Cyclooxygenase-2 Inhibitors Attenuate Increased Blood Pressure in Renovascular Hypertensive Models, but Not in Deoxycorticosterone-Salt Hypertension

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COX-2 is an inducible cyclooxygenase (COX) that has been reported to be expressed in the macula densa and surrounding cortical thick ascending limb in normotensive rats. The present study assessed the contribution of COX-2 in three different rat models of hypertension, each characterized by a different activation of the renal renin-angiotensin system. Mean blood pressure (MBP) in the rat 2 kidney-1 clip (2K1C) model was significantly reduced with a COX-2 selective inhibitor, NS-398 (10 mg/kg, p.o., twice a day) (vehicle-administered rats \((n = 8): 154 \pm 6 \text{ mmHg}; \) NS-398-administered rats \((n = 5): 128 \pm 10 \text{ mmHg}) . By contrast, a COX-1 selective inhibitor, mofezolac, did not lower MBP. Increased plasma renin activity \((23 \pm 8 \text{ ng/kg/h (n = 6)} \text{ vs. sham operation, } 2.4 \pm 0.9 \text{ ng/kg/h (n = 4)})\) was markedly reduced to \(6.8 \pm 2.7 \text{ ng/ml/h (n = 5)}\) by NS-398, but not by mofezolac. The development of 1 kidney-1 clip (1K1C) hypertension was also inhibited by NS-398 \((n = 12): 133 \pm 1 \text{ mmHg}; \) NS-398 \((n = 7): 122 \pm 3 \text{ mmHg}) accompanied by a reduction in plasma renin activity \((3.0 \pm 0.3 \text{ ng/ml/h, n = 4}) \text{ to } 1.0 \pm 0.2 \text{ ng/ml/h (n = 5})\) . The COX-2 inhibitor increased urinary excretions in the 1K1C model, but not in the 2K1C model. In a deoxycorticosterone acetate (DOCA)-salt model, plasma renin activity was markedly suppressed to less than \(0.3 \text{ ng/ml/h}.\) The COX-2 inhibitor caused no significant changes in MBP, plasma renin activity, or urinary excretion, suggesting that COX-2 made a lesser contribution in this model. Increased expression of COX-2 mRNA and protein was observed in the kidneys of 1K1C and 2K1C rats, but not in DOCA-salt rats. These results suggest that COX-2 plays a significant role in the development of 2K1C and 1K1C renovascular hypertension, in addition to making a substantial contribution to the diuretic effect in the 1K1C model. (Hypertens Res 2002; 25: 927–938)

Key Words: hypertension, renovascular, renin, cyclooxygenase

Introduction

Prostaglandins have wide-ranging effects in the body and are thought to be important mediators of inflammation. In the kidney, they also play important roles as autocrine/paracrine regulators to control several renal functions, such as renal blood flow and hemodynamics (1) and transepithelial NaCl transport.
in several nephron segments (2, 3). Cyclooxygenase (COX) plays a key regulatory role in prostaglandin synthesis, and occurs in both constitutive (COX-1) and inducible (COX-2) isoforms (4, 5). COX-1 is thought to provide cytoprotective effects (6), whereas COX-2 is both inducible and the major isoform of inflammatory cells (7). Reduction of prostaglandin production by inhibition of COXs appears to be the main mechanism of action of most non-steroidal anti-inflammatory drugs (NSAID) (8). The constitutive isoform (COX-1) is expressed in the kidney under normal conditions (9), and abundant levels of immunoreactive COX-1 have been localized in arterial vascular endothelial cells, medullary and cortical collecting ducts, medullary interstitial cells, and epithelial cells lining the Bowman’s capsule (10). This indicates that, at least to a certain degree, the enzyme is expressed constitutively, suggesting a physiological role. The inducible form of COX-2 is also expressed in the kidney (9). The presence of COX-2 has been reported in the thick ascending limb and medullary interstitial cells, and in a minority of macula densa cells (11). In a complex structure like the kidney, which contains several cell types with highly specialized morphology and function, the possible contribution of COX-2 to renal function can be elucidated by the knowledge of its precise cellular localization.

Both experimental models and clinical experience have indicated that prostaglandins are involved in the regulation of renal renin expression and release (12–16). In addition to physiological regulation of renin in response to alterations in intravascular volume status, prostaglandins have also been implicated in the mediation of increased renin production by the affected kidneys in renovascular hypertension (17, 18). Previous studies have suggested the baroreceptor reflex or abnormal sympathetic tone or both were the primary mediators of renin production and release in renovascular hypertension (12), thereby implicating the local release of prostaglandin from afferent arterioles (19). However, more recent studies have suggested that macula densa signaling may also contribute to the increased renin production in renovascular hypertension (15, 16).

