Quercetin attenuates cyclooxygenase-2 expression in response to acute ureteral obstruction

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Carlsen I, Frøkiær J, Nørregaard R. Quercetin attenuates cyclooxygenase-2 expression in response to acute ureteral obstruction. Am J Physiol Renal Physiol 308: F1297–F1305, 2015. First published March 25, 2015; doi:10.1152/ajprenal.00514.2014.—Unilateral ureteral obstruction (UUO) is associated with increased hydrostatic pressure, inflammation, and oxidative stress in the renal parenchyma. Previous studies have demonstrated marked cyclooxygenase (COX)-2 induction in renal medullary interstitial cells (RMICs) in response to UUO. The aim of the present study was to evaluate the effect of quercetin, a naturally occurring antioxidant, on COX-2 induction in vivo and in vitro. Rats subjected to 24 h of UUO were treated intraperitoneally with quercetin (50 mg·kg⁻¹·day⁻¹). Quercetin partly prevented COX-2 induction in the renal inner medulla in response to UUO. Moreover, RMICs exposed to conditions associated with obstruction, inflammation (produced by IL-1β), oxidative stress (produced by H₂O₂), and mechanical stress (produced by stretch) showed increased COX-2 expression. Interestingly, quercetin reduced COX-2 induction in RMICs subjected to stretch. Similarily, PGE₂ production was markedly increased in RMICs exposed to stretch and was reversed to control levels by quercetin treatment. Furthermore, stretch-induced phosphorylation of ERK1/2 was blocked by quercetin, and inhibition of ERK1/2 attenuated stretch-induced COX-2 induction in RMICs. These results indicate that quercetin attenuates the induction of COX-2 expression and activity in RMICs exposed to mechanical stress as a consequence of acute UUO and that the MAPK ERK1/2 pathway might be involved in this quercetin-mediated reduction in COX-2.

Cyclooxygenase-2; quercetin; unilateral ureteral obstruction; renal medullary interstitial cells; heat shock protein

Quercetin, a plant-based antioxidant, is one of the most common flavonoids. The primary sources of dietary quercetin are fruits and vegetables, with red wine and tea being rich sources as well (11). Quercetin is known for having beneficial pharmacological functions, such as anti-inflammatory and antiapoptotic effects (7, 16, 22). In vivo and in vitro studies have demonstrated that quercetin attenuates inflammation by suppression of inflammatory mediators such as MAPKs and NF-κB, thereby reducing expression and release of cytokines as TNF-α and IL-1β (7, 22, 38). Quercetin also functions as an inhibitor of heat shock proteins (HSPs) by inhibiting the activity of HSF transcription factor (HSF)-1 (12). Importantly, it has been recently shown that quercetin accumulates in the kidney (3), suggesting that the kidneys may be considered a target for the potential beneficial effects of quercetin. In combinational therapy with another plant-derived flavonoid, quercetin has shown to reduce unilateral ureteral obstruction (UUO)-induced renal fibrosis in rats (38).

During UUO, urine transport is impaired, which leads to increased hydrostatic pressure, inflammation, and oxidative stress in the renal parenchyma. These effects can be detrimental to the kidney and lead to progressive loss of function. In vivo studies have demonstrated marked induction of cyclooxygenase (COX)-2 located to renal medullary interstitial cells (RMICs) and in the inner medulla (IM) in rats subjected to both UUO and bilateral ureteral obstruction (4, 26, 27). COX-2 is the transiently expressed isofrom of two COXs (COX-1 and COX-2) and is induced in response to inflammation and oxidative stress (4, 27). COX converts arachidonic acids into prostaglandins, and PGE₂ is the most abundant prostaglandin in the kidney (10). PGE₂ is essential for the regulation of renal hemodynamics as well as renal handling of salt and water (10). One of the key characteristics of acute ureter obstruction is that RMICs are exposed to exaggerated stress as a consequence of mechanical, inflammatory, and oxidative changes in the renal parenchyma (17). In vitro studies have revealed increased COX-2 expression in RMICs subjected to the above-mentioned stress conditions (4, 27). In addition, previous studies have indicated that quercetin prevented COX-2 induction in response to inflammation and oxidative stress (2, 22, 24, 29, 34).

