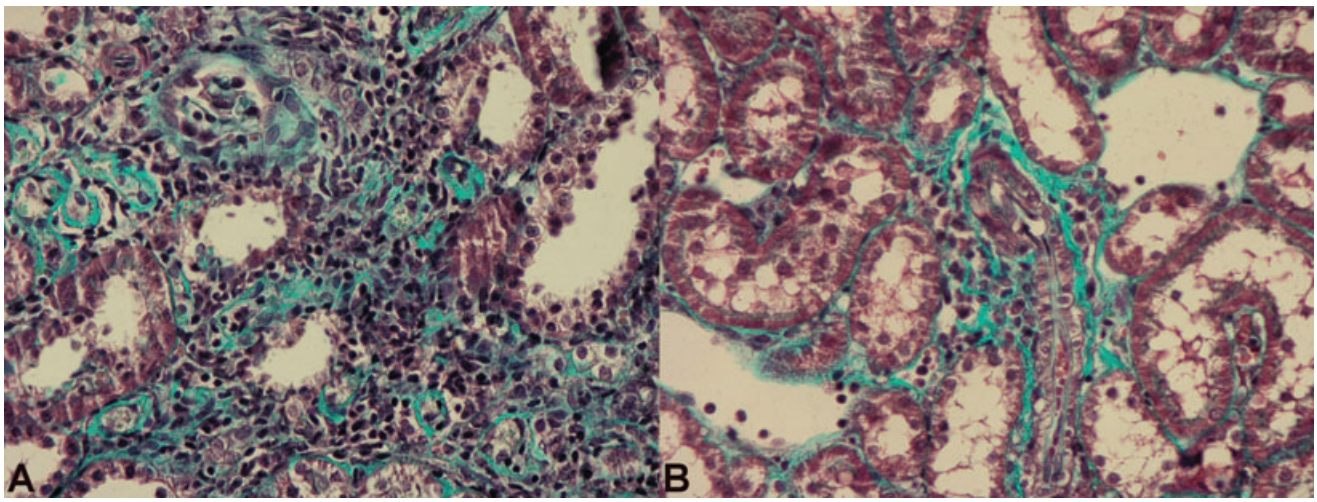

Fig. 2 (A) Correlated with the leukocyte infiltration, increased fibrosis was seen in non-treated rat kidneys from ureteral obstruction (UUO). (B) PPC treatment significantly reduced the development of fibrosis in UUO rat kidneys. (Masson-trichrome stain, reduced from original magnification ×400).

(Th) cells CD8(+) cytotoxic T cells (Fig. 4C) was seen to be obstructed by day 10 in both cortical and medullar areas of the kidney. Treatment with PPC caused a clear reduction in the interstitial monocyte/macrophage number (Figs 3B,D, 4B,D, and 5, $P < 0.05$).

Effects of PPC on SMA and NF-κβ expression in UUO kidneys

Induction of interstitial myofibroblast was assessed by immunohistochemical detection of α-SMA. Positive cells for α-SMA were seen in vascular smooth muscle cells, but not in the interstitial space in the sham-operated kidney (Fig. 6A). Trace or spot-like immunostaining of α-SMA was observed at the periglomerular and peritubular interstitium in addition to the vascular smooth muscle cells of the arterioles in untreated rat kidneys on the 30th day of UUO (Fig. 6B). Polyenylphosphatidylcholine treatment reduced the appearance of α-SMA-positive myofibroblasts in both cortex and medulla (Fig. 6C, Table 3).

Control kidneys had weak staining for NF-κβ that was mainly located in the glomerular endothelial and tubular epithelial cells (Fig. 7A). NF-κβ immunostaining of renal cells was intense (grades 2 and 3) and evenly distributed between both periglomerular and peritubular cells of untreated rats (Fig. 7B). Polyenylphosphatidylcholine treatment significantly suppressed this expression in all renal tissue in UUO rats (Fig. 7C, Table 3).

