Urol Res (2010) 38:223–228
DOI 10.1007/s00240-009-0243-z

ORIGINAL PAPER

Preventive effects of COX-2 inhibitor, celecoxib on renal tubular injury induced by shock wave lithotriptor

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Received: 3 February 2009 / Accepted: 11 November 2009 / Published online: 1 December 2009

Abstract Our study was designed to investigate the protective effect of the COX-2 inhibitor, celecoxib, on renal tubules against shock wave lithotripsy (SWL). Sprague–Dawley rats were randomly divided into three groups: sham, control, and COX-2 groups. The control group was administrated normal saline. The COX-2 group was administered celecoxib (10 mg/kg). After administration for 1 week, the control and COX-2 groups received 1,000 shock waves. Before and after SWL, 24-h urine was collected. CCr was measured to assess renal function. To determine the renal tubular injury, *N*-acetyl glucosaminidase (NAG) and β-2 microglobulin levels in urine were quantified. The COX-2 gene expression was compared between the three groups. Prior to SWL, all groups had similar levels of NAG and β-2 microglobulin. After SWL, all groups showed similar CCr. Compared with the sham group, control and COX-2 groups produced increase of NAG and β-2 microglobulin excretion. However, NAG and β-2 microglobulin excretions were significantly lower in the COX-2 group than control group. The COX-2 gene expression did not increase in the sham group. However, the COX-2 gene expression was significantly increased in the control group, which was prevented by celecoxib in COX-2 group. Biochemical findings supported a renal protective effect of celecoxib on SWL. This study suggests that celecoxib would be useful prior and after SWL because of renal protective effects.

Keywords Kidney · COX-2 inhibitors · Extracorporeal shockwave lithotripsy

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Introduction

Urinary stone is one of the most common medical conditions in the field of urology, and shock wave lithotripsy (SWL) is now the treatment of choice in many cases, especially cases involving renal and upper ureteral stones. The many advantages of SWL, such as short procedure time, non-invasiveness, proven safety, and high success rate, have led to urologists treating patients with urinary stones with SWL rather than surgery. More than 16,000 cases of SWL are performed annually in tertiary referral centers in Korea.

However, SWL is not absolutely safe. When treating renal stones, transient renal dysfunction will occur due to tubular damage by shock waves. Although calcium channel blockers, such as verapamil or nifedipine, have been recommended to prevent tubular damage by SWL, calcium channel blockers are not used routinely because of the possibility of side effects and no additional pain relief.

Pain during SWL is not negligible. It is reported that SWL is more painful than cystoscopy and even more painful than ureteroscopic stone removal under local anesthesia [1]. Pain is the most common cause of limiting the increase

in power and frequency of the shock wave and often the cause of stopping the procedure. To reduce pain, opioid analgesics are generally recommended. But side effects, such as nausea, dizziness, and costs limit the use of opioid analgesics. The use of non-steroidal anti-inflammatory drugs (NSAIDS) is also limited because of renal toxicity to patients, especially patients who have reduced renal function due to obstruction and who would have transient renal dysfunction due to the effect of SWL on the kidney.

Cyclooxygenase-2 (COX-2) inhibitor is known to have renal toxicity like other NSAIDS. However, recently, it has been reported that COX-2 inhibitor ameliorates renal and ureteral damage in rats with obstructive uropathy [2]. Moreover, the specific COX-2 inhibitor has been to shown to protect several organs, including the kidney, from ischemia–reperfusion injury [3–5]. Ischemia–reperfusion injury is one of the causes of tubular damage by shock waves [6].

If COX-2 inhibitors could reduce the pain of patients and renal tubular damage simultaneously, it would have much benefit over other drugs. Our study was designed to investigate the potential protective effect of the COX-2 inhibitor, celecoxib, on renal tubules against shock wave.

Materials and methods

SWL model

All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by the Clinical Research Institute of Seoul National University Hospital. Twenty male Sprague–Dawley rats, weighing 200–220 g, were anesthetized with Zoletil (1 mg), and laparotomy was performed. The left kidney was identified and a very small round needle (vicryl 8-0) was inserted in the kidney. After surgery, all rats were randomly divided into three groups: (1) sham group ($N = 4$), (2) control group ($N = 8$), and (3) COX-2 group ($N = 8$). All rats were maintained on standard rat chow and water ad libitum for the study period.

After a 2-week recovery period, the control group was administrated normal saline (NS; 1 ml once a day for 1 week) by lavage tube. The COX-2 group was administered orally celecoxib (10 mg/kg body weight) suspended in NS (1 ml once a day for 1 week).

After administration for 1 week, 1 day before SWL, all rats were transferred to metabolic cages for a 24-h urine collection.