The aim of the present study was to investigate the effect of a natural antioxidant, quercetin, on COX-2 expression in response to acute UUO and explore COX-2 expression and activity in quercetin-treated RMICs exposed to obstruction-associated conditions. We hypothesized that quercetin may prevent increased COX-2 expression and activity in rats subjected to 24-h UUO and in RMICs exposed to mechanical, inflammatory, and oxidative stress. Attenuated COX-2 induction through quercetin treatment may reduce inflammation in the obstructed kidney. In addition, the effects of quercetin on HSP expression were examined.

METHODS

Experimental animals. Animal protocols were approved by the Animal Experiments Inspectorate under the Danish Veterinary and Food Administration (license no. 2010/561-1830). Experiments were performed on male Wistar rats (250 ± 5 g) that were allowed free access to a standard rodent diet and tap water. During the experiments, rats were housed in cages with a 12:12-h light-dark cycle, temperature of 21 ± 2°C, and humidity of 55 ± 2%. Animals were divided into four groups: 1) sham operation (sham), 2) sham with quercetin, 3) UUO, and 4) UUO with quercetin (n = 8 animals/group). The quercetin dose of 50 mg·kg⁻¹·day⁻¹ (dissolved in 25% ethanol) was based on previous in vivo studies in rats (22, 24). Treatment started 1 day before surgery. Sham and UUO rats were treated with 25% ethanol intraperitoneally daily as a vehicle.

During surgery, rats were anesthetized with sevoflurane and placed on a heating pad to maintain body temperature at 37–38°C. Through a midline abdominal incision, the left ureter was exposed and occluded with a 5-0 silk ligature. Sham control rats were prepared in
parallel. Kidneys were prepared for quantitative PCR, semiquantitative immunoblot analysis, and immunohistochemical experiments.

**Blood sampling.** Twenty-four hours after UUO, rats were anesthetized with sevoflurane. The abdomen was opened by a midline incision allowing free dissection of the aortic bifurcation. A blood sample of ~5 ml was collected, and plasma analysis of creatinine, urea, and osmolality levels was performed using a Roche Cobas 6000 analyzer (Roche Diagnostics).**

**Cell culture and in vitro experiments.** Primary cell cultures of RMICs were obtained from Dr. C. Maric (University of Mississippi Medical Center). Passages 10–20 were used for experiments after cells were cultured in supplemented RPMI medium as previously described (4). Cells were incubated at 37°C in humidified atmosphere containing 5% CO₂. At 80–90% confluence, cells were treated with quercetin (30 g/ml) and stimulated for 6 h with IL-1β (5 ng/ml) or H₂O₂ (200 μM) or stretched for 4 h to expose cells to inflammatory, oxidative, or mechanical stress, respectively. For ERK1/2 inhibition, cells were treated with PD-98059 (30 μM).

**Flexcell apparatus.** The effect of stretch on RMICs was studied using the Flexcell FX-5000T system (Dunn Labotechnik, Asbach, Germany), which applies stretch to adhering cell types. RMICs were cultured on collagen-coated BioFlex six-well plates (Dunn Labotechnik) and exposed to 20% uniform static stretch for 4 h. The determination of optimal stretch conditions was performed as previously described by Carlsen et al. (5). Cells not subjected to stretch were used as controls. The complete system was placed in an incubator to maintain correct conditions during the stretch experiment.

**PGE₂ assay.** PGE₂ in serum-free culture media was measured using a Prostaglandin E₂ ELISA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s protocol. RMICs were subjected to 4 h of 20% uniform static stretch using the Flexcell FX-5000T system. Control cells were not exposed to stretch. The PGE₂ concentration in RMICs was measured by a DetectX PGE₂ Multi-Format EIA Kit (Arbor Assays, Ann Arbor, MI) according to the manufacturer’s protocol.

