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Renal effects on a solitary kidney of specific inhibition of cyclooxygenease-2 after 24 h of complete ureteric obstruction in rats

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Abstract The purpose of this study is to show whether selective (celecoxib) and non-selective (piroxicam) inhibitors of COX-2 can alter the morphological and functional changes after the release of a 24 h complete ureteric obstruction in tissue from solitary rat kidney.

Forty male Sprague-Dawley rats weighing 225–250 g were used. The animals were divided into four groups. In group 1 rats (control, $n=10$), only right nephrectomy was performed. Group 2 rats (untreated, $n=10$) underwent right nephrectomy and the left ureter was completely obstructed. In group 3 rats (celecoxib), the same operation was performed as described for group 2 and than celecoxib was administered by gavage for a period of 24 h. Group 4 rats (piroxicam) underwent the same operation as described for group 2, then piroxicam was administered intramuscularly at least 1 h before the release of the for 24 h complete ureteric obstruction. All animals were then prepared for functional and histopathological studies. The administration of celecoxib produced a significant decrease in blood urea nitrogen levels when compared to the animals receiving piroxicam and the animals with no treatment. Moreover, celecoxib caused a significant decreased in creatinine levels when compared to the untreated group. Urine volume and the urinary sodium values were increased in the celecoxib group when compared with the other groups. The administration of celecoxib and piroxicam caused a significant decrease in the number of interstitial macrophages when compared to the untreated group. The Bowman space was significantly increased in the untreated group when compared with the celecoxib and the piroxicam groups. These studies indicate that celecoxib may be an important factor affecting renal morphological and functional changes after the release of a 24 h complete ureteric obstruction.

Keywords Cyclooxygenease-2 · Celecoxib · Piroxicam · Ureteric obstruction · Rat

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Introduction

The prostaglandins (PGs) are a diverse group of autocrine and paracrine hormones that mediate many cellular and physiological processes [12]. In the kidney, PGs represent important physiological modulators of renal hemodynamics and salt and water homeostasis [18]. Cyclooxygenase (COX), an enzyme with two isoforms, catalyzes the formation of endoperoxides from arachidonic acid. The isoforms are known as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). They are similar in amino-acid sequence and enzymatic function [1, 10], although their physiological functions are thought to be quite different [16]. COX-1 is expressed constitutively in most tissues and appears to be involved in the basal physiological production of prostaglandins. In contrast, COX-2 is mainly cytokine inducible and is expressed in inflammatory cells [10, 11, 12]. The discovery of these two COX isoforms has led to the hypothesis that selective inhibition of COX-2 could have the anti-inflammatory and analgesic effects of non-steroidal anti-inflammatory drugs (NSAIDs) without affecting COX-1.

In the normal kidney, COX-1 is present in the endothelial and smooth muscle cells of the preglomerular and postglomerular vessels, as well as in the collecting duct and interstitial cells [7]. COX-2 is constitutively present in the kidney (where it has been localized in the thick ascending limb, in interstitial cells of the papilla, and in the cells of the macula densa of the rat) [6, 7, 17].

The intrarenal distribution of COX-2 and the observation that salt depletion increases the expression of COX-2 in the kidney both suggest that, in addition to its role in inflammation and growth, COX-2 may contribute to the regulation of vascular tone and salt and volume homeostasis, particularly during salt depletion and hypovolemia [11]. However, the role of COX-2 in the physiological regulation of renal hemodynamics and sodium and water balance in humans is far from being clear, and whether the selective inhibition of COX-2 enables the avoidance of renal side effects of non-selective cyclooxygenase inhibition in humans is still unknown.

In this experimental study of unilaterally nephrectomized rats, we aimed to show whether the administration of selective (celecoxib) and non-selective (piroxicam) inhibitors of COX-2 would alter the morphological and functional changes after the release of a 24 h complete ureteric obstruction in solitary rat kidney tissue.

Materials and methods

Forty male Sprague-Dawley rats weighing 225–250 g were used. The animals were divided into four groups. Each rat was anesthetized with ketamine (50 mg/kg) and xylazine (4 mg/kg). In group 1 rats (control, $n=10$), only a right nefrectomy was performed. Group 2 rats (untreated, $n=10$) underwent a right nephrectomy and the left ureter was completely obstructed between the bladder and spermatic cord through a small suprapubic incision. The abdomen was then closed in layers with 5–0 silk. In group 3 rats, the same operation was performed as described for group 2 and then the COX-2 inhibitor celecoxib (200 mg/kg, Celebrex, Istanbul, Turkey) was administered by gavage for a period of 24 h. Group 4 rats again underwent the same operation as described for group 2 and then the COX-1 inhibitor piroxicam (20 mg/kg, Felden, Istanbul, Turkey) was administered intramuscularly at least 1 h before the release of the 24 h complete ureteric obstruction. Following surgery, the animals were allowed free access to water and standard rat chow. After 24 h, the animals were prepared for studies of morphological and functional changesto the kidney tissue.