On the day of SWL, all rats were anesthetized with Zoletil (1 mg intramuscularly). After careful shaving of the dorsal skin, the control and COX-2 groups received 1,000 shock waves at intensity 2 to the left kidney for 10 min using a Dornier compact delta lithotripter (Dornier MedTech, GA, USA). The focus point was easily identified by a previously inserted needle under fluoroscopy.

After SWL, all rats were transferred back to metabolic cages for a 24-h urine collection, and then killed. A midline laparotomy was performed, and blood samples were collected from the vena cava by syringe. After the kidneys had been excised, they were longitudinally bisected; one hemi-section was fixed in formalin solution and stained with hematoxylin-eosin (H&E) for microscopic examination, and the other hemi-section was frozen in liquid nitrogen, and stored at −80°C for Western blot evaluation of COX-2 gene expression.

Urine chemistry and enzymes

Creatinine clearance was assessed using 24-h urine collections and blood samples to evaluate kidney function. Creatinine clearance (CCr) was measured using the formula: CCr = urine creatinine × 24-h urine volume/serum creatinine.

To determine the presence of renal tubular epithelial cell injury, *N*-acetyl glucosaminidase (NAG) and β-2 microglobulin levels in urine were quantified.

COX-2 gene expression

To confirm the expression of the COX-2 gene after SWL and inhibition of the expression of the COX-2 gene after administration of celecoxib, we compared the expression of the COX-2 gene using Western blotting between the three groups.

Kidney homogenates were obtained by vortexing with glass beads at 6,000 rpm for 15 s twice in 1.3 vol homogenate buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and phenylmethylsulphonyl fluoride) and then centrifuging at 12,000*g* for 10 min. Homogenates dissolved in SDS sample buffer were separated on 10% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. Membranes were blocked by incubating the membranes for 1 h in 5% skim milk—TBST (50 mM Tris–Cl (pH 7.5), 200 mM NaCl, 0.1% Tween 20). The membranes were then incubated with a goat polyclonal anti-Cox-2 antibody (1:500 dilutions, santacluz sc-1745) and a mouse monoclonal anti-β-actin antibody (1:10,000, Sigma) in 5% skim milk—TBST for 1 h. The resulting membranes were washed three times with 0.1% TBST, incubated with horseradish peroxidase-conjugated anti-mouse IgG and anti-goat IgG antibodies (Zymed, Invitrogen, CA, USA), respectively, at 1:3,000 dilution in 5% skim milk—TBST for 1 h. After washing three times with 0.1% TBST, the membranes were incubated with SuperSignal west pico solution (Pierce, IL, USA) and exposed to X-ray film.

Statistical analysis

Results are given as the mean ± standard deviation (SD). The statistical significance of data was evaluated by ANOVA analysis. P values <0.05 were considered significant.

Results

24-h urine chemistry and enzyme results in pre- and post-SWL

Prior to SWL, all of the groups of rats had similar levels of NAG and β-2 microglobulin in the 24-h urine collections (Table 1). After SWL, the rats in all groups retained relatively normal renal function, as assessed by a lack of significant differences in CCr levels.

However, compared with the sham group, rats that had the SWL procedure produced a significant increase in excretion of the urinary tubular enzyme, i.e., urinary NAG and β-2 microglobulin excretions were significantly higher in the control group than in the sham group, indicating the presence of renal tubular injury.

Urinary NAG excretions were also significantly higher in the COX-2 groups than in the sham group. However, NAG and β-2 microglobulin excretions were significantly lower in the COX-2 group than in the control group (Table 1; Fig. 1a, b).

Analyzing in the same group cohort, sham group showed no significant difference in regard to CCr, NAG excretion, and β-2 microglobulin excretions. Control group showed no significant difference in regard to CCr. However, NAG excretion and β-2 microglobulin excretions were significantly increased after SWL. COX-2 group showed no significant difference in regard to CCr. NAG excretion and β-2 microglobulin excretions were also increased but not significant.

