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ORIGINAL ARTICLE

Winnie Chan · Richard J. Krieg, Jr. · Kenley Ward
Fernando Santos, Jr. · Kwei-Chi Lin
James C.M. Chan

Progression after release of obstructive nephropathy

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Abstract Progressive glomerular and tubulointerstitial fibrosis develop in 1-year-old rats even after relief (R) of unilateral ureteral obstruction (UUO) at 5 days of age. The present study investigated whether a progressive renal injury model of UUO could be achieved after reversal of UUO (RUUO) in adult instead of neonatal rats. The potential for α-tocopherol modulation of mRNA for the fibrogenic cytokine, transforming growth factor-β1 (TGFβ1), apoptosis (TUNEL assay), and the presence of the stress protein, heat shock protein-70 (HSP-70), was also studied in this post-obstructive model. Male Sprague-Dawley rats weighing 125–150 g were randomly assigned to groups of 4 animals each for durations of 7 or 14 days of α-tocopherol supplementation after RUUO. The groups included: (i) sham, regular chow; (ii) RUUO, regular chow; (iii) RUUO, contralateral nephrectomy (NX); and (iv) RUUO, NX plus α-tocopherol supplementation. We found a significant increase in the ratio of kidney weight/body weight in the RUUO+NX group at 14 days compared with the sham and RUUO groups. This rise in the RUUO+NX group was significantly reduced after 14 days of α-tocopherol administration. The elevated level of kidney TGFβ1 mRNA in the RUUO+NX group was only partially reduced at 7 days. But at 14 days this became significantly reduced with the continued α-tocopherol treatment. The HSP-70 staining and the apoptosis of the kidney showed results parallel to those of TGFβ mRNA at 14 days. To separate the effects of hypertrophy after unilateral NX from the RUUO studies, we carried out a second experiment in control animals subjected to NX, with and without α-tocopherol supplementation. Fourteen days after NX, the apoptosis and TGFβ1 mRNA showed no significant differences from the control animals. Our data suggest that a model of progressive renal injury in RUUO can be established in adult rats. After contralateral NX, the progressive injury is evidenced by the increase in the ratio of kidney weight/total body weight, the apoptotic counts, as well as fibrogenic cytokine TGFβ1 mRNA in the post-obstructed kidney. Finally, our data also support the concept that α-tocopherol is renal protective, as judged by TGFβ1 mRNA, apoptosis, and HSP-70 staining, even in the progressive disease process of the post-obstructed model.

Keywords Unilateral ureteral obstruction · Relief of obstruction · α-Tocopherol · Transforming growth factor-β1 · Apoptosis · HSP-70

W. Chan · K. Ward · F. Santos, Jr. · K.-C. Lin · J.C.M. Chan
Department of Pediatrics, Virginia Commonwealth University, Richmond, VA 23298, USA

W. Chan · J.C.M. Chan
Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond, VA 23298, USA

R.J. Krieg, Jr.
Department of Anatomy, Virginia Commonwealth University, Richmond, VA 23298, USA

J.C.M. Chan (✉)
MCV Campus, P.O. Box 980498, Richmond, VA 23298–0498, USA
e-mail: jchan@hsc.vcu.edu
Tel.: +1-804-8289608, Fax: +1-804-8286455

Introduction

It has been recently reported that despite relief of 5 days of unilateral ureteral obstruction (RUUO) in the neonatal rat, when these rats grew into adulthood and were studied at 1 year of age, progressive renal injury and chronic renal insufficiency were clearly demonstrable [1]. The 40% reduction of glomerular numbers, coupled with the 80% reduction of glomerular filtration rate in these rats may lead to hyperfiltration and result in significant increases in glomerulosclerosis, tubular atrophy, and interstitial fibrosis documented 1 year after release of the obstruction [1]. To explore the feasibility of an adult form of this neonatal rat model, we carried out these experiments using temporary UUO in adult rats coupled with contralateral nephrectomy (NX). Because we [2, 3] have previously reported the amelioration of acute oxidative injury after UUO by α-tocopherol, in this report we also examined the effect of α-tocopherol on the progressive

injury at two intervals of 7 and 14 days, after relief of the UUO (RUUO) by quantitating kidney apoptosis [4] and mRNA for the fibrogenic cytokine, transforming growth factor-β1 (TGFβ1) [5]. We also focused on the effects of hypertrophy after unilateral NX by examining these parameters after unilateral NX alone.