On the second day of the study, all animals were anesthetized with 100 mg/kg inactin i.p. and placed on a thermoregulated table. The abdomen was reopened in all rats and a PE-50 catheter was inserted into the left ureter above the ligature for calculation of the urine flow rate. During this period of surgical preparation, the rats in all groups received 1% of their body weight of Ringer's lactate. Urine collection began 60 min after inserting the left ureteric catheter and continued for 2–3 h thereafter. Urinary excretion of Na^+ and K^+ was measured in samples diluted 1:5 by use of a Flame Photometer (Instrumentation Laboratory). Renal function was assessed by measurements of blood urea nitrogen (BUN; mg/dl) and serum creatinine (mg/dl) levels. Biopsies were obtained from the remaining left kidneys before killing the rats.

Immunohistochemistry

Tissues were fixed in 10% formaldehyde overnight and embedded in paraffin. Sections were cut to 4 μm thickness, dewaxed in xylene, and incubated for 20 min in 0.3% H_2O_2 to block endogenous peroxidase activity. Sections were than microwaved for 4 min in phosphate buffer saline (PBS), and incubated with primary antibody (macrophage CD 68, monoclonal mouse antihuman, DAKO, England) overnight at room temperature. The primary antibody was visualized with diaminobenzidine (DAB) as chromogen. The sections were counterstained in hematoxylin and eosin, cleared with xylene and coverslipped.

The measurement of the Bowman space was performed at the 3, 6 and 9 o'clock positions and the count of macrophages in the interstitial area of the renal cortex in each specimen was performed by using an objective mounted micrometer (200× magnification, Olympus Eyepiece Micrometer) and an light microscope (100× magnification, Olympus, BH4)

Statistics

All data were calculated as mean ± SD. The comparisons between the groups were analyzed by the Mann-Whitney U-test. The level of statistical significance was set at $P<0.05$.

Results

The results of BUN, creatinine and the excretory function for all groups after the release of the 24 h of complete ureteric obstruction are shown in Table 1.

Mean BUN and creatinine levels were significantly increased in the untreated, the celecoxib and the piroxicam groups in comparison with the sham-control group ($P<0.0001$, $P<0.0001$, $P<0.0001$, respectively). The BUN value was decreased significantly in the celecoxib group when compared with the untreated and the piroxicam groups ($P<0.001$, $P<0.001$). Creatinine values were decreased in the celecoxib group when compared with the untreated group ($P=0.03$). There was no significant difference in the creatinine values between the celecoxib and piroxicam groups.

The urine volume also increased in the untreated, the celecoxib and the piroxicam groups when compared to

Table 1. Effect of piroxicam and celecoxib on excretory function after the release of 24 h of complete ureteric obstruction. Values are means ± SD, n is number of experiments, BUN is blood urea nitrogen; $U_{Na}+$, U_K+, are urinary excretion of sodium and potassium

Groups	n	BUN, ml/dl	Creatinine, ml/dl	Urine flow, $\mu l min^{-1} kg^{-1}$	$U_{Na}+$, $\mu eq min^{-1} kg^{-1}$	U_K+, $\mu eq min^{-1} kg^{-1}$
Control	10	39.70 ± 0.94	0.62 ± 0.02	27.7 ± 3.94	1.75 ± 0.21	1.12 ± 0.02
Untreated	10	272.60 ± 25.69	4.58 ± 0.44	30.6 ± 4.71	2.39 ± 0.25	1.11 ± 0.02
Celecoxib	10	215.60 ± 26.22*	4.23 ± 0.33**	36.9 ± 5.85†	3.92 ± 0.19§	1.12 ± 0.01
Piroxicam	10	261.90 ± 10.22	4.46 ± 0.36	31.4 ± 5.12	1.88 ± 0.17‡	1.10 ± 0.02

* $P<0.001$, $P<0.001$ compared with untreated and piroxicam groups
** $P<0.05$ compared with untreated
† $P<0.05$ compared with untreated and piroxicam groups
§ $P<0.0001$, $P<0.001$ compared with untreated and piroxicam groups, respectively
‡ $P<0.001$ compared with untreated

the control group. This increase was not significant between the control group and the untreated, and the piroxicam groups. However, the urine volume was increased significantly in the celecoxib group when compared to other groups ($P < 0.001$, $P < 0.05$, $P < 0.05$).