The above findings led us to the hypothesis that prostaglandins synthesized by inducible COX-2 in the macula densa may trigger renin release and regulate blood pressure in renovascular hypertension. This hypothesis, in turn, suggests that COX inhibitors will have an anti-hypertensive effect on renovascular hypertension. In this study, we therefore evaluated the effects of COX-1 and COX-2 inhibitors on the blood pressure in three rat models in vivo: the 1 kidney-1 clip (1K1C) and 2 kidney-1 clip (2K1C) renovascular hypertensive models and the deoxycorticosterone acetate (DOCA)-salt induced hypertensive model.

Methods

Animals

Male Sprague-Dawley strain (SD) rats (specific pathogen-free; SLC Japan, Shizuoka, Japan) aged 5 weeks (body weight: 140 g) or 7 weeks (body weight: 210 g) were used. All animals were housed at constant humidity (60 ± 5%) and temperature (25 ± 1°C), and kept on a 12-h light/12-h dark cycle throughout the experiments. The rats were maintained on regular rat chow containing 0.5% (w/w) sodium chloride (Clea Japan Inc., Osaka, Japan). Tap water was available ad libitum throughout the study. The number of animals used for each experiment is stated in the corresponding section. This study was performed in accordance with the guidelines for animal experiments of Kitasato University School of Medicine.

Renovascular Hypertension

The 1K1C model and 2K1C model were used to investigate renovascular hypertension. Rats were anesthetized with diethyl ether. The kidney was exposed through a small flank incision, externalized, and carefully maintained with ophthalmic chalazion forceps. For clipping, a short section of the renal artery of the left kidney was separated by blunt dissection with a cotton pin, and a clip was placed closed to the aorta. Silver ribbon (NITOFLEX PIPESEAL No. 95; 1.5-mm width and 0.1-mm thickness; Nitto Denko Corp., Osaka, Japan) was used to form 8 1.5 mm clips with a 0.1-mm-wide slit for the 2K1C model or with a 0.3-mm-wide slit for the 1K1C model. The kidney was then gently pushed back into the retroperitoneal cavity. For right nephrectomy, two ligatures were passed around the renal vascular pedicle and the ureter was tied. The kidney was removed without the adrenal gland. The muscle layer was sutured, and the skin incision was closed with surgical staples. Control rats underwent a sham procedure that consisted of the entire surgery without the artery clipping. Blood pressure in rats of the 1K1C model was measured by the tail-cuff plethysmography method at 12 days and by intra-arterial catheter at 13 days after the clipping. For rats of the 2K1C model, the systolic blood pressure was measured at 7 days and the mean blood pressure was measured at 8 days after the clipping.

Deoxycorticosterone Acetate-Salt Hypertension

At 7-weeks-of-age, SD rats underwent unilateral nephrectomy under light diethyl ether anesthesia. The left renal artery and vein were partially exposed and ligated. The right kidney was removed after a flank incision as described above. The drinking water was replaced with 1% NaCl solution after the nephrectomy, and 10 mg/ml of deoxycorticosterone acetate (DOCA) (Nacalai Tesque, Kyoto, Japan) suspended in physiological saline containing 50 mg/ml of gum arabic was subcutaneously administered (50 mg/kg) twice a week as reported previously (20). Systolic blood pressure (SBP) was measured at 12 days after the nephrectomy and 12 days after the first administration of DOCA, and mean arterial blood pressure (MBP) was measured at 13 days after these...
Measurement of Blood Pressure

SBP of unanesthetized rats was determined twice a week using the tail-cuff plethysmography method (model UR-1000; Ueda Seisakusho, Tokyo, Japan). Each pressure value was obtained by averaging eight individual readings. Systemic MBP was also determined in conscious, unrestrained rats, as reported previously (21, 22). The rats were anesthetized with light diethyl ether, and a polyethylene cannula (PE-10; Becton Dickinson & Co., Parsippany, USA) was inserted into the abdominal aorta by way of the femoral artery. The tip of the cannula was placed between the bifurcation of the femoral arteries and the branching of the renal arteries. The cannula was connected to a PE-50 cannula (Becton Dickinson & Co.) and exteriorized in the interscapular region, filled with heparinized saline (1,000 units/ml), and plugged with stainless steel pins. The rats were allowed to recover in individual cages with free access to food and water. The day after cannula insertion, the intra-arterial catheter was attached to a blood pressure transducer (TP-400T; Nihon Kohden, Tokyo, Japan). MBP was monitored on a polygraph (Thermal Array Recorder RTA-1100M; Nihon Kohden, Tokyo, Japan). Measurement began at 30 min after connection of the transducer and was continued for more than 30 min; rats were housed in separate cages during the recording. The MBP values were taken as the means over the 30-min measurement period.

Collection of Urine and Measurements of Urinary Sodium and Creatinine

Twenty-four-hour urine samples from individual rats were collected using metabolic cages. The volume of urine was recorded at the end of the 24-h period. Urinary sodium levels were determined using a polarized Zeeman atomic absorption spectrophotometer (180-80, Hitachi, Tokyo, Japan) with a hollow cathode Na lamp (absorbance lines of 330.2 and 589.0 nm, Hitachi) and a data processing unit (180-0205, Hitachi, Tokyo, Japan). MBP was monitored on a polygraph (Thermal Array Recorder RTA-1100M; Nihon Kohden). The recording began at 30 min after connection of the transducer and was continued for more than 30 min; rats were housed in separate cages during the recording. The MBP values were taken as the means over the 30-min measurement period.