**Immunoblot analysis.** During preparation of proteins for immunoblot analysis, RMICs were collected and lysed using mammalian protein extraction reagent (Thermo Scientific, Vedbaek, Denmark) as previously described (4). IM tissue was dissected, homogenized, and centrifuged at 1,000 g at 4°C for 15 min. The supernatant was used for immunoblots. Samples were run on a 12% polyacrylamide gel, transferred to a nitrocellulose membrane, and immunolabeled (4). The primary antibodies used are shown in Table 1. GAPDH was used as a housekeeping gene. Proteins were expressed relative to total protein.

**RNA extraction, cDNA synthesis, and quantitative PCR.** Total RNA extraction from the IM was performed as previously described (4). Briefly, cDNA was synthesized from 0.5 μg total RNA using a Fermentas RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, Burlington, ON, Canada) according to the manufacturer’s instructions. Quantitative PCR was performed using 100 ng cDNA as a template for PCR amplification. We used Maxima Hot Star PCR Master Mix according to the manufacturer’s instructions (Thermo Scientific). Each sample, including the standards, was prepared in duplicate in 96-well plates, and PCR was performed for 40 cycles consisting of denaturation for 30 s at 95°C followed by annealing and polymerization at 60°C for 1 min. Emitted fluorescence was detected during the annealing/extension step in each cycle. Specificity was assured by postrun melting curve analysis, and reaction products were separated on agarose gels and imaged. 18S rRNA was used as a housekeeping gene. The primer sequences used are shown in Table 2.

**Immunohistochemistry.** After rats had been euthanized, the kidneys were fixed with 4% paraformaldehyde in 0.1 M PBS (pH 7.4) by retrograde perfusion through the abdominal aorta. The kidneys were fixed for 1 h and washed three times. Fixed kidneys were then dehydrated, embedded in paraffin, and cut into 2-μm slices on a rotary microtome. Labeling and antigen exposure were performed as previously described by Nilsson et al. (25) using COX-2 antibodies. Hematoxylin and eosin (H&E) staining was performed using a standard procedure. Tissue was deparaffinized in xylene and rehydrated in ethanol (100% down to 70%). Nuclei were stained with counterstain before mounting.

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**Table 1. Primary antibodies used for immunoblot experiments**

<table>
<thead>
<tr>
<th>Target</th>
<th>Dilution</th>
<th>Catalog Number</th>
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<tr>
<td>COX-2</td>
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<td>160126</td>
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<td>p44/42 (ERK)</td>
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<td>4695</td>
<td>Cell Signaling</td>
<td>Rabbit</td>
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<tr>
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<td>4370</td>
<td>Cell Signaling</td>
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<td>Rabbit</td>
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<tr>
<td>Phosphorylated p38 MAPK</td>
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<td>4631S</td>
<td>Cell Signaling</td>
<td>Rabbit</td>
</tr>
<tr>
<td>HSP27</td>
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Levels of proteins involved in the mediation of cellular responses to unilateral ureteral obstruction (UUO) and the effects of quercetin were measured by immunoblot analysis. COX, cyclooxygenase; HSP, heat shock protein.

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**Table 2. Primer sequences**

<table>
<thead>
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<th>Molecular Size, bp</th>
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<td>5'-CTCTGCAGCTTCGTGCTCAGAAGC-3'</td>
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<td>5'-GGCAATTAGACAGATGCTGCCGAGGAAAGC-3'</td>
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</tr>
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<td>IL-1β</td>
<td>5'-CCGACACAGATTGCTGCTCAGAAGC-3'</td>
<td>5'-CCGACACAGATTGCTGCTCAGAAGC-3'</td>
<td>M98820.1</td>
</tr>
<tr>
<td>MCP-1</td>
<td>5'-TCCGACACAGATTGCTGCTCAGAAGC-3'</td>
<td>5'-TCCGACACAGATTGCTGCTCAGAAGC-3'</td>
<td>M98820.1</td>
</tr>
<tr>
<td>KIM-1</td>
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<td>AF035965.1</td>
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<td>5'-TCCGACACAGATTGCTGCTCAGAAGC-3'</td>
<td>M98820.1</td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemoattractant protein-1; KIM-1, kidney injury molecule-1.
hematoxylin followed by cytosolic staining with eosin and finally dehydrated in ethanol (95% up to 100%).