Discussion

Polyenylphosphatidylcholine is a strong antioxidant that protects several cells and/or tissues from oxidative stress. Although we know relatively little about the pharmacokinetics of PPC's antioxidant properties, it has been proven to be a useful therapeutic agent in different pathological events.[10,14] These studies ensure the ability of PPC to either scavenge or inhibit the production of reactive oxygen species (ROS). It was suggested that this incorporation of PPC increased the resistance of

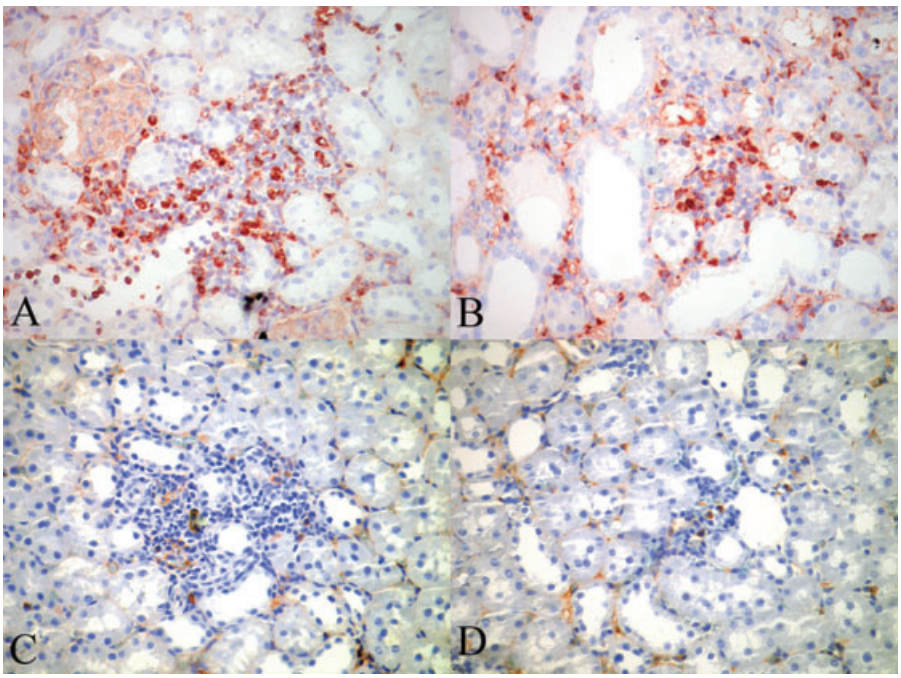

Fig. 3 Effect of PPC treatment on renal infiltration of monocyte/macrophage. (A) Dense ED-1-positive and (C) ED-2-positive cell infiltration is observed in the non-treated UUO rat kidneys. Decreased infiltration of (B) ED-1-positive and (D) ED-2-positive cells is seen in PPC-treated UUO rats.