Heat shock proteins (HSPs) are differentially expressed in the kidney in response to various challenges, including oxidative stress. It is postulated that HSP-70 probably folds proteins during stress into a configuration that can be refolded to a normal state more rapidly after reversal of the injury. Currently no data are available on induction of HSP-70 protein in obstructive nephropathy after release of UUO and α-tocopherol administration. For these reasons, kidney samples of the 14-day group were stained for HSP-70.

Materials and methods

The procedures described in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and adhered to the guidelines of minimal usage of animals to achieve statistical significance. Forty-eight male Sprague-Dawley rats weighing 125–150 g were purchased from Charles River Laboratories (Wilmington, Mass., USA) and assigned to the groups as described below.

UUO and RUUO procedures

UUO and RUUO were created by methods previously described by this laboratory [2–5]. Briefly, to create the UUO, a 4-mm segment of silastic tube was slit longitudinally and fitted over the mid-section of the left ureter. A silk ligature was tied around the silastic tube (and ureter) and left in place for 3 days [4]. To release the UUO (RUUO), the suture was untied and the silastic tube removed. Methylene blue dye was used to confirm patency of the ureter [4]. The contralateral NX procedure [6] was performed 24 h after the RUUO. Dietary α-tocopherol supplementation (250 IU/kg chow) was begun for the assigned group on the day of the NX procedure.

Assignment of animal groups

(A) First experiment

One day after arrival, the animals were assigned to eight groups of 4 animals each. The first set of animals consisted of four groups, with one assigned to α-tocopherol (vitamin E) supplementation for 7 days; at the end of this period, all the animals were killed to provide the following four groups of animals: (1) sham, regular chow; (2) RUUO, regular chow; (3) RUUO, NX, regular chow and (4) RUUO, NX plus α-tocopherol (vitamin E) supplementation to regular chow. The second set of animals also consisted of four groups and differed from the first set only in that α-tocopherol (vitamin E) supplementation was for 14 days. These animals were studied at the end of this period. Body weights were tracked during the experiment. All animals were sacrificed by exsanguination under general anesthesia, and the kidney was collected and weighed as previously described [3].

(B) Second experiment

To distinguish the effort of compensatory hypertrophy following unilateral right NX alone, we carried out a second set of experiments: (I) a sham group (n=5); (II) a unilateral NX-only control group (n=5) and (III) an unilateral NX-only control group treated with α-tocopherol, 250 mg/kg chow (n=6). All animals were sacrificed 14 days after the sham or unilateral NX procedure.

TGFβ1 mRNA isolation and Northern blotting

Total RNA was isolated from renal cortical tissue using Trizol reagent (Life Technologies, Gaithensburg, Md., USA) and quantitated spectrophotometrically at 260 nm. The RNA was then applied to a denaturing 0.8% agarose gel and its integrity was assessed by ethidium bromide staining of the 28-S and 18-S ribosomal RNA bands. The abundance of the mRNA for TGFβ1 was determined using an RNAse protection assay [7] with a rat TGFβ1 probe (catalogue no. 63197, ATCC, Rockville, Md., USA). Cyclophilin was used as an internal standard. Values were expressed in arbitrary densitometric units (ADU).