**Immunofluorescence of the actin cytoskeleton.** RMICs were treated and stretched as described above and then fixed for 15 min in 4% paraformaldehyde in PBS. Afterward, cells were washed with 0.1% Triton X-100 in PBS for 5 min followed by three washes in PBS and incubated for 30 min with TRITC-phalloidin (Sigma-Aldrich, St. Louis, MO, 1:200) and 4’,6-diamidino-2-phenylindole (1:200) in PBS. After incubation, cells were washed with PBS. The silicone membranes were removed from the six-well plates and mounted on slides using Vector mounting media. To quantify the potential changes induced by quercetin treatment for analysis of actin stress fiber organization, we developed a scoring system, which includes an established actin scoring system (20, 36). Evaluation and counting of cells displaying actin filament characteristics and distribution was scored as follows: 1 = >90% thick filaments, 2 = at least two thick filaments at the center of the cell, and 3 = fine filaments at the center of the cell. To determine the actin filament distribution, 50 cells for each condition were analyzed under fluorescence microscopy in a blinded fashion.

**Statistical analysis.** Values are expressed as means ± SE. For comparisons between experimental groups, one-way ANOVA and Student’s t-test with Bonferroni adjustment were performed. Statistical significance was accepted at \( P < 0.05 \).

**RESULTS**

Quercetin attenuated COX-2 induction in the renal IM in response to 24-h UUO. To examine whether quercetin prevented COX-2 induction in response to 24-h UUO, expression of COX-2 in the IM was studied. COX-2 protein and mRNA expression were increased in rats subjected to UUO compared with sham rats (Fig. 1, A and B). Quercetin attenuated COX-2 protein induction in rats subject to UUO (Fig. 1A). To investigate COX-2 activity, we measured PGE2 production in the IM. PGE2 production increased in UUO rats compared with sham rats. Quercetin administration did not change the IM production of PGE2 in rats subjected to UUO (Fig. 1C).

Immunohistochemical analysis showed stronger COX-2 label-
Quercetin did not change kidney function or renal damage in response to 24-h UUO. To document the effects of 24-h UUO, plasma creatinine, urea, and osmolality as well as kidney weight were measured in the four groups (Table 3). Kidney weight and plasma creatinine, urea, and osmolality were increased in UUO rats compared with sham rats. Quercetin treatment attenuated the increase in plasma urea.

H&E staining was performed to study renal morphology and tubular dilatation. The data showed no visible difference in tubular dilatation or tissue damage between UUO and sham rats. Quercetin treatment did not affect renal morphology (Fig. 3A). To examine the effect of quercetin on the regulation of inflammation and injury markers, we measured mRNA levels of IL-1β, TNF-α, kidney injury molecule-1, monocyte chemoattractant protein-1, and the macrophage marker CD68 (Table 4). All measured markers showed increased expression in UUO rats compared with sham rats. Quercetin did not change the expression of any of the markers in rats subjected to 24-h UUO. Furthermore, we examined the development of oxidative stress by analyzing the expression of the antioxidants SOD1 and SOD2. SOD1 expression was decreased in UUO rats compared with sham rats, whereas SOD2 was not changed. Quercetin administration did not change SOD1 or SOD2 expression in response to 24-h UUO (Fig. 3B).

Quercetin reduced COX-2 expression and PGE₂ production in RMICs exposed to mechanical stress. In response to ureteral obstruction, RMICs are subjected to oxidative, inflammatory, and mechanical stress (17). To mimic these stress factors, RMICs were subjected to H₂O₂, IL-1β, or mechanical stretch. RMIC subjected to stretch were cultured on collagen-coated Bio-flex plates and exposed to stretch for 4 h using the Flexcell FX-5000T system. COX-2 expression was induced in RMIC during oxidative, inflammatory, and mechanical stress. Interestingly, quercetin attenuated COX-2 induction in RMICs exposed to stretch. However, no effect was observed in RMICs exposed to oxidative and inflammatory stress (Fig. 4, A–C). Increased COX-2 expression was accompanied by a corresponding increase in COX enzyme activity. As shown in Fig. 4D, PGE₂ production markedly increased during stretch. This increase was normalized to the control level by quercetin. Furthermore, HSP32 protein expression was increased in RMICs exposed to mechanical stress, and this effect was