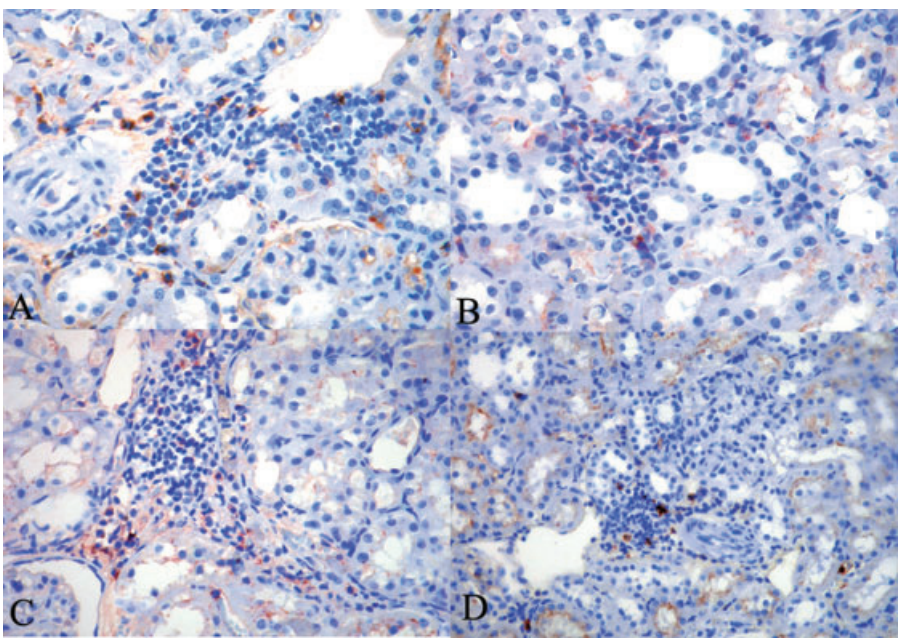

Fig. 4 Effect of PPC treatment on renal infiltration of lymphocyte. (A) Increased CD-4-positive and (C) CD-8-positive cells are seen in the non-treated group 2 rats. Decreased infiltration of (B) CD-4-positive and (D) CD-8-positive cells is seen in PPC-treated UUO rats.

low density lipoproteins against lipoperoxidation. It was also suggested that DLPC, the major and active component of PPC, could also provide a 'trap' for the free radicals.[20,21] Therefore, by scavenging free radicals and preserving cell integrity, PPC might be renoprotective against oxidative stress induced by UUO.

Previous studies have demonstrated that progressive renal injury in UUO is associated with cell infiltration of the glomerular and tubulointerstitial compartments.[22,23] Elimination of leukocyte infiltration in rats with ureteral obstruction resulted in a higher post-obstructive glomerular filtration rate and renal plasma flow compared with controls indicating that leukocyte infiltrate may play an important role in obstructive uropathy.[24] This present study demonstrates a significant increase in leukocyte (mainly monocytes and macrophages) infiltration and proinflammatory cytokine release 30 days after UUO. The grade of leukocyte infiltration and the release of cytotoxic mediators were significantly reduced when animals were treated with PPC. Because oxidants greatly activate leukocyte migration and infiltration into ischemic tissue, this anti-inflammatory effect of PPC in UUO is partly explained by the inhibitory effect on oxidative stress. Moreover, increased ROS concentrations are one of the most important mechanisms promoting activation of the NF-κβ. This expression initiates or enhances the expression of not only genes for inflammatory cytokines (IL-1, IL-8, TNF-α) and their receptors, but also other inflammatory mediators and adhesion molecules.[25,26] In our study, PPC treatment resulted in

significant suppression of NF-κβ expression in UUO. In an *in vivo* study, DLPC, the main and active component of PPC, has been demonstrated to diminish the TNF-α generation in Kupffer cells of ethanol-fed rats by inhibiting NF-κβ activation.[27] We think that this suppressive effect of PPC on UUO-induced leukocyte infiltration is a direct action (via inhibition of NF-κβ activation) and a reflection of the decreased level of oxidative stress.

Local production of TGFβ-1 either by intrinsic renal cells or by macrophages invading the kidney, has been implicated as a key mediator of renal fibrosis in ureteral obstruction.[27–30] It activates renal fibroblasts into myofibroblasts and stimulates the synthesis of extra cellular matrix proteins such as fibronectin, collagens and proteoglycans by these cells, and it is also chemotactic for monocytes and macrophages. In our study, PPC treatment reduced the level of TGFβ-1 and suppressed the degree of immunostaining of α-SMA in UUO kidneys. The decreased interstitial fibrosis along with a decreased oxidative stress marker and cell infiltration degree in the UUO kidneys strongly suggests the existence of a close relationship between them. We think that, because PPC treatment decreased the oxidative stress and reduced the leukocyte infiltration, and hence decreased TGFβ-1 levels and α-SMA, expressions were obtained in rats with UUO. Furthermore, it has been shown that PPC has a strong anti-fibrogenic effect in cultured hepatic stellate cells (HSC) and it decreased acetaldehyde-stimulated collagen accumulation in the culture media. The inhibition was attributed, in part, to the stimulation of collagenase activity by its active component DLPC, which constitutes 45–50% of the PPC extract. It has been also shown that DLPC prevents TGFβ-1-induced increase in collagen mRNA by inhibiting generation of oxidative stress and this effect has also been associated with the inhibition of p38 mitogen activated protein kinases (MAPK) activation in HSC.[31] All these actions exerted by PPC might play an important role in reducing the tubulointerstitial fibrosis in UUO, and need further investigation.