The mean urinary sodium value was significantly increased in the celecoxib ($P < 0.0001$) and decreased in the piroxicam group ($P < 0.0001$) when compared with the untreated groups. There was no significant difference in urinary potassium values between the celecoxib and the untreated and the piroxicam groups.

No histopathological changes were observed in specimens of control rats by light microscopic examination. Light microscopic examination of untreated rats with complete ureteric obstruction showed hydropic degeneration of the tubular epithelium and widening of Bowman space. The mean count of macrophages in the control group was < 1 in the interstitial area of the kidney tissue. It was, however, 21.4 ± 7.5, 2.1 ± 0.73 and 2.3 ± 0.82 in groups 2, 3 and 4, respectively. The increase in the number of macrophages in the untreated group was significant ($P < 0.0001$) compared to the control, celecoxib, and piroxicam groups. However, the increase in the number of macrophages in the celecoxib and the piroxicam groups was not significantly different. Moreover, the administration of celecoxib and piroxicam caused a significant decrease in the number of interstitial macrophages when compared to the untreated group ($P < 0.001$). Fig. 1 shows the immunohistochemical localization of macrophages in the interstitial space of the kidney tissues of an untreatment rat (a), and of a celecoxib treated rat (b).

The measurement of the Bowman space was 400 ± 66.6, 790 ± 99.4, 510 ± 56.7 and 560 ± 84.3 μm (mean ± SD) in groups 1, 2, 3 and 4, respectively. The mean value of the Bowman space was increased significantly in the untreated, celecoxib and piroxicam groups in comparison with that of the control group ($P < 0.0001$, $P < 0.002$, $P < 0.001$). The Bowman space was significantly increased in the untreated group when compared with the celecoxib and piroxicam groups ($P < 0.0001$). There was no significant difference for the Bowman space between the celecoxib and piroxicam treated groups.

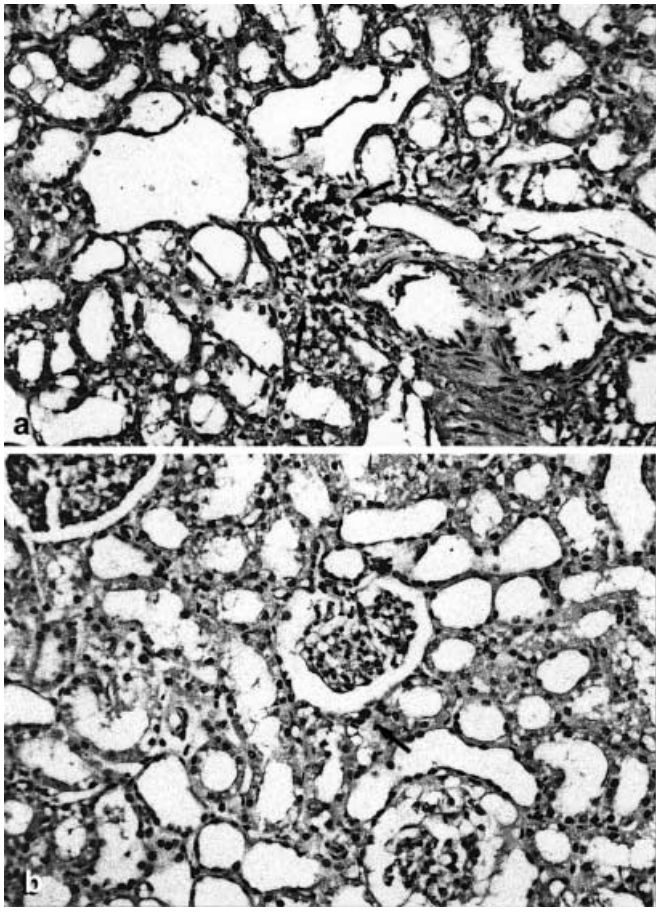

Fig. 1. Immunohistochemical localization of macrophages in the interstitial space of the kidney tissues of an untreated group rat (*arrow*) **(a)**, and of a celecoxib treated rat **(b)**. Please note the decrease in the number of macrophages after celecoxib treated (*arrow*) (×200 immun-peroxidase)

Discussion

Our results suggest that the administration of a selective (celecoxib) and a non-selective (piroxicam) inhibitor of COX-2 protect from the degenerative effects of acute, complete obstruction in the solitary kidney tissue of rats. Moreover, the selective inhibitor provided more protection from kidney damage.