Apoptosis by TUNEL assay

Apoptosis was localized at the cellular level by in situ labelling of the apoptosis-induced nuclear DNA strand breaks using the *t*erminal deoxynucleotidyl transferase-mediated deoxy*u*ridine triphosphate *n*ick *e*nd-*l*abelling method (TUNEL assay). With this technique, fluorescein-labelled DNA precursors and the Klenow fragment of DNA polymerase I were used to repair the gaps generated in the DNA from apoptosis. Kidney tissues were fixed in 10% buffered formalin at 4°C for 24 h. Tissues were transferred to 70% alcohol, processed, and embedded in paraffin; 5-µm sections were dewaxed using xylene and rehydrated with graded alcohol washes. Proteinase K was used to digest the tissue for 15 min at room temperature, followed by a rinse with phosphate-buffered saline (PBS). Endogenous peroxidases were blocked with 3% hydrogen peroxide in methanol and 30 min at room temperature. The tissues were rinsed with PBS and then digested with a 0.1% Triton in 0.1% sodium citrate solution for 2 min on ice. Following another rinse with PBS, 1% bovine serum albumin in PBS was used for 10 min at room temperature to reduce background staining. The tissues were incubated in 50 µl of TUNEL reaction mixture ("in situ cell death detection kit, Fluorescein", Boehringer Mannheim, Los Angeles, Calif.„ USA) for 1 h at 37°C to label the 3'-hydroxy termini of the nicked nuclear DNA. Unincorporated dNTPs were removed by a PBS rinse and the slides were mounted with 20% glycerol in PBS. A fluorescent microscope was used to analyze the results. Apoptotic bodies were counted in ten non-overlapping, randomly selected fields within the renal cortex at 400× magnification.

HSP-70 staining

Kidney tissues were fixed in 10% buffered formalin for 24 h at 4°C and transferred to 70% alcohol before being processed and embedded in paraffin. Sections, 5 µm-thick, were deparaffinized with a xylene wash and rehydrated by a series of degraded alcohol washes.

Immunohistochemistry was performed using the streptavidin-biotin peroxidase method (Dako LSAB2 rat kit, Dako, Carpinteria, Calif., USA). The primary antibody used was mouse monoclonal anti-HSP-70 (W27 clone, 1:50, Santa Cruz Biotechnology, Santa Cruz, Calif., USA) which binds to both inducible and constitutive HSP-70. Staining was completed with diaminobenzidine/hydrogen peroxide chromogen solution. Sections were then counterstained with Gill's hematoxylin, dehydrated, and mounted for examination by light microscopy.

Statistical analysis

Values were calculated as mean±standard error of the mean (SEM). For comparison between two groups, unpaired t-test was performed. For more than two groups, analysis of variance was used. A P value <0.05 was considered significant.

Results

Figure 1 shows that no differences were found in the ratio of kidney weight (KW)/body weight (BW) between the sham versus 7 days RUUO or 7 days RUUO+NX groups. There was a significant increase in KW/BW in the 14 days RUUO+NX group compared with sham or RUUO groups, respectively (Fig. 2). Compared with sham and RUUO groups, the KW/BW of both groups of RUUO+NX showed statistically significant increases, but subsequent administration of α-tocopherol significantly reduced the increment ($P<0.05$).

Figure 3 shows that the kidney TGFβ1 mRNA was significantly elevated in the RUUO+NX animals compared with sham and RUUO groups. The TGFβ1 mRNA partially dropped after 7 days of α-tocopherol administration in the RUUO+NX+E group, but did not reach statistical significance.

Figure 4 shows that the TGFβ1 mRNA, normalized to cyclophilin, was elevated (0.276±0.013 ADU) in the RUUO+NX normal chow rats compared with the sham group (0.122±0.015 ADU, $P<0.01$) and was reduced to 0.212±0.008 ADU in the 14 days α-tocopherol (RUUO+NX+E) treated rats ($P<0.05$). This value was still higher than the sham and RUUO groups.

Figure 5 shows HSP-70 to be lightly stained material in the distal tubular cells (mainly cytoplasmic staining) of the sham animal's kidney. In the RUUO+NX animals, the HSP-70 staining of the distal tubular cells was markedly darker and both nuclear and cytoplasmic. In the α-tocopherol-treated RUUO+NX+E animals, the HSP-70 staining was markedly decreased.