The effects of quercetin on HSP27, HSP32, and HSP70 expression in the IM in response to 24-h UUO were examined. Immunoblot analysis demonstrated that both phosphorylated HSP27 and HSP32 were increased in rats subjected to UUO compared with sham rats (Fig. 2, A–C). HSP27 and HSP70 protein levels were not changed (Fig. 2A). Quercetin treatment did not significantly change the expression of any of the HSPs examined (Fig. 2, A–C).

Table 3. Kidney weight and functional plasma parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + Quercetin</th>
<th>UUO</th>
<th>UUO + Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney weight, g/g body wt</td>
<td>3.69 ± 0.08</td>
<td>3.55 ± 0.05</td>
<td>5.88 ± 0.13</td>
<td>5.91 ± 0.06</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>16.5 ± 0.5</td>
<td>17.5 ± 0.8</td>
<td>28.4 ± 1.1*</td>
<td>28.5 ± 0.8</td>
</tr>
<tr>
<td>Plasma urea, mmol/l</td>
<td>6.1 ± 0.2</td>
<td>6.3 ± 0.3</td>
<td>8.4 ± 0.3*</td>
<td>7.5 ± 0.2†</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kg H₂O</td>
<td>309 ± 4.3</td>
<td>311 ± 6.2</td>
<td>333 ± 5.6*</td>
<td>351 ± 2.7</td>
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</table>

Values are mean ± SE; n = 8. Kidney weights are listed as average weights of left kidneys. *P < 0.05 compared with sham operation (sham); †P < 0.05 compared with UUO.

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abolished by quercetin treatment (Fig. 4E). Taken together, these findings indicate that quercetin attenuates COX-2 expression and activity as well as HSP32 expression in RMICs subjected to mechanical stress.

Regulation of MAPK pathway in quercetin-treated RMICs exposed to mechanical stress. We (27) have previously shown that the MAPK pathway plays a role in the regulation of COX-2 expression in RMICs subjected to mechanical stress. Therefore, we investigated whether quercetin may act via MAPK. To test this possibility, we determined the effect of quercetin on the activation of ERK1/2 and p38, which have been previously shown to be activated in RMICs exposed to stretch (27). Activation of ERK1/2 and p38 was determined by immunoblot analysis using antibodies specific for the phosphorylated proteins, as previously described. These data demonstrated that stretch increased the phosphorylation of ERK1/2 compared with control cells and that quercetin abolished the stretch-induced activation of ERK1/2 in RMICs (Fig. 5A). Likewise, we examined the effect of quercetin on the regulation of ERK1/2 in vivo, but quercetin did not change the increased phosphorylation of ERK1/2 in response to 24-h UUO (data not shown). However, p38 phosphorylation was not increased during 4 h of stretch; nevertheless, quercetin treatment increased the level of phosphorylation (Fig. 5A).

To investigate whether the effects of quercetin on the ERK1/2 pathway could play a role in COX-2 induction in RMICs exposed to mechanical stress, RMICs were exposed to stretch and the ERK1/2 inhibitor PD-98059. Immunoblot analysis demonstrated that quercetin combined with inhibition of the ERK1/2 signaling cascade had a greater inhibitory effect on stretch-induced COX-2 expression in RMICs than quercetin alone (Fig. 5B). In summary, these results indicate that quercetin might act on the ERK1/2 pathway, which could play an important role in the induction of COX-2 in response to mechanical stress.