Measurement of Plasma Renin Activity

EDTA-treated plasma was incubated at 37 °C for 90 min, and the amounts of angiotensin I generated were measured by radioimmunoassay using a Gamma Coat 125I Plasma Renin Activity Kit (Baxter Healthcare Corp., Cambridge, USA) (23). Plasma renin activity was expressed as ng of angiotensin I generated per ml plasma over a period of 1 h.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Approximately 100 mg of kidney tissues were removed from each rat and homogenized in 1 ml of TRIZOL Reagent (GIBCO BRL, Rockville, USA). Total RNA was prepared according to the manufacturer’s instructions. Single-stranded cDNA from 250 ng of the RNA was synthesized using 0.4 µg of oligo-p(dT)15 primer and four units of AMV reverse transcriptase (Roche Diagnostics, Basel, Switzerland). PCR was performed in 20 µl of 20 mmol/l Tris-HCl (pH 8.7) containing 10 mmol/l KCl, 5 mmol/l (NH4)2SO4, 1.5 mmol/l MgCl2, 0.2 mmol/l dNTP mix, 0.5 µmol/l of the forward and reverse primers, and 0.5 unit of Taq DNA polymerase (Qiagen, Hilden, Germany). Complementary DNA from 25 ng of total RNA was used as the template. The oligonucleotide primers used were: for rat COX-1, 5'-TCTGATGCTCTTCTCCACGATCTG-3' (bases 897–920) and 5'-CCACCCAATCCACATCG-3' (bases 1327–1350); for COX-2, 5'-CCATGTCACAAAAAGGTGGTAATGC-3' (bases 73–95) and 5'-ATGGGAGTTGGGCAGTCATCA-3' (bases 446–465); and for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-CCCTTCTAAGGATCCTCAACTGGAATG-3' (bases 100–125) and 5'-GGAGGGCCATCCACAGTCTCTG-3' (bases 569–574). The polymerase chain reaction (PCR) cycling conditions were as follows: 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s followed by a final extension for 10 min at 72 °C. The PCR products were analyzed on 1.5% agarose gel electrophoresis and the product sizes were predicted from the sequence.

Immunohistochemistry for COX-2

Kidneys were obtained from sham-operated, 1K1C, 2K1C, and DOCA-salt rats. After overnight fixation in methyl-carnoy solution (60% methanol, 30% chloroform, 10% glacial acetic acid), the tissues were dehydrated by bathing in increasing concentrations of methanol and 100% isopropanol, and embedded in paraffin. They were then sectioned at a thickness of 3 µm and mounted on glass slides. Immunohistochemical staining was performed by the avidin-biotin peroxidase method using a DAKO LSAB2 Kit (DAKO Corp., Carpinteria, USA). Briefly, endogenous peroxidase activity was blocked with 1% H2O2 in methanol for 30 min at room temperature and the tissue was sequentially incubated with 10% (v/v) of normal swine serum. Murine COX-2 polyclonal antibody (1:400 dilution, No. 160126; Cayman Chemicals, Ann Arbor, USA) was used for the detection. Sections were layered with the primary antibody and incubated at 4 °C overnight. Biotinylated anti-mouse IgG (1:200 dilution) was then incubated on the slide for 10 min at room temperature followed by reaction with streptavidin.
Fig. 1. Development of renovascular and DOCA-salt hypertension in rats. The systolic blood pressure (SBP) of 1K1C (closed circles), 2K1C (closed triangles), and DOCA-salt (closed squares)-treated rats were measured by the tail-cuff plethysmography method at the indicated number of days after treatment. The SBPs of sham-operated rats of the 1K1C, 2K1C, and DOCA-salt models are indicated by open circles, open triangles, and open squares, respectively. Values are the means SEM of the SBP measured in 5 to 7 rats. *p < 0.05, **p < 0.01, ***p < 0.01, †p < 0.05, ‡p < 0.01, ANOVA followed by post-hoc Dunnett’s multiple test; c.f., the sham-operated group.

horseradish peroxidase (HRP). Each slide was immersed in DAB solution (0.02% 3,3-diaminobenzidine and 0.3% nickel ammonium sulfate dissolved in 50 mmol/l Tris-HCl buffer, pH 7.6) and counterstained with hematoxylin.