Apoptotic bodies per 400× field at 7 days after RUUO in all groups (mean±SEM) were no different from each other: sham (0.3±0.1); RUUO (0.20±0.1); RUUO+NX (0.2±0.1); RUUO+NX+E (0.1±0.1). Figure 6 shows the apoptosis at 14 days to be significantly elevated in RUUO+NX animals compared with the sham and

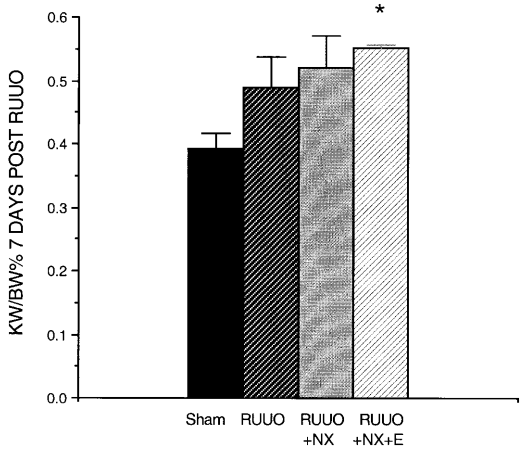

Fig. 1 Kidney weight (*KW*)/body weight (*BW*) (%) in sham, relief of unilateral ureteral obstruction (*RUUO*), RUUO with contralateral nephrectomy (*NX*), and RUUO+NX+α-tocopherol (*E*) treatment. *$P<0.05$ compared with sham

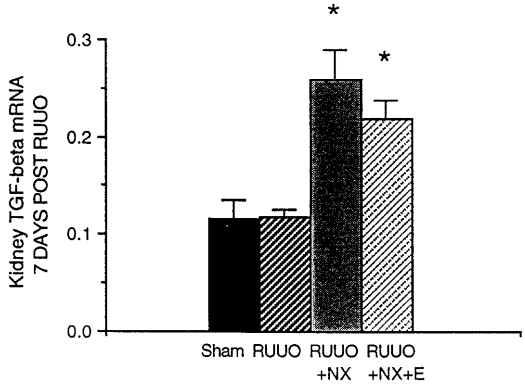

Fig. 3 Kidney transforming growth factor (*TGF*) beta 1 mRNA in arbitrary densitometry units (*ADU*) at 7 days post RUUO. *$P<0.05$ compared with sham and RUUO groups

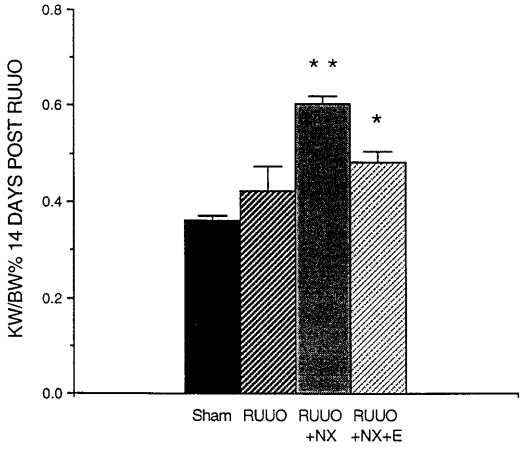

Fig. 2 KW/BW (%) in sham, RUUO, RUUO+NX, and RUUO+NX+E. **$P<0.05$ comparing RUUO+NX with sham and RUUO+NX+E groups. *$P<0.05$ comparing RUUO+NX+E with sham

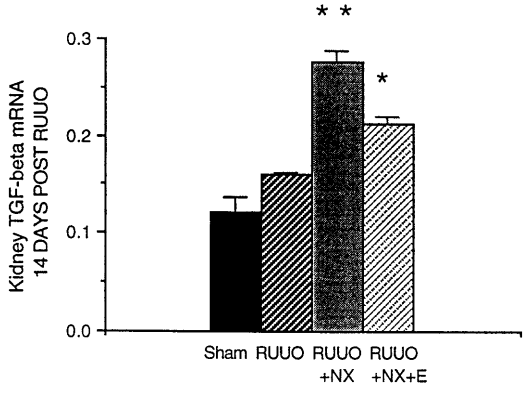

Fig. 4 Kidney TGF beta 1 mRNA in ADU. Data at 14 days post RUUO. **$P<0.05$ compared with all other groups; *$P<0.05$ compared with all other groups