**Distribution of actin filaments during mechanical stress in RMICs.** Previous studies have demonstrated an interaction between quercetin and actin filaments (23) and that cytoskeletal alterations are linked to increased COX-2 expression (8) and activity (32). To determine the effect of quercetin on the distribution of actin filaments, RMICs were exposed to stretch for 4 h. Actin filaments were visualized with TRITC-phalloidin under fluorescence microscopy. We used a scoring system to quantify the distribution of actin filaments into categories according to the criteria defined above in METHODS. Non-stretched control cells showed a large number of cells with thick, well-aligned stress fibers running parallel in one direction on the cell (Fig. 6A). Stretched cells demonstrated a large number of cells with thin fine actin filaments and had a significantly reduced number of thick actin filaments compared with control cells (Fig. 6, B and D). Quercetin treatment did not significantly change the distribution or thickness of actin filaments in RMICs exposed to stretch (Fig. 6, C and D).

**DISCUSSION**

In the present study, renal COX-2 regulation in vivo and in vitro was evaluated in response to quercetin treatment. Our findings demonstrated that quercetin attenuated COX-2 induc-

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**Table 4. mRNA levels of inflammatory and injury markers**

<table>
<thead>
<tr>
<th>Relative mRNA Expression</th>
<th>Sham</th>
<th>Sham + Quercetin</th>
<th>UUO</th>
<th>UUO + Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-α</strong></td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td><strong>IL-1β</strong></td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.9 ± 0.7</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td><strong>KIM-1</strong></td>
<td>1.0 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>1871 ± 131</td>
<td>2031 ± 347</td>
</tr>
<tr>
<td><strong>MCP-1</strong></td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>6.7 ± 0.7</td>
<td>5.1 ± 0.7</td>
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<tr>
<td><strong>CD68</strong></td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.4 ± 0.1</td>
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</table>

Values are means ± SE. 18S rRNA was used as a reference gene, and all data were normalized to 18S. *P < 0.05, UUO compared with sham.
tion in the IM in rats subjected to 24-h UUO and in RMICs exposed to inflammatory, oxidative, and mechanical stress. Furthermore, quercetin normalized stretch-induced COX-2 expression in RMICs exposed to inflammatory, oxidative, and mechanical stress. Quercetin attenuated the stretch-induced COX-2 expression. PGE2 production was increased in response to 4-h stretch, which was completely abolished by quercetin treatment. E: Immunoblot analysis of HSP32 expression in RMICs subjected to stretch. HSP32 was increased in RMICs exposed to stretch, and this increase was prevented by quercetin treatment. Densitometric analysis and representative immunoblots are shown. Values are means ± SE; n = 4–6. *P < 0.05 compared with control cells; #P < 0.05 compared with stretched cells.

Fig. 4. Effect of quercetin on COX-2 expression and activity in RMICs exposed to inflammatory, oxidative, and mechanical stress. A–C: Immunoblot analysis for COX-2 expression in RMICs subjected to 6 h of 5 ng/ml IL-1β stimulation (A), 6 h of 200 μM H2O2 stimulation (B), or 4 h of static stretch (C). Densitometric analysis and representative immunoblots demonstrated increased COX-2 expression in RMICs exposed to inflammatory, oxidative, and mechanical stress. Quercetin attenuated the stretch-induced COX-2 expression. D: PGE2 production was increased in response to 4-h stretch, which was completely abolished by quercetin treatment. E: Immunoblot analysis of HSP32 expression in RMICs subjected to stretch. HSP32 was increased in RMICs exposed to stretch, and this increase was prevented by quercetin treatment. Densitometric analysis and representative immunoblots are shown. Values are means ± SE; n = 4–6. *P < 0.05 compared with control cells; #P < 0.05 compared with stretched cells.