**Drugs and Chemicals**

Aspirin aluminium (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) and NS-398 (N-2-cyclohexyloxy-4-nitrophenyl) methane-sulfonamide (Cayman Chemical Co., Ann Arbor, USA) were purchased. Mofezolac (3,4-di-(4-methoxyphenyl)-5-isoxazoyl) acetic acid) and JTE-522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonyamide) were the generous gifts of Yoshitomi Pharmaceutical Industries (Fukuoka, Japan) and Japan Tobacco Inc. (Osaka, Japan). Other reagents were all of analytical grade and were obtained from commercial sources. For administration, aspirin (6 mg/ml), NS-398 (2 mg/ml), mofezolac (2 mg/ml), and JTE-522 (2 mg/ml) were suspended in 5% gum arabic. Aspirin (30 mg/kg), NS-398 (10 mg/kg), mofezolac (10 mg/kg), and JTE-522 (10 mg/kg) were administered orally every 12 h. As the vehicle control, 5% gum arabic (5 ml/kg, p.o.) was given.

**Statistical Analysis**

All statistical analyses were performed using the StatView program (version 4.5-J; Abacus Concepts Inc., Berkeley, USA). The significance of differences among 4 or 5 groups was assessed by analysis of variance (ANOVA) followed by Dunnett’s multiple test. Results are shown as the means SEM with the number of observations indicated in parentheses. A probability (p) value of less than 0.05 was taken to indicate statistical significance.

**Results**

Effects of COX Inhibitors on Blood Pressure in Renovascular and DOCA-Salt Hypertension

In each of the 1K1C, 2K1C, and DOCA-salt rat models, the development of hypertension and the effects of COX inhibitors on blood pressure were evaluated by SBP using the tail-cuff plethysmography method (Figs. 1 and 2a–c). The SBP of 1K1C rats receiving vehicle solution increased to 151 ± 7 mmHg at 7 days and 160 ± 2 mmHg at 12 days after the uninephrectomy and the clipping, and these values were significantly higher than that for the sham-operated rats (122 ± 1 mmHg; p < 0.05 at 7 days and p < 0.01 at 12 days). Daily oral administration of aspirin (30 mg/kg, p.o.) as a COX inhibitor from the first day of the clipping did not affect the SBP (163 ± 4 mmHg). The SBP values of 1K1C rats treated with one of the selective COX-2 inhibitors, NS-398 (10 mg/kg, p.o., 133 ± 5 mmHg) or JTE-522 (10 mg/kg, p.o., 146 ± 6 mmHg), were significantly lower than that in vehicle-administered rats (p < 0.01). In contrast, the SBP increased slightly to 172 ± 7 mmHg in 1K1C rats treated with mofezolac, a relatively selective COX-1 inhibitor. In the 2K1C model, the SBP of rats receiving vehicle solution increased to 157 ± 6 mmHg at 3 days, 170 ± 6 mmHg at 7 days, and 185 ± 11 mmHg at 12 days after the clipping, and these values were significantly higher than those of sham-operated rats (135 ± 6 mmHg, p < 0.05 at 3 days and p < 0.01 at 7 and 12 days). The elevation of SBP in the 2K1C model occurred earlier than that in the 1K1C model. Oral administration of aspirin did not affect the SBP (166 ± 8 mmHg). The SBP values of 1K1C rats treated with NS-398 (133 ± 5 mmHg) or mofezolac (153 ± 4 mmHg) were significantly lower than that in vehicle-administered rats (p < 0.01 for NS-398 and p < 0.05 for mofezolac). DOCA-salt treatment increased the SBP of vehicle-administered rats to 150 ± 2 mmHg at 3 days, 171 ± 3 mmHg at 7 days, and 187 ± 5 mmHg at 12 days after the start of the treatment, and these values were significantly higher than those of uninephrectomized rats without DOCA-salt treatment (109 ± 3 mmHg, p < 0.05 at 3 days and p < 0.01 at 7 and 12 days). Compared to the SBP of vehicle-administered DOCA-salt rats, administration of aspirin (186 ± 6 mmHg), mofezolac (176 ± 4 mmHg), or NS-398 (173 ± 7 mmHg) did not affect the blood pressure (p < 0.178 for aspirin, p < 0.874 for mofezolac, and p < 0.990 for NS-398).

The results from the direct determination of blood pressure in conscious, unrestrained rats confirmed those from the
tail cuff determination (Fig. 2d–f). MBP was significantly reduced by NS-398 (122 ± 3 mmHg) and JTE-522 (122 ± 2 mmHg) compared to that in the vehicle-administered rats (133 ± 1 mmHg) in the 1K1C model at 13 days after the clipping (p < 0.01). Administrations of aspirin (131 ± 5 mmHg) and mofezolac (136 ± 5 mmHg) had no significant effects on the elevated MBP. In the 2K1C model, MBP was significantly reduced by NS-398 (128 ± 10 mmHg) compared to that in the vehicle-administered group (154 ± 6 mmHg) at 8 days after the clipping (p < 0.05). Treatment of 2K1C rats with aspirin (133 ± 10 mmHg) or mofezolac (135 ± 6 mmHg) resulted in a weak suppression of the MBP elevation in hypertension, but this effect was not significant (p = 0.320 for aspirin and p = 0.167 for mofezolac). MBP increased to 154 ± 2 mmHg in the rats treated with DOCA-salt at 13 days after the treatment. Administration of aspirin resulted in a slight but not significant increase in MBP (163 ± 9 mmHg, p = 0.157). Mofezolac (158 ± 2 mmHg) and NS-398 (160 ± 10 mmHg) did not affect the MBP.