Table 1 Fourteen days after unilateral nephrectomy (NX) alone, kidney apoptotic bodies/400x field and transforming growth factor (TGF)-β1 mRNA in arbitrary densitometry units (ADU), and ratio of kidney weight (KW) to body weight (BW). Mean±SEM (*E* vitamin E supplementation)

	n	Apoptosis	*P*	TGFβ1 mRNA	*P*	KW/BW%	*P*
Group I: sham	5	0.2±0.2		0.11±0.01		0.38±0.02	
Group II: NX	5	0.1±0.1	NS	0.13±0.01	NS	0.56±0.03	<0.05
Group III: NX+E	6	0.2±0.1	NS	0.14±0.01	NS	0.55±0.01	<0.05

P values compared with group I

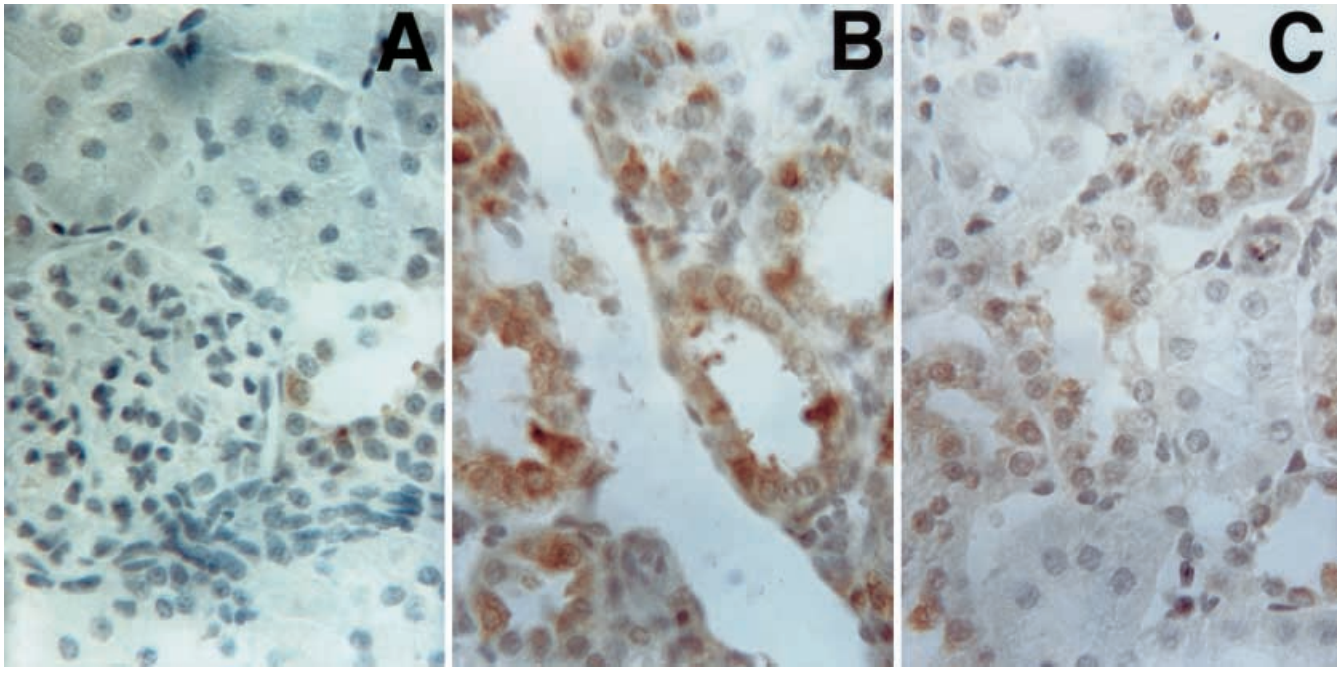

Fig. 5 **A** Heat shock protein-70 (HSP-70) was lightly stained in the distal tubular cells in the sham animal's kidney. **B** In the RUUO+NX animals, the HSP-70 staining of the distal tubular cells was markedly darker and included cytoplasmic and nuclear staining. **C** In the α-tocopherol-treated RUUO+NX+E animals, the HSP-70 staining became markedly decreased compared with the untreated RUUO+NX animals