Quercetin attenuated COX-2 expression in vivo and in vitro. Our data showed that quercetin prevented COX-2 induction in the IM in rats subjected to UUO and in RMICs exposed to stretch. Previous in vivo and in vitro studies have demonstrated that quercetin attenuated COX-2 induction (2, 22, 24, 34). As a consequence of UUO, RMICs are subjected to mechanical, inflammatory, and oxidative stress (17), and, consistent with our previous data, we demonstrated increased COX-2 expression in RMICs subjected to these stressful conditions (4, 27). Quercetin attenuated the induction of COX-2 in RMICs exposed to mechanical stress. As a result of decreased COX-2 expression, quercetin reduced PGE2 production in RMICs exposed to mechanical stress, suggesting that quercetin might reduce PGE2 biosynthesis via reduced COX-2. However, we cannot rule out that other mechanisms in the quercetin-mediated suppression of PGE2 can be involved, and a potential effect in blocking the release of arachidonic acid could play a role for the reduction in the synthesis of PGE2. Previous in vitro studies have shown that quercetin suppressed PGE2 production (34, 37). However, in vivo quercetin did not reduce UUO-induced PGE2 production in IM tissue. These apparently different findings in vivo relative to in vitro increase the possibility that different signaling pathways can be involved in PGE2 production. COX-1 plays a role in the production of
PGE2 in the collecting ducts (10). We observed no change in COX-1 expression among the four groups, suggesting that COX-1 could be responsible for the production of PGE2 in quercetin-treated rats subjected to UUO. These results indicate that quercetin might regulate COX-2 expression and activity in RMICs exposed to mechanical stress. The following experiments were conducted to study the underlying mechanisms for the regulation of COX-2 of quercetin.

**Quercetin attenuated stretch-induced COX-2 expression and phosphorylation of ERK1/2.** MAPKs are involved in many cellular processes, and some of quercetin’s effects are mediated through the MAPK pathway (9, 22, 34). In rats exposed to lead-induced inflammation, quercetin had renoprotective effects by modulating MAPK ERK1/2, p38, and JNK (22). In IL-1β-stimulated fibroblasts, quercetin prevented increased COX-2 expression and activity by inhibiting the phosphorylation of ERK1/2, p38, and JNK (34). Furthermore, we demonstrated that p38 MAPK is important for the regulation of COX-2 in RMICs subjected to 6-h pressure (4) and that both ERK1/2 and p38 MAPK pathways were central for COX-2 induction in RMICs exposed to stretch for 2 h (27). To explore some of the potential underlying mechanisms behind the quercetin-mediated effects on COX-2 regulation, we focused on ERK1/2 and p38 MAPK, which we have previously shown are involved in COX-2 regulation in RMICs (4, 27). In vitro experiments showed that quercetin reduced the stretch-induced phosphorylation of ERK1/2 after 4 h, although we did not observe any effect in vivo. These differences between the in vivo and in vitro findings could be caused by the mixture of different cell types in the protein homogenate that might mask the regulation of the ERK1/2 pathway in RMICs. Our in vitro findings are consistent with previous studies in neurons (33) and endothelial cells exposed to mechanical stress (14, 33). Furthermore, treatment with an ERK1/2 inhibitor attenuated COX-2 induction in RMICs exposed to mechanical stress. This indicated that quercetin could target the ERK1/2 pathway to inhibit COX-2 expression and activity in RMICs exposed to mechanical stress. However, we cannot discard the possibility that the reduction in COX-2 expression and reduction in ERK1/2 phosphorylation could be parallel events as inhibition of ERK1/2 alone is able to reduce COX-2 as well. We have previously shown activation of the p38 pathway in RMICs exposed to 2-h stretch (27). However, we were not able to induce phosphorylation of p38 after 4-h stretch in this study. Several studies have demonstrated that p38 phosphorylation is not only dependent on the force applied; time is also a very important factor, since p38 phosphorylation fluctuates over time (15, 28). In lipopolysaccharide-treated macrophages, the expression of phosphorylated p38 peaked after 30 min, declined gradually thereafter, and, after 4-h lipopolysaccharide exposure, very low expression was observed (19). This could indicate that 4-h stretch might be in the time period when p38, after a period of activation, is inactivated by phosphatases, leaving phosphorylated p38 levels low. Quercetin induced p38 phosphorylation in RMICs exposed to stretch. A recent study (21) has also shown that quercetin increased phosphorylated p38 in β-cells exposed to a cytokine cocktail consisting of IL-1β, TNF-α, and interferon-γ, suggesting that quercetin might have both stimulatory and inhibitory effects on the activation of the p38 MAPK pathway.