Effects of COX Inhibitors on Plasma Renin Activities in the 1K1C, 2K1C, and DOCA-Salt Models

Renin activity was measured in the plasma of 1K1C, 2K1C, and DOCA-salt rats at 13 days, 8 days, and 13 days after each treatment, respectively (Fig. 3). Plasma renin activity was significantly increased to 3.00 ± 0.34 ng/ml/h in 1K1C rats at 13 days after the clipping when compared to that in control rats (1.60 ± 0.37 ng/ml/h, p < 0.05). The renin activity in rats administered NS-398 (1.00 ± 0.22 ng/ml/h) or JTE-522 (1.60 ± 0.37 ng/ml/h) was significantly less than that in the vehicle-administered rats in the 1K1C model (p < 0.01 for NS-398 and p < 0.05 for JTE-522). Treatment of 1K1C rats with aspirin resulted in a slight but not significant increase in the plasma renin activity (3.24 ± 0.50 ng/ml/h, p = 0.726). Mofezolac did not change the activity in the 1K1C model (3.03 ± 1.27 ng/ml/h, p = 0.886). At 8 days after the clipping in the 2K1C model, the plasma renin activity (23.08 ± 7.75 ng/ml/h, p < 0.01) was significantly higher than that in the control group (2.367 ± 0.857 ng/ml/h) or that in the 1K1C-treated rats (3.00 ± 0.34 ng/ml/h). The renin activity in rats administered NS-398 (6.80 ± 2.68 ng/ml/h) was significantly less than that in the vehicle-administered rats in the 2K1C model (p < 0.05). Treatment of 2K1C rats with aspirin or mofezolac tended to decrease the activity, but the effect was not significant (16.53 ± 3.72 ng/ml/h, p = 0.108 for aspirin and 16.10 ± 3.50 ng/ml/h, p = 0.090 for mofezolac). In DOCA-salt rats, the plasma renin activity markedly...
Effects of COX Inhibitors on Urine Volume and Urinary Sodium Excretion in Renovascular and DOCA-Salt Hypertensions

The urine volume in the 1K1C, 2K1C, and DOCA-salt models was measured for 24 h at 11, 6, and 11 days, respectively (Fig. 4a–c). The volume in vehicle-administered rats (14.8 ± 0.8 ml) of the 1K1C model was almost the same as that of the sham-operated group (15.6 ± 1.9 ml). The urine volume increased significantly in rats administered NS-398 (21.8 ± 2.8 ml), JTE-522 (29.3 ± 3.9 ml), or aspirin (18.3 ± 2.6 ml) (p < 0.01 for NS-398 and JTE-522, p < 0.05 for aspirin). Treatment of 1K1C rats with mofezolac increased the urine volume slightly but not significantly (17.0 ± 1.4 ml). In the 2K1C model, the treatment significantly increased the urine volume (13.8 ± 1.6 ml, p < 0.01) compared to that in the sham-operated group (7.0 ± 0.6 ml). Administration of aspirin (12.9 ± 1.3 ml), mofezolac (14.8 ± 2.3 ml), or NS-398 (16.8 ± 3.8 ml) to 2K1C rats had no significant effect on urine volume (p = 0.994, p = 0.990, and p = 0.836, respectively) when compared to that in vehicle-administered rats. The urine volume in DOCA-salt rats increased markedly to 69.1 ± 4.8 ml, which was significantly greater than that in the sham-operated rats (13.1 ± 0.9 ml, p < 0.01). Administration of aspirin (65.6 ± 18.1 ml), mofezolac (73.7 ± 11.3 ml), or NS-398 (65.2 ± 13.1 ml) to the DOCA-salt-treated rats did not change the volume (p = 0.998, p = 0.996, and p = 0.997, respectively).

The amounts of sodium excreted into urine over a 24-h collection period were measured to examine the effects of COX inhibitors on the excretion (Fig. 4d–f). The sodium excretion into urine of vehicle-administered rats (39.5 ± 1.6 mg) in the 1K1C model was almost identical to that of the sham-operated group (39.1 ± 2.1 mg). The excretion did not change by the administration of NS-398 (43.3 ± 1.2 mg, p = 0.140) or JTE-522 (39.6 ± 4.5 mg, p = 0.997). Treatment of rats with aspirin (28.5 ± 4.9 mg) significantly reduced the excretion (p < 0.05). Administration of mofezolac tended to decrease the sodium excretion slightly, but this effect was not significant (34.2 ± 3.7 mg, p = 0.125). In the 2K1C model, aspirin (36.2 ± 11.1 mg), mofezolac (33.5 ± 8.9 mg), and NS-398 (33.8 ± 18.6 mg) had no significant effect on the excretion (p = 0.999, p = 0.966, and p = 0.829, respectively) when compared with that in the vehicle-received rats (20.1 ± 3.2 mg). The level of sodium excreted into the urine of DOCA-salt rats increased markedly to 316.9 ± 26.7 mg, which value was significantly greater than that in the sham-operated rats (44.2 ± 14.8 mg, p < 0.01). Administration of aspirin (247.7 ± 61.2 mg) or NS-398 (257.3 ± 47.0 mg) to the DOCA-salt-treated rats tended to decrease the sodium excretion, but this effect did not achieve statistical significance (p = 0.161 for aspirin and p = 0.146 for NS-398). Mofezolac did not change the excretion (302.9 ± 47.5 mg, p = 0.803). Twenty-four-hour excretion of creatinine into urine was 8.15 ± 1.73 g/day (n = 5) in 1K1C rats at day 11, 8.92 ± 1.47 g/day (n = 5).
g/day in 2K1C rats at day 7, and 13.73 ± 1.47 g/day in DOCA-salt rats at day 11. The amounts of creatinine excretion in 1K1C and 2K1C rats were not significantly different from that in control rats (7.14 ± 0.52 g/day, n = 5).