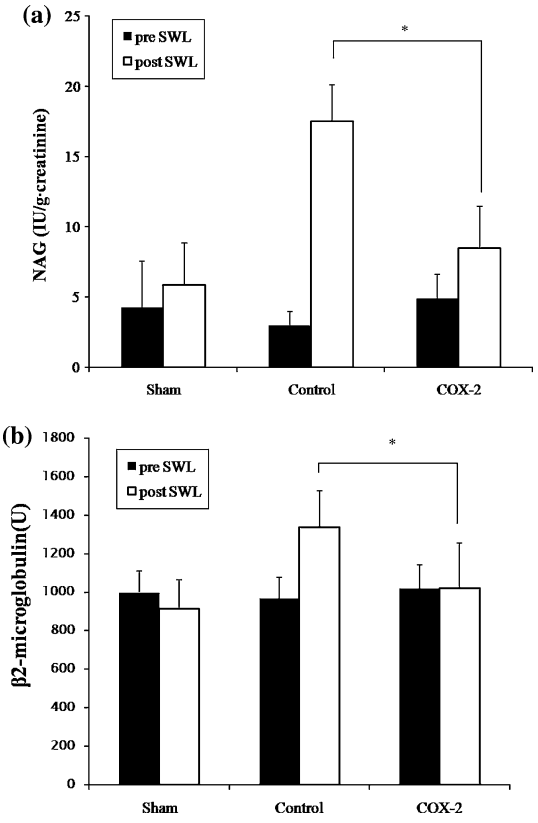

Fig. 1 **a** Prior to SWL, control and COX-2 group had similar levels of NAG. After SWL, urinary NAG excretions were significantly lower in the COX-2 group than in the control group. *$P < 0.05$ versus control post-SWL group. **b** Prior to SWL, control and COX-2 group had similar levels of β-2 microglobulin. After SWL, urinary β-2 microglobulin excretions were significantly lower in the COX-2 group than in the control group. *$P < 0.05$ versus control post-SWL group

COX-2 gene expression

Western blot showed no increase in the expression of the COX-2 gene in any of the rats in the sham group. However, immunoblot showed a significant increase in the expression

Table 1 24-h urine chemistry and enzyme results

	Pre-SWL			Post-SWL		
	Sham group	Control group	COX-2 group	Sham group	Control group	COX-2 group
CCr (ml/min)	1,761 ± 414	1,942 ± 293	1,368 ± 300	1,860 ± 480	2,073 ± 345	1,675 ± 240
NAG (IU/g creatinine)	4.3 ± 3.3	3.0 ± 1.0	4.9 ± 1.7	5.9 ± 3.0	17.5 ± 2.6*,‡	8.5 ± 3.0*,†
β-2 microglobulin (U)	1,000 ± 110	968 ± 109	1,020 ± 123	916 ± 150	1,337 ± 190*,‡	1,022 ± 233†

Value presented as Mean ± SE

CCr creatinine clearance, *NAG N*-acetyl glucosaminidase

* $P < 0.05$ versus sham post-SWL group

† $P < 0.05$ versus control post-SWL group

‡ $P < 0.05$ versus pre-SWL same group

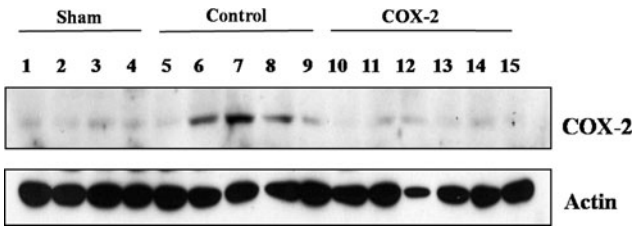

Fig. 2 Western blot showed a significant increase in the expression of the COX-2 gene in the control group, which was prevented by celecoxib treatment

of the COX-2 gene in the control group, which was prevented by celecoxib treatment (Fig. 2).

Discussion

With improvements in the shock wave lithotripter, the number of patients undergoing SWL treatment has markedly increased. Usually SWL is known to be safe and does not do significant harm to the kidneys. However, even when the shock wave is focused on renal stones, the adjacent normal tissues surrounding the renal stones are included in the focus area and are vulnerable to injury.

Renal injury during SWL has been demonstrated in various studies using radiologic and histologic examinations [7–10]. Renal injuries related to SWL are usually transient, but permanent dysfunction has also been reported [11].

Since Akdas et al. [12] showed that renal dysfunction does not recover until 1 week after SWL, many clinicians have treated patients at 1-week intervals. To reduce renal injury and dysfunction, various drugs and chemicals have been evaluated [7, 13–15]. Recent studies [7, 14] have shown the efficacy of calcium channel blockers and free radical scavengers selenium. However, these drugs have no additional effect on pain and also have hypotension as a side effect, so these drugs are not routinely used by urologists.

Another side effect of SWL is pain. To reduce pain, opioid analgesics and NSAIDS have generally been recommended. NSAIDs are among the most commonly prescribed drugs worldwide because of their effectiveness in the treatment of pain, inflammation, and fever. Their anti-inflammatory action is due to the inhibition of activity of cyclo-oxygenase-1 (COX-1) and COX-2 enzymes, which are responsible for the synthesis of prostaglandins (PGs). Most traditional NSAIDs are non-selective inhibitors of COX-1 and COX-2, and result in gastrointestinal and renal side effects. Therefore, the use of NSAIDS is also limited because of renal toxicity to patients, especially those who have reduced renal function due to obstruction and those who would have transient renal dysfunction due to SWL.

Selective inhibitors of COX-2 are the coxibs, which are superior to classical NSAIDs because of less gastrointestinal side effect. COX-2 is constitutively expressed in organs like the brain and kidneys. It is responsible for the synthesis of PGs from arachidonic acid. PGs have a role in inflammation, maintenance of gastric mucosal integrity, and renal microvascular hemodynamics.