In summary, obstructive nephropathy is a major cause of end-stage renal disease, with many patients progressing unrelentingly to end-stage renal disease not related to early surgical correction. We made the novel observation that PPC treatment protects against the renal

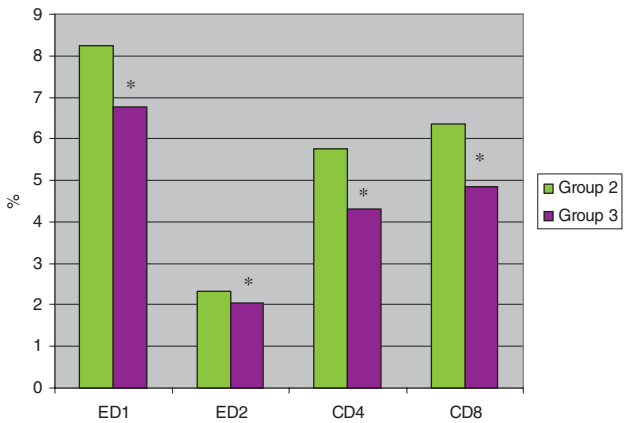

Fig. 5 Effects of PPC treatment on renal infiltration of monocyte/macrophages.

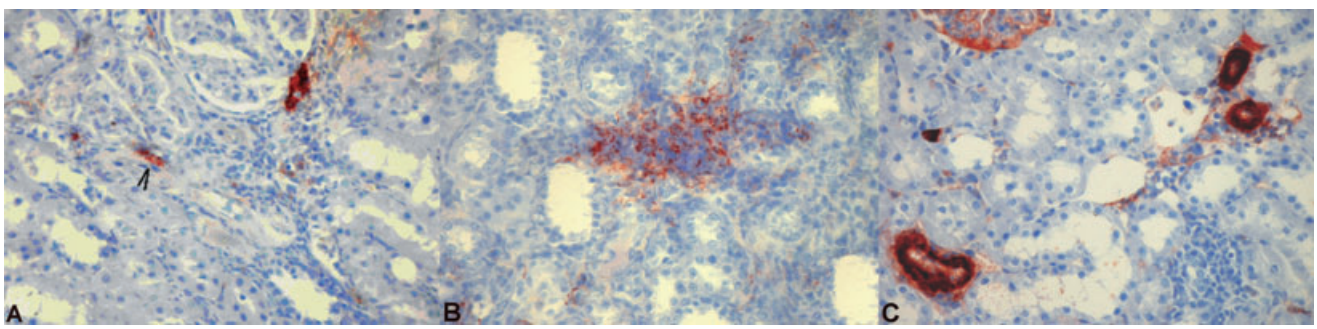

Fig. 6 Immunohistochemical findings for alpha smooth muscle actin (α-SMA). (A) Control. (B) In untreated rat kidneys with UUO, there is a marked increase (trace and spot-like) in α-SMA staining. (C) In PPC treated kidneys no or trace staining are seen.