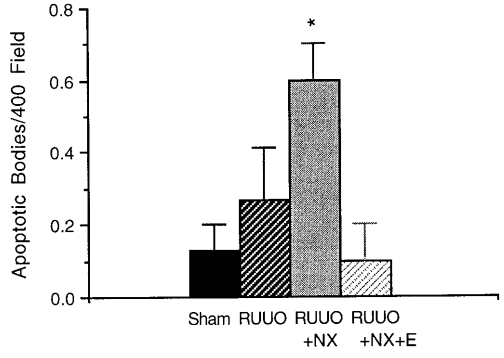

Fig. 6 Kidney apoptotic bodies per 400x field at 14 days after RUUO. Apoptosis of RUUO+NX was significantly elevated (**P<0.05) compared with all other groups. Supplementation with α-tocopherol (RUUO+NX+E group) reduced this elevated apoptosis to sham, RUUO levels

RUUO groups. This index of programmed cell death was suppressed with α-tocopherol, as documented by the significant reduction of apoptosis in the RUUO+NX+E group.

Table 1 shows the results of our second experiment to delineate the effects of right NX alone. There are no differences in apoptosis or in TGFβ1 mRNA among the three groups. In this second experiment (Table 1), the KW/BW ratio was significantly increased compared with the sham. The administration of vitamin E did not lower this parameter.

Discussion

Despite neonatal pyeloplasty to relieve congenital obstructive uropathy, unrelenting progression to end-stage renal disease is encountered in the majority of affected children by 8–11 years of age [8–11]. The mechanism of this unrelenting progression after release of obstruction is unknown. There is no effective therapy to counter this progression after release of the ureteral obstruction in the neonatal period [1]. It is unknown whether such progression occurs after relief of temporary ureteral obstruction in the adult period. This has particular relevance, be-

cause of the frequency of obstructive uropathy in elderly men from prostatic hypertrophy, and for adult men and women to obstruction by tumors and stones in the urogenital system. The question of whether there is ongoing renal injury in adults after release of temporary ureteral obstruction of a few days is unsettled, and thereby represents a matter of some concern.

The UUO model of kidney injury has established that the angiotensin system is pivotal to renal parenchymal damage [12–16]. HSP activation [17] may also contribute to the angiotensin-related response to UUO. These investigations provided the rationale for the successful use of converting enzyme inhibitors to halt the tubulointerstitial fibrosis of UUO, which may be mediated by decreasing the chronic inflammatory mediator, nuclear factor-kappa β (NF-kβ) [18] and a decrease in intraglomerular hyperfiltration. Orally administered anti-fibrotic agents, such as pirfenidone [19], may also have a role in preventing and reversing the tubulointerstitial fibrosis of UUO. Even after several days' delay, treatments with converting enzyme inhibitors, enalapril or lisinopril, could still ameliorate the tubulointerstitial fibrosis in rats with UUO [20].

Despite this large body of work concerning UUO [12–16], it was only recently that investigations concerning the mechanism of the unrelenting progression to renal failure after RUUO became available [21]. Thus, the recent publications on neonatal rats by Chevalier et al. of persistent tubular injury at 28 days [21] and 1 year [1] after release of 5 days of neonatal UUO are particularly important. Hyperfiltration of the post-obstructed kidney is the postulated mechanism and has special relevance in the exaggerated renin-angiotensin system, characteristic of neonatal rat kidneys [22]. To study whether there is unrelenting progression in the adult kidneys after release of UUO, we carried out the present experiments. The TGFβ1 mRNA in adult rats after RUUO did not differ from that of sham, at 7 days (Fig. 3) or at 14 days (Fig. 4) after RUUO. This suggests that, contrary to neonatal rats with persistently elevated tubular TGFβ1 mRNA 14 days after RUUO, elevated TGFβ1 expression did not occur unless RUUO was accompanied by contralateral NX.

The apoptosis followed exactly the same pattern as the TGFβ1 mRNA data, with no difference between the groups at 7 days, but programmed cell death became markedly elevated in RUUO+NX 14 days after the procedure (Fig. 6). Such data support our contention that we have a model of progressive renal disease, if RUUO is accompanied by contralateral NX. Finally, the data also support our contention that α-tocopherol reverses apoptosis in RUUO+NX.

In recognition of the severe renal vasoconstriction in the 24 h after release of UUO [23], we did not carry out the contralateral NX procedure until the 2nd day post release of obstruction. It remains to be determined whether the use of imidiazole [23], which blocks the renal vasoconstriction in the post-obstructive kidney, may obviate the need for the 24-h delay and allow completion of the NX procedure concurrent with the RUUO procedure.