In the past, it has been reported that COX-2 inhibitors also have a negative effect on the kidney. Kitahara et al. [16] reported that COX-2 inhibitors impair glomerular capillary repair in experimental membranous glomerulonephritis.

However, many studies have reported that COX-2 inhibitors do not have renal toxicity and can even prevent renal injury. Reichman et al. [17] reported that renal function and morphology were not affected in vivo by COX-2 inhibitors, as opposed to the non-selective COX inhibitor, indomethacin. Dey et al. [18] reported that COX-2 inhibition decreases pro-inflammatory chemokines and tubulointerstitial fibrosis. They suggested that altered vascular arachidonic acid enzymes contribute to renal damage, and that COX-2 inhibition decreases glomerular injury. Other studies have shown that COX-2 inhibition is associated with modest nephroprotection in DM animal and partial nephrectomy models [19].

Our results also demonstrated that COX-2 inhibition is not associated with decreased renal function. The CCr levels were not different between the NS and COX-2 groups, and these results are consistent with previous reports [17–19]. However, we did not expect any significant differences in CCr in control and COX-2 inhibitor group compared with sham group. As NAG and β-2 microglobulin are the markers of renal tubular injury, urinary excretion of these enzymes was increased after SWL in control and COX-2 groups. However, creatinine clearance showed no significant change compared with sham group. We thought that opposite kidney would compensate the reduced function in kidney injured by SWL.

In this study, COX-2 group showed reduced urinary excretion of NAG and β-2 microglobulin compared with control group. This is the first study that has shown renal tubular injury after SWL could be attenuated by a COX-2 inhibitor. Therefore, this study suggests that celecoxib would be very useful prior and after SWL because of the renal protection effect, as well as the analgesic effect.

The mechanism of tissue injury by SWL is thought be a direct force injury, cavitation effect, shearing effect, and cohesive force effect. Recently, hemodynamic changes and subsequent biochemical injury by oxygen radicals are now thought be the mechanism of injury. In other words, microvessel injury by SWL causes renal tissue ischemia, and oxygen radicals from the ischemia–reperfusion pathway induce renal tissue injury. Ischemia–reperfusion injury is

induced by activation of the arachidonic acid cascade following the induction of COX-2, and our results showed increased COX gene expression after SWL compared to the sham operation group. Therefore, our study supports the ischemia–reperfusion tissue injury theory of SWL-induced renal injury. This is the first study that has shown renal tubular injury after SWL is mediated by COX gene expression.

Recently, several studies have reported that COX-2 inhibitors could reduce ischemia–reperfusion injuries in various organs, including the small bowel, liver, and kidneys [3–5]. Feitoza et al. [5] reported that blockade of COX-2 resulted in a decrease in tubular necrosis and improvement in renal function in an ischemia reperfusion animal model. These studies are consistent with our results that the COX-2 inhibitor, celecoxib, reduced biochemical parameters.

Our study has some limitations. First, although we postulated the mechanism of renal injury by SWL as ischemia and reperfusion by the COX-2 expression pathway, we did not evaluate the oxidative injuries in renal tissues. A further biochemical study evaluating oxidative injuries between the control and COX-2 inhibitor groups by measuring malondialdehyde (MDA) and superoxide dismutase (SOD) levels in renal tissues would validate the SWL injury pathway hypothesis. We also did not perform PCR to determine Cox-2 gene expression and investigate the level of prostaglandin E2 to show increased activity of Cox -2 and its reduction by treatment with the COX-2 inhibitor.

Second, we did not compare the COX-2 inhibitor with other NSAIDS (non-selective inhibitors of COX-1 and COX-2). If the renal injury is mediated by the COX-1 gene as well as the COX-2 gene, non-specific NSAIDS also might reduce renal tubular injuries. But non-specific NSAIDS could be the cause of gastric ulcers and our focus was the effect of the COX-2 inhibitor on renal tubular injury, so we did not perform experiments comparing the two drugs.

Finally, we did not examine the tissue injury in histology or immunohistochemistry that would provide more confirmative information.

In spite of these limitations, this preliminary study is the first study showing that renal tubular injury after SWL could be attenuated by a COX-2 inhibitor. Following preclinical and clinical studies will give more information about the effect of the COX-2 inhibitor on renal tubular injury.

Conclusion

SWL increased COX-2 gene expression in renal tissues and COX-2 inhibition reduced renal tubular injury after SWL. Biochemical findings also supported a renal protective effect of celecoxib on SWL. Therefore, this study suggests that celecoxib may be useful prior and after SWL because of the renal protective and analgesic effects. However, to apply to clinical practice, following studies must be needed.

Acknowledgment This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2007-331-E00151).

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