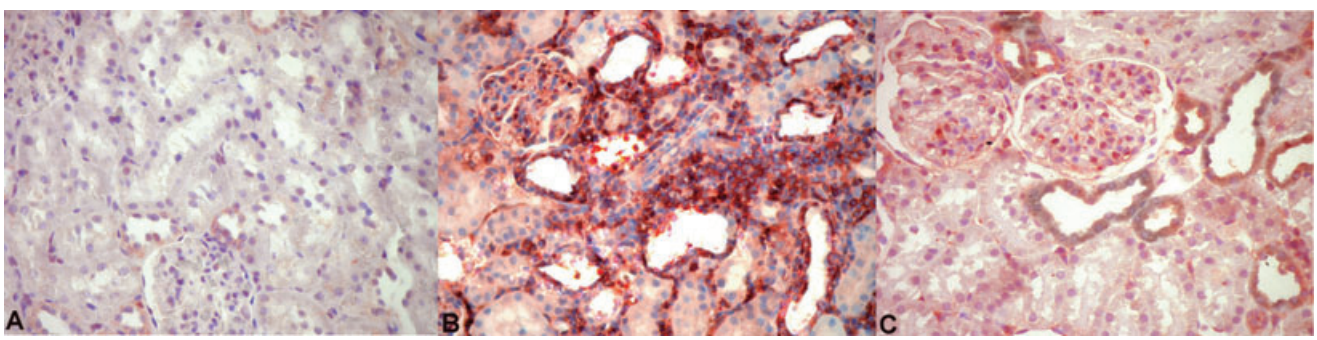

Fig. 7 Immunohistochemical detection of nuclear factor kappa beta (NF-κβ) expression. (A) Control (sham-operated) kidneys showing trace NF-κβ staining are present in endothelial and tubular cells. (B) Untreated rat kidneys showing strong diffuse immunostaining are seen in endothelial and tubular cells. (C) PPC-treated rat kidneys showing spot like immunostaining in tubular cells.

interstitial inflammation and fibrosis elicited by ureteral occlusion. Because PPC is already widely used, it could easily be applied for kidney protection in patients with UUO.