Several investigators [3, 14, 19] have also examined the protective effects of a number of selective and non-selective COX-2 inhibitors in kidney tissue after complete ureteric obstruction. We examined, for first time, the protective effects of a selective (celecoxib) and a non-selective (piroxicam) COX-2 inhibitor in the complete ureteric obstruction of solitary kidneys in the rat. Our study eliminated any compensatory effects of the contralateral kidney by surgically removing it, so that all observed changes in the kidney tissue occurred in kidneys with complete ureteric obstruction.

Arachidonic acid metabolites (eicosanoids) are actively produced by the kidneys and are involved in control of renal blood flow (RBF), glomerular filtration rate (GFR), free water clearance, tubular transport, and renin release [1, 5]. Under normal conditions, inhibition of prostanoid synthesis has no effect on either RBF or GFR [15]. Under conditions in which the vasoconstrictor tone is increased, vasodilator eicosanoids are released in an effort to compensate for the vasoconstriction [5]. After the release of as little as 24 h of unilateral ureteric obstruction (UUO), there is intense renal vasoconstriction in the postobstructive kidney (POK), accompanied by a decrease in RBF and GFR [19]. Nishikawa et al [9] reported that, after 72 h UUO, the in vitro perfused hydronephrotic rabbit kidney has an increased production of prostaglandin E2 (PGE2) when

stimulated with either bradykinin or angiotensin II (ANGII), as compared with the intact contralateral kidney. In Yarger et al.'s [19] study, the indomethacin (a COX-1 and COX-2 inhibitor) was infused intravenously after the release of UUO also failed to improve renal function. However, a significant improvement in renal function was observed with infused imidazole, (an inhibitor of thromboxane synthetase) in the POK. It has recently been hypothesized that the selective inhibition of COX-2 could provide the potent anti-inflammatory and analgesic effects of NSAIDs with fewer gastrointestinal and renal side effects, the latter being attributed primarily to the inhibition of COX-1 [10, 11, 16, 18]. Lopez Sanches et al. [12] suggested that COX-2 dependent vasodilatory prostaglandins were critical for the increases in renal blood flow after renal ablation and that COX-2 partially prevented the renal functional changes elicited by renal ablation. Gross et al. [4] demonstrated that the infusion of piroxicam, but not the COX-2 inhibitors NS-398 and meloxicam, blunted the natriuretic response to increased renal interstitial hydrostatic pressure, suggesting that the natriuretic response to this increased pressure may be preserved during the inhibition of COX-2. In our experimental study, after the release of a complete ureteric obstruction in the solitary kidney at 24 h, the administration of celecoxib produced a significantly greater protective effect on kidney function than piroxicam. The BUN and creatinine value was decreased and the urine volume and urinary sodium were significantly increased in the celecoxib group when compared to other groups.

The role of macrophages in the response to obstruction has been studied since the early 1970s. The relationship between macrophages and the fibrotic response of UUO is being actively investigated. Nagle et al. [8] showed that macrophages were present in obstructed rabbit kidneys. Schreiner et al. [13] described the presence of macrophages in cortical and medullary tissue isolated from rats with a UUO of 24 h; relief of the UUO was associated with the disappearance of macrophages from the tissue isolates. In addition, Diamond and co-workers [2] found transforming growth factor-β (TGF-β) in association with interstitial macrophages in early obstruction. In our study, there was an intense concentration of macrophages in the cortical tissue isolated from untreated rats with complete a ureteric obstruction of 24 h,in contrast to few immunoreactive macrophages in the interstitial area of the renal cortex in the celecoxib and piroxicam treated rats. Light microscopic examination of untreated rats with ureteric obstruction showed hydropic degeneration of the tubular epithelium and widening of the Bowman space.

Our study suggests that both celecoxib and piroxicam make contributions to the determination of post-obstructive renal function after the release of a 24 h complete ureteric obstruction in rats. However, celecoxib may be the most important determinant of post-obstructive renal function in the rat. Finally, the present findings suggest areas of exploration for the therapeutic manipulation of post-obstructive renal function in humans.

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