**Quercetin attenuated stretch-induced HSP32 expression in RMICs.** Quercetin reduces expression of HSPs by inhibiting HSF-1 (12, 13). In endothelial cells subjected to heat, HSF-1 plays a role in the induction of COX-2 (30), and HSP27 is required for this COX-2 induction (1). These studies have indicated a link between HSPs and COX-2 expression, and one might speculate that quercetin may inhibit COX-2 expression through inhibition of HSPs. We have previously shown that phosphorylated HSP27 was increased in response to 6- and 12-h UUO (5). Our data also demonstrated the induction of phosphorylated HSP27 and HSP32 after 24-h UUO. HSP32 was similarly increased in RMICs exposed to mechanical stretch. Surprisingly, we only observed that quercetin attenuated stretch-induced HSP32 expression in vitro. No significant effect of quercetin was observed in vivo. The lack of effect in our in vivo experiments could be caused by the low dose of quercetin or the short treatment period, since other studies have used higher quercetin doses and longer treatment periods (18, 35). These findings reveal that quercetin had no inhibitory effect on HSP induction, indicating that the inhibitory effect of quercetin on COX-2 may not involve HSPs.
Fig. 6. Effect of quercetin on actin filament distribution in RMICs exposed to 4 h of static stretch. Labeling of F-actin in cultured RMICs with TRITC-phalloidin is shown. Magnification: × 40. A–C: control (A), stretched RMICs (B), and stretched RMICs treated with quercetin (C). D: cells were examined by fluorescence microscopy and sorted into the following categories: category 1, >90% thick filaments (blue bars); category 2, at least two thick filaments at the center of the cell (red bars); and category 3, fine filaments at the center of the cell (green bars). Stretched cells demonstrated a large number of cells with thin fine actin filaments and had a significantly reduced number of thick actin filaments compared with control cells. Fifty cells in each group were analyzed under fluorescence microscopy in a blinded fashion. Values are means ± SE. *P < 0.05 compared with control cells.

Actin stress filaments distribution in RMICs during stretch. The present study showed that quercetin reduced COX-2 expression and activity that was increased by mechanical stress in RMICs. A previous study (23) has investigated the relationship between quercetin and actin filaments, and studies in endothelial cells have demonstrated that cytoskeletal alterations were linked to increased COX-2 expression (8) and activity (32). This suggests that quercetin could reduce COX-2 by modulating cytoskeletal and actin filaments.

In our study, F-actin staining in control RMICs demonstrated thick, well-aligned actin filaments throughout the cytoplasm. After 4 h of stretch, actin filaments became fine and thin. Quercetin treatment did not significantly change the distribution or thickness for actin filaments in RMICs exposed to stretch, suggesting that the regulation of COX-2 may not be through cytoskeleton changes.

Impact of UUO on renal function and kidney biochemical parameters. UUO is associated with impairment of renal functions (4, 6, 39). Previous studies have shown that quercetin has anti-inflammatory and antioxidant effects (7, 31, 34). However, our data demonstrated no protective actions of quercetin in our acute 24-h UUO model. We observed that quercetin attenuated the increased plasma urea levels in response to UUO, consistent with a previous observation in rats with cadmium-induced nephrotoxicity (24). The lack of effects in our UUO model could be due to the quercetin dose used, since previous studies have shown that the effect of quercetin is dose dependent (37, 40). Likewise, one might consider that quercetin could be protective in a long-term UUO study using 3- or 7-day UUO models. However, this is beyond the scope of this study, since here we wanted to focus on the acute actions of quercetin.

Conclusions. In summary, the results of this study showed both in vivo and in vitro that quercetin inhibited COX-2 expression in RMICs and rats subjected to acute UUO. The MAPK ERK1/2 pathway may be involved in the quercetin-induced reduction in COX-2 expression in RMICs exposed to stretch. Our findings provide mechanistic insights into the action of the flavonoid quercetin, which attenuate the induction of COX-2 that occurs in response to mechanical stress as a consequence of ureteral obstruction.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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