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References

1 Bohle A, Mackensen-Haen S, von Gise H *et al.* The consequences of tubulo-interstitial changes for renal function in glomerulopathies. A morphometric and cytological analysis. *Pathol. Res. Pract.* 1990; **186**: 135–44.
2 Klahr S. New insight into consequences and mechanism of renal impairment in obstructive nephropathy. *Am. J. Kidney Dis.* 1991; **18**: 689–99.
3 Kuncio GS, Neilson EG, Haverty T. Mechanisms of tubulo-interstitial fibrosis. *Kidney Int.* 1991; **39**: 550–6.
4 Misseri R, Rink RC, Meldrum DR, Meldrum KK. Inflammatory mediators and growth factors in obstructive renal injury. *J. Surg. Res.* 2004; **119**: 149–59.
5 Klahr S. Urinary tract obstruction. *Semin. Nephrol.* 2001; **21**: 133–45.
6 Ricardo SD, Ding G, Eufemio M, Diamond JR. Anti-oxidant expression in experimental hydrnephrosis: role of mechanical stretch and growth factors. *Am. J. Physiol. Ren. Physiol.* 1997; **41**: F789–F798.
7 Saborio P, Krieg RJ Jr, Kuemmerle NB *et al.* α-Tocopherol modulates lipoprotein cytotoxicity in obstructive nephropathy. *Pediatr. Nephro.* 2000; **14**: 740–6.
8 Kawada N, Moriyama T, Ando A *et al.* Increased oxidative stress in mouse kidneys with unilateral ureteral obstruction. *Kidney Int.* 1999; **56**: 1004–13.
9 Mourella M, Guarner F, Molageloda J. Polyunsaturated phosphatidylcholine prevents stricture formation in rat model colitis. *Gastroenterolgy* 1996; **110**: 1033–7.
10 Demirbilek S, Gurses I, Sezgin N *et al.* Protective effect of polyunsaturated phosphatidylcholine pretreatment on stress ulcer formation in rats. *J. Pediatr. Surg.* 2004; **39**: 57–62.
11 Niederau C, Strohmeyer G, Heintges T, Peter K, Gopfert E. Polyunsaturated phosphatidyl-choline and interferon alpha for treatment of chronic hepatitis B and C: a multi-center, randomized, double-blind, placebo-controlled trial. Leich Study Group. *Hepatogastroenterology* 1998; **45**: 797–804.
12 Karaman A, Demirbilek S, Sezgin N *et al.* Protective effect of polyunsaturated phosphatidylcholine on liver damage induced by biliary obstruction in rats. *J. Pediatr. Surg.* 2003; **38**: 1341–7.
13 Aabdallah DM, Eid NI. Possible neuroprotective effects of lecithin and alpha-tocopherol alone or in combination against ischemia/reperfusion insult in rat brain. *J. Biochem. Mol. Toxicol.* 2004; **18**: 273–8.
14 Carlson SE, Montalto MB, Ponder DL, Werkman SH, Korones SB. Lower incidence of necrotizing enterocolitis in infants fed a preterm formula with egg phospholipids. *Pediatr. Res.* 1998; **44**: 491–8.
15 Wen JG, Chen Y, Frokiaer J *et al.* Experimental partial unilateral ureter obstruction I. Pressure flow relationship in a rat model with mild and severe acute ureter obstruction. *J. Urol.* 1998; **160**: 1567–71.
16 Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.* 1978; **36**: 271–8.
17 Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium- reduction method. *Clin. Chem.* 1990; **36**: 1440–3.
18 Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* 1982; **78**: 206–9.
19 Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* 1988; **34**: 497–500.
20 Aleynik SI, Leo MA, Takeshige U *et al.* Dilinoleoylphosphatidylcholine is the active antioxidant of polyenylphosphatidylcholine. *J. Invest. Med.* 1999; **47**: 507–12.
21 Aleynik SI, Lieber CS. Polyenylphosphatidylcholine corrects the alcohol-induced hepatic oxidative stress by restoring s-adenosylmethionine. *Alcohol* 2003; **38**: 208–12.
22 Harris KP, Klahr S, Schreiner G. Obstructive nephropathy: from mechanical disturbance to immune activation? *Exp. Nephrol.* 1993; **1**: 198–204.
23 Schreiner GF, Harris KP, Purkerson ML, Klahr S. Immunological aspects of acute ureteral obstruction: immune cell infiltrate in the kidney. *Kidney Int.* 1988; **34**: 487–93.
24 Harris KP, Schreiner GF, Klahr S. Effect of leukocytes depletion on the function of the postobstructed kidney in the rat. *Kidney Int.* 1989; **36**: 210–15.
25 Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Radic Biol. Med.* 1996; **21**: 335–48.
26 Ferran C, Millan MT, Csizmadia V *et al.* Inhibition of NF-kappa B by pyrrolidine dithiocarbamate blocks endothelial cell activation. *Biochem. Biophys. Res. Commun.* 1995; **214**: 212–23.
27 Cao Q, Mak KM, Lieber CS. Dilinoleoylphosphatidylcholine decreases LPS-induced TNF-α generation in Kuppfer cells of ethanol-fed rats: respective roles of MAPKs and NF-κB. *Biochem. Biophys. Res. Commun.* 2002; **294**: 849–53.
28 Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N. Engl. J. Med.* 1994; **331**: 1286–92.
29 Sharma K, Ziyadeh FN. The transforming growth factor-beta system and the kidney. *Semin. Nephrol.* 1993; **13**: 116–28.
30 Klahr S, Morisey JJ. The role of growth factor, cytokines and vasoactive compounds in obstructive nephropathy. *Semin. Nephrol.* 1998; **18**: 622–32.
31 Cao Q, Mak KM, Lieber CS. DLPC decreases TGF-β1-induced collagen mRNA by inhibiting p38 MAPK in hepatic stellate cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2002; **283**: G1051–G1061.