Our data showing the significant rise in KW/BW and significant elevation in TGFβ1 mRNA in both the 7 days after RUUO+NX (Fig. 3) and 14 days after RUUO+NX (Fig. 4) are evidence of ongoing fibrogenesis in this kidney model in adult rats. Our data also imply that temporary UUO in the adult kidney is not likely to be associated with ongoing injury in the post-obstructed kidney, in contrast to the neonatal RUUO kidney. In adult kidneys, unless there is further compromise by the removal of the contralateral kidney, the fibrogenic cytokine, TGFβ1 mRNA, remained the same as the sham (Figs. 3 and 4).

To separate the effect of NX alone from the effect of relief of obstruction followed by NX, the lack of change in apoptosis and in TGFβ1 mRNA in the NX-only experiments effectively exclude compensatory hypertrophy as a cause of the observed changes in our RUUO+NX experiments. These observations, together with the increased ratio of KW/BW after RUUO+NX, support our contention of a progressive renal disease in our RUUO+NX model.

α-Tocopherol provides protection against lipid peroxidation and is an effective non-enzymatic free radical quencher. α-Tocopherol-deficient animals have increased concentrations of peroxides, which lead to increased susceptibility to oxidative injury in many organ systems [24]. An α-tocopherol transfer protein mutation in man [24] gives rise to low serum α-tocopherol concentrations in retinitis pigmentosa and ataxia. α-Tocopherol supplementation has been given to ameliorate the progression of retinitis pigmentosa. Trachtman et al. [25, 26] demonstrated that antioxidants, specifically α-tocopherol [27] and taurine [25], effectively attenuate glomerulosclerosis in the puromycin-induced nephrotic rat. In experimental IgA nephropathy, we [26] have shown indirect evidence of increased free radical release and have shown that α-tocopherol reduces the incidence of hematuria, the degree of proteinuria, the kidney malondialdehyde (MDA) content, and TGFβ1 mRNA expression. We have also shown that α-tocopherol reverses the glomerulosclerosis seen in remnant kidney rats [6]. The use of an antioxidant, such as α-tocopherol, in a tubulointerstitial disease, specifically UUO, has only recently been published [2].

In a previous publication [2], we showed that oxidative stress in the kidney after 3 days of UUO led to a 25% increase in renal parenchymal MDA content from UUO animals compared with sham. α-Tocopherol supplementation significantly reduced this MDA elevation in the renal parenchyma. A concomitant 66% rise in TGFβ1 mRNA in the UUO kidney was also significantly reduced by α-tocopherol [2]. We carried out the current study in order to determine whether the elevated TGFβ1 mRNA, 7 and 14 days after release of the UUO, in the RUUO+NX model of progressive nephropathy, is reversible by α-tocopherol. Our data indicated that 14 days' administration of a diet that is enriched in α-tocopherol clearly ameliorates the rise in TGFβ1 mRNA of this

model (Fig. 4), although at 7 days after RUUO+NX, the α-tocopherol supplementation (Fig. 3) only partially lowers this fibrolytic cytokine. The protective effect was demonstrated only at 14 days of α-tocopherol supplementation (Fig. 4) with statistical significance ($P<0.05$). The percentage increase in KW/BW ratio being reduced with α-tocopherol supplementation ($P<0.05$) also suggests that the increased kidney mass with RUUO+NX can be reversed by 14 days of α-tocopherol at this late stage. But this reduction was not apparent in the second experiment (Table 1) with unilateral right NX alone. The reason for this lack of response to α-tocopherol in this later situation is not apparent.

In response to injury during metabolic stress, all mammalian cells increase the synthesis of a class of stress proteins [28], commonly referred to as heat shock proteins, HSP. The HSP system protects the cell and promotes more rapid recovery after reversal of the initial metabolic stress. It is speculated that during such metabolic stress, HSP-70 folds proteins into configurations which resist denaturation [28]. Other groups of HSPs, especially ubiquitin, are involved with degradation of denatured proteins [28, 29]. Endogenous HSP-70 is inadequate to accomplish cytoprotection [30]. Induction of HSP-70 has been examined in puromycin aminonucleoside nephropathic injury [31].