Effects of COX Inhibitors on the Heart Mass in Renovascular and DOCA-Salt Hypertensive Rats

Table 1 shows the effects of COX inhibitors on the heart mass in 1K1C, 2K1C, and DOCA-salt hypertensive rats at 13, 8, and 13 days after the treatment, respectively. A significant increase in the weight of the heart was observed in vehicle-administered 1K1C rats (p < 0.05, when compared to the sham-operated group). The 1K1C rats treated with NS-398 showed a significant decrease in heart mass (p < 0.01). Other COX inhibitors had no effect on heart mass in the 1K1C model. In 2K1C rats, administration of mofezolac significantly reduced the heart mass (p < 0.05). None of the COX inhibitors had any significant effects on heart mass in the DOCA-salt model.

Expressions of COX Isolezymes in the Kidneys of Renovascular and DOCA-Salt Hypertensive Rats

The expressions of COX-1 and COX-2 in the kidneys from renovascular and DOCA-salt hypertensive rats were analyzed by RT-PCR. Although constitutive expression of COX-1 mRNA was detected in the normal rats, the expression of COX-2 mRNA in these animals was lower (Fig. 5, lane 1). The kidneys of both 1K1C and 2K1C rats expressed COX-2 mRNA in addition to COX-1 mRNA (Fig. 5, lanes 2 and 3). In DOCA-salt rats, the expression of COX-2 mRNA
COX-2 positive cells in the macula densa (Fig. 6e). Histo-
model. In the DOCA-salt model, there were only a few
neys of 2K1C rats was much stronger than that in the 1K1C
6d). The expression of COX-2 proteins in the clipped kid-
-in the contralateral, non-clipped kidneys in 2K1C rats (Fig.
6a). The staining became intense in the macula densa of
distal tubules. Moderate immunological staining of COX-2
expression was mainly localized in the macula densa cells of
Both renovascular hypertensive models was comparable to
ative to that in the 1K1C model. The creatinine excretion in
blood pressure in the 2K1C model was also increased rela-
tional insult to the kidneys. Use of a clip with a 0.3 mm-long
cleft in the 2K1C model did not change the blood pressure
increase in the number of glomerular mesangial cells in the
inflammatory cell infiltration.

Discussion

In the present study, we demonstrated that selective COX-2
inhibitors had anti-hypertensive effects on two renovascular
hypertensive models, 1K1C and 2K1C, but not on a DOCA-
salt model. The 2K1C and 1K1C models were previously es-
ablished as models of renovascular hypertension in the rat
24, 25). These models were used extensively to clarify the
relationship among the renin-angiotensin system, hyperten-
sion, and cardiovascular disorders (26, 27). Irrespective of
species, the 2K1C model is characterized by an increase in
blood pressure immediately after clipping, which parallels
the release of active renin in the blood stream. Different lev-
evels of hypertension can be obtained depending on the diame-
ter of the clip opening (28). In the present study, plasma
renin activity in the 1K1C model increased several-fold after
stenosis of the right renal artery by a clip with a 0.3 mm-long
cleft. On the other hand, the renin activity in the 2K1C mod-
el was elevated 20-fold by a clip with a 0.1 mm-long cleft. In
conjunction with this increase in plasma renin activity, the
blood pressure in the 2K1C model was also increased relative
to that in the 1K1C model. The creatinine excretion in
both renovascular hypertensive models was comparable to
that in normal rats, suggesting that, under the present experi-
mental conditions, clipping resulted in minimal or no func-
tional insult to the kidneys. Use of a clip with a 0.3 mm-long
cleft in the 2K1C model did not change the blood pressure
for 2 weeks after the clipping (data not shown).