Our previous data [3] in UUO obtained by Western blot analysis indicate a marked 3.7-fold increase in expression of HSP-70 10 days after UUO, followed by a return to baseline (sham) values 7 days after release of the obstruction (R-UUO). In our current study, the immunohistochemical studies of sham kidney for HSP-70 (Fig. 5 A) showed that distal tubules were lightly stained. The staining pattern was uniform and mainly cytoplasmic. In 14-day RUUO+NX kidney (Fig. 5B), the staining intensity increased in both cytoplasm and some nuclei of tubular cells of distal tubules. The number of darkly stained cells in tubules also drastically increased, suggesting that the NX procedure has caused sustained stress to the RUUO kidney. In the RUUO+NX+E kidney (Fig. 5C), the 14 days' supplementation of α-tocopherol markedly decreased the intensity of HSP-70 immunohistostaining in distal tubules and reduced the number of darkly stained cells in tubules compared with RUUO+NX, possibly an indication of reduced stress.

Our original data on oxidative stress, as evidenced by increased MDA content and HSP-70 in the contralateral unobstructed rat kidney following UUO [3], have been supported by the recent data from Chevalier et al. [1] showing that the contralateral, unobstructed rat kidney suffers from fibrosis even 1 year after release of temporary UUO of a few days.

In summary, we propose a model of progressive nephropathy after relief of temporary obstructive uropathy in the rat. Our data showed that RUUO in adult rats was not associated with continued elevation of TGFβ1 mRNA, as established in neonatal rats. Progressive injury in adult rats after relief of UUO was encountered only when contralateral NX accompanied relief of this injury. In this adult rat model of progressive nephropathy, α-tocopherol ameliorated this fibrogenic cytokine as well as apoptosis, suggesting a role of α-tocopherol in halting progression. Finally, this model may be useful in studying adaptive or reparative responses in obstructive nephropathy, providing a venue to examine how converting enzyme inhibitors exert renal protective effects beyond their effect of abrogating hyperfiltration alone. This will have implications on how the long-term inhibition of renin-angiotensin-aldosterone system and the reversal of oxidative stress protects against proliferation, hypertrophy, collagen deposition, and fibrosis [32].

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LITERATURE ABSTRACT

H. Xu · K. Iijima · T. Shirakawa · S. Shiozawa · M. Miwa
K. Yamaoka · N. Kawamura · H. Nakamura · N. Yoshikawa

Platelet-activating factor acetylhydrolase gene mutation in Japanese children with *Escherichia coli* O157-associated hemolytic uremic syndrome

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Platelet-activating factor (PAF) may be involved in the pathogenesis of *Escherichia coli* O157-associated hemolytic uremic syndrome (HUS). PAF is degraded to inactive products by PAF acetylhydrolase. In this study, we investigated whether a PAF acetylhydrolase gene mutation (G→T transversion at position 994) is involved in HUS in Japanese children. A point mutation in the PAF acetylhydrolase gene (G994T) was identified using polymerase chain reaction in 50 Japanese children with *E. coli* O157-associated HUS and 100 healthy Japanese. We then determined the relationship between the PAF acetylhydrolase G994T gene mutation and clinical features of HUS. There was no difference in genotype and allele frequencies between patients with HUS and healthy controls. The mean duration of oligoanuria was significantly longer in patients with the GT genotype than in those with the GG genotype (P=0.012). Although 11 of 15 patients (73%) heterozygous for the mutant allele (GT) required dialysis, only 13 of the 35 wild-type homozygotes (GG; 37%) required dialysis (P=0.030). Mean plasma PAF acetylhydrolase activity was significantly less in patients with the GT genotype than in those with the GG genotype (P<0.0001). In conclusion, we have shown an association between the G994T PAF acetylhydrolase gene mutation and the severity of renal damage in *E. coli* O157-associated HUS. Our study suggests that analysis of the PAF acetylhydrolase gene mutation in Japanese children with *E. coli* O157-associated HUS may allow the prediction of the severity of HUS.