NS-398, a COX-2 inhibitor, successfully suppressed the
elevations of both blood pressure and plasma renin activity

Table 1. Effects of COX Inhibitors on Heart Weight in Renovascular and DOCA-Salt Induced Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>1K1C</th>
<th>2K2C</th>
<th>DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.905 ± 0.050 (6)</td>
<td>0.723 ± 0.026 (6)</td>
<td>0.876 ± 0.041 (5)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.016 ± 0.019* (13)</td>
<td>0.803 ± 0.047 (8)</td>
<td>0.980 ± 0.034 (7)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1.019 ± 0.031 (5)</td>
<td>0.699 ± 0.037 (4)</td>
<td>0.995 ± 0.040 (7)</td>
</tr>
<tr>
<td>Mofezolac</td>
<td>1.028 ± 0.056 (7)</td>
<td>0.676 ± 0.019* (11)</td>
<td>0.925 ± 0.027 (7)</td>
</tr>
<tr>
<td>NS-398</td>
<td>0.876 ± 0.021** (7)</td>
<td>0.720 ± 0.034 (5)</td>
<td>0.948 ± 0.022 (7)</td>
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Aspirin aluminium (aspirin, 30 mg/kg), mofezolac (mofezolac, 10 mg/kg), and NS-398 (NS-398, 10 mg/kg), or 5% gum arabic
(vehicle, 5 ml/kg) was administered orally twice a day from day 0. Heart were excised and weighed at 13 days, 8 days, and 13 days after
the treatments with 1K1C, 2K1C, and DOCA-salt models, respectively. Values are means ± SEM of heart wet weight in various
numbers (n) of rats. * p < 0.05, ** p < 0.01, ANOVA followed by post-hoc Dunnett’s multiple test; c.f., vehicle solution-administered
animals (vehicle). * p < 0.05 between sham-operated (sham) and vehicle administered groups.

Fig. 5. Expressions of COX-1 and COX-2 mRNAs in the
kidneys of renovascular and DOCA-salt hypertensive rats. Kidneys were collected at 13 days, 8 days, and 13 days after
the treatments with 1K1C (lane 2), 2K1C (lane 3 for left kid-
ney and lane 4 for right kidney), and DOCA-salt (lane 5)
models, respectively, as well as from normal rats (lane 1).
Total RNA was extracted followed by first-stranded cDNA
synthesis. The cDNAs were used as template for PCR. The
products were subjected to electrophoresis on 1.5% agarose
gel, and DNA was visualized by staining with ethidium bro-
mide. The RT-PCR product sizes for COX-1, COX-2, and
GAPDH were 431, 374, and 470 bp, respectively.

was reduced by a degree comparable to that in normal rats
(Fig. 5, lane 4). The expression of COX-2 protein was also
confirmed by immunohistochemical analysis. This expres-
sion was mainly localized in the macula densa cells of the
distal tubules. Moderate immunological staining of COX-2
protein was seen in the macula densa of the control rats (Fig.
6a). The staining became intense in the macula densa of 1K1C and 2K1C rats (Fig. 6b and c), but it was diminished in the
contralateral, non-clipped kidneys in 2K1C rats (Fig.
6d). The expression of COX-2 proteins in the clipped kid-
neys of 2K1C rats was much stronger than that in the 1K1C model. In the DOCA-salt model, there were only a few
COX-2 positive cells in the macula densa (Fig. 6e). Histo-
logical evaluation of the kidneys in 1K1C, 2K1C, and DO-
CA-salt hypertensive rats was also made at 13, 8, and 13
days after treatment to induce hypertension, respectively. No
obvious histological changes were observed in the kidneys
of any of the hypertensive models relative to the kidneys in
control rats. There was also no evidence of tubulorrhexis or
increase in the number of glomerular mesangial cells in the
inflammatory cell infiltration.
in the 1K1C and 2K1C models. The decreases in plasma renin correlated with concomitant decreases in systemic blood pressure by the COX-2 inhibitor. The results following administration of another selective COX-2 inhibitor, JTE-522, confirmed that the suppressive effect was COX-2-selective. These findings indicate that the prostaglandin(s) produced by inducible COX-2 augment renin release, which could lead to the development of renovascular hypertension. We also attributed the blood pressure-lowering effect of COX-2 inhibitor to a decrease in the renin release mediated by COX-2. Similarly, Wang et al. reported that clipping led to a significant increase in plasma renin activity, and that selective inhibition of COX-2 significantly attenuated the increase (29). In their study, there was a partial but significant amelioration of the hypertension in response to COX-2 inhibition. In contrast, in previous studies using rats with DOCA-salt-induced hypertension, a model of angiotensin-independent hypertension (20, 30), none of the COX inhibitors affected the blood pressure significantly. And in another study using this model, the plasma renin activity was markedly reduced compared to that in sham-operated rats, confirming the renin-angiotensin-independency (31). This result supports the explanation given above in that COX-2 inhibitors prevented the new production of prostaglandin(s) mediated by inducible COX-2, resulting in the depression of blood pressure via a decrease in renin release in renovascular hypertensive models.

In the present study, RT-PCR confirmed that the COX-2 mRNA levels were increased in the 1K1C and 2K1C rat models, but not in the DOCA-salt rat model. Furthermore, the levels of COX-2 protein as revealed by immunological staining increase in the macula densa in the two renovascular hypertensive models. The latter observation may exclude the possibility that the increased mRNA for COX-2 in the renovascular hypertensive rats originated from migrated leukocytes due to inflammatory insult to the kidney. COX-2 has been shown to localize mainly in the macula densa (32). In addition, double-immunostaining studies have revealed the coexpression of COX-2 and renin protein in the same juxtaglomerular apparatus (14, 15, 33). These findings indicate the important role of COX-2 and prostaglandins in mediating the increased renin expression in the 1K1C and 2K1C models. In chronically salt-depleted animals, COX-2 expression significantly increases in the macula densa and surrounding cortical thick ascending limb of Henle cells (11). Furthermore, in rats treated with angiotensin converting enzyme (ACE) inhibitors, elevations in plasma and kidney renin have been shown to be significantly inhibited by simultaneous treatment with a selective COX-2 inhibitor (34). And administration of a selective COX-2 inhibitor was reported to sig-
nificantly inhibit increases in renin mRNA expression and renal renin activity in response to a low salt diet (35). Thus, the macula densa-derived prostaglandins generated by COX-2 could play a crucial role in the regulation of renal renin expression and the release under physiological and pathophysiological conditions.

It is of interest that the administration of mofezolac tended to decrease sodium excretion into urine, and that a significant increase was observed in the rats treated with aspirin in the 1K1C model. This suggests that prostaglandins produced by COX-1 may act to induce sodium reabsorption in 1K1C renovascular hypertension. In contrast, decreased sodium excretion was not observed in the 2K1C model. The elevation of blood pressure caused by a higher plasma renin level and resulting in subsequent aldosterone release and vasconstriction of the renal arteries in the present 2K1C model may have overcome the depressor effect of COX-1-mediated prostaglandin on the sodium excretion. One of the primary effects of endogenous prostaglandin is the induction of natriuresis via increases in sodium and chloride excretion into the kidneys. In addition, the thick ascending limb of Henle cells contains significant numbers of prostaglandin E2 (PGE2) receptors (36), and COX-1 antigenicity has been demonstrated in cortical collecting tubules (10). It is thus likely that constitutively expressed COX-1 plays a role in sodium handling in renal tubules under mild, but not severe, renovascular hypertension.

Treatment of 1K1C rats with NS-398 cancelled a significant increase in the heart mass. Sustained hypertension causes the myocardium to adapt to an increased load. The adaptive response is characterized by an overall increase in protein synthesis, eventually resulting in increased cardiac mass (37). Therefore, the attenuated hypertension by the COX-2 inhibitor in the 1K1C model may result in the prevention of hypertrophy. Cardiac hypertrophy has been shown in a murine model of pressure overload by constriction of the aortic arch (38) and in two Goldblatt models in different species with a characteristically hypertrophic feature (39, 40).

In the present study, we did not examine the dose-dependent effect of COX inhibitors on increased blood pressure and renin release by clipping of the renal artery. All of the doses used here—10 mg/kg of NS-398 (41, 42) and JTE-522 (43), and 30 mg/kg of aspirin (44, 45)—were selected because they were previously shown to be effective for other experimental models with specificity. However, mofezolac is a relatively, but not highly, selective inhibitor for COX-1 (46). The ratio of the 50% inhibitory concentration of mofezolac against purified COX-1 to that against COX-2 has been reported to be 0.03 (47). Therefore, it is likely that administration of mofezolac at a higher dose would result in inhibition of COX-2 in addition to COX-1. Although aspirin, which inhibits both COX-1 and COX-2, showed a tendency to attenuate the blood pressure elevation in the 2K1C model, it failed to do so in the 1K1C model. In the 1K1C model, prostaglandins produced by COX-1 may contribute to the diuretic action. Thus, inhibition of COX-1 by aspirin could counteract the anti-hypertensive effect by the COX-2 inhibition. The discrepant effects of aspirin and COX-2 inhibitors on 2K1C hypertension should be examined in the future. Recently, nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to exert anti-inflammatory and anti-tumor activities due to mechanisms other than COX inhibition (48, 49). Thus the discrepant effects of aspirin may be due to some unknown mechanism.

It is well known that administration of aspirin or other NSAIDs can sometimes aggravate essential hypertension in humans (18, 50, 51). In contrast, in humans with renovascular hypertension, intravenous aspirin significantly reduced systemic blood pressure, while blood pressure was increased in patients with essential hypertension. Intravenous aspirin also decreased plasma renin activity in a subset of subjects with hyperreninemic essential hypertension (18). Together, these data and the present results suggest that while NSAIDs may aggravate hypertension in renin-independent, sodium-sensitive hypertension, possibly in part by inhibition of the COX-1 responsible for sodium excretion, selective COX-2 inhibition would decrease renin levels and ameliorate hypertension in renin-dependent, renovascular hypertension (52, 53). We thus conclude that selective COX-2 inhibitors may have significance as potent anti-hypertensive drugs for patients with renovascular hypertension.

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