Original Article

Disruption of elastic lamellae in aorta and dysfunction of vaso-regulation by rofecoxib in rats

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ABSTRACT — We assessed the effects of rofecoxib on cross-linkage formation in elastin and vaso-regulatory function in rats. After administration of rofecoxib at a dose of 10 mg/kg for 7 weeks to young rats and for 7 and 10 weeks to adult rats, thoracic aortas were isolated. The elastic lamellae in the aortas were disrupted histopathologically in all the treated groups. However, the content of cross-linkages in elastin, i.e. desmosine and isodesmosine, which give elasticity to the aortic wall, was not significantly different between the rofecoxib treated and control groups. On the other hand, although the baseline blood pressure was not changed during the treatment period in both young and adult rats, after several weeks of treatment with rofecoxib the change between systolic blood pressure before and after sympathetic stimulation by L-epinephrine was 2 to 3-fold larger than that in the control group. Similar results were obtained using angiotensin II instead of L-epinephrine. The exposure to rofecoxib (area under the plasma concentration-time curve) of rats after single administration was a few times higher than that of humans in clinical use. These findings indicate that rofecoxib did not directly inhibit formation of cross-linkages in elastin of the aorta in rats. However, the treatment with rofecoxib for several weeks disrupted elastic lamellae and caused depression of vaso-regulatory function in rats, which could bring on an increased risk of cardiovascular events in human.

Key words: Rofecoxib, Cardiovascular event, Elastin, Blood pressure, Vaso-regulation

INTRODUCTION

A highly selective cyclooxygenase-2 (COX-2) inhibitor, rofecoxib [3-phenyl-4-[4-(methylsulfonyl)phenyl]-2-(5H)-furanone, VIOXX], was marketed by Merck & Co. beginning in 1999 and was used widely in many countries by virtue of fewer gastrointestinal side effects and strong medicinal potency for the treatment of acute pain and osteoarthritis. However, in 2004, rofecoxib was withdrawn from the worldwide market due to an increased risk of cardiovascular (CV) events such as heart attack and stroke among patients taking 25 mg rofecoxib for more than 18 months, in the Adenomatous Polyp Prevention on VIOXX (APPROVe) trial, which was conducted to evaluate prevention of the recurrence of colorectal polyps (Bombardier et al., 2000; Ray et al., 2002; Bresalier et al., 2005). In the period after the withdrawal, it was reported that other selective COX-2 inhibitors (e.g. etoricoxib, parecoxib and valdecoxib) may also have the potential for increased CV risk (Aldington et al., 2005; Nussmeier et al., 2005). However, rofecoxib differed from the others in having a significantly greater frequency of, and higher odds ratio for CV events (Mamdani et al., 2004; Graham et al., 2005; Kimmel et al., 2005; Solomon et al., 2004), suggesting that rofecoxib could have a distinct mechanism leading to the increased CV risk compared to other selective COX-2 inhibitors.

Several hypotheses for the increased CV risk by rofecoxib have been reported. Before its launch, McAdam et al. (1999) proposed the following hypothesis and were concerned for the safety due to its high selectivity to COX-2. That is, selective COX-2 inhibitors diminished the production of prostaglandin (PG) I₂, a vasodilator and inhibitor of platelet aggregation, in the vascular endothelium, but not that of thromboxane (TX) A₂, a vasoconstrictor and promoter of platelet aggregation, in the platelets. Accordingly, in the presence of selective COX-2 inhibitors the action of TXA₂, would be relatively dominant compared to that of PGI₂, in the blood vessels, and this imbalance might increase the risk of CV events (McAdam et al., 1999).
et al., 1999). On the other hand, focusing on the chemical structure of rofecoxib, Walter et al. (2004) and Mason et al. (2006) proposed that the pro-oxidant effect of rofecoxib would promote oxidative damage to low-density lipoproteins (LDLs) and phospholipids leading to atherogenesis. In 2006, Oitate et al. demonstrated that rofecoxib covalently binds to the elastic protein “elastin” and causes lesions in elastic lamellae of the thoracic aortic wall in rats (Oitate et al., 2006, 2007a and 2007b), and suggested that the dysfunction of vaso-regulation caused by the disruption of elastin in arterial walls could increase the risk of CV events in humans.

Elastin is a major component of elastic lamellae, and is found in the extracellular matrices of connective tissues, providing elasticity and resilience to tissues which require the ability to deform repetitively and reversibly (Rosenbloom et al., 1993; Kielty et al., 2002). In rats, elastin occupies approximately 40% of the thoracic aorta on a dry-weight basis (Looker and Berry, 1972; Starcher and Galione, 1976) and gives the aorta abundant elasticity to act as a vaso-regulator. In the course of elastin biosynthesis, specific cross-linkages are formed in intra- and inter-tropeolastin, which is secreted from fibroblasts as a precursor protein (Vrhovski and Weiss, 1998). Structures of the cross-linkages in elastin, which are referred to as desmosine and isodesmosine, give elastin abundant elasticity (Thomas et al., 1963; Partridge et al., 1966).

When blood is ejected from the heart and then flows through the thoracic aorta, the elasticity of the aortic wall weakens the pressure caused by the intermittent nature of ventricular ejection to keep the blood pressure normal. If, however, the elastin has been disrupted and this induces a loss of elasticity in the aorta (Martyn and Greenwald, 1997; D’Armiento, 2003; Sandberg et al., 1981), hypertension can develop, which is a risk factor for CV events. Furthermore, a long lasting deterioration of the elasticity in arterial walls might increase the risk of arteriosclerosis and arterial aneurysm.

It was reported that elastin biosynthesis is more active in the juvenile period, such as a few weeks after birth in rats (Looker and Berry, 1972). If the disruption of elastic lamellae were initiated by binding of rofecoxib, the disruption might be more severe in young rats than in adult rats. Thus, in the present study using young and adult rats after repeated administration of rofecoxib for several weeks, we investigated the change in the content of the cross-linkages, desmosine and isodesmosine in the aorta, and the effects on the capacity for vaso-regulation, especially after stimulation with L-epinephrine or angiotensin II.

### MATERIALS AND METHODS

**Chemicals and reagents**

Rofecoxib (Fig. 1) and valdecoxib were kindly donated by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Isodesmosine (Fig. 1), pyridylethyl-cysteine, acetonitrile [high-performance liquid chromatography (HPLC) grade], 1 M ammonium acetate solution (HPLC grade), formic acid [liquid chromatography-mass spectrometry (LC/MS) grade], paraformaldehyde, 20% glutaraldehyde solution (electron microscopy grade), HCl (35-37%) and heparin sodium were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pentobarbital sodium and 0.5 M heptafluorobutyric acid (LC/MS grade) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). L-Epinephrine and human angiotensin II were purchased from Sigma-Aldrich. (St. Louis, MO, USA). Desmosine (Fig. 1) was purchased from Toronto Research Chemicals (Toronto, ON, USA). Isoflurane was purchased from Intervet Co., Ltd. (Tokyo, Japan). All other reagents and solvents in this study were commercially available and were either extra pure, guaranteed, HPLC or LC/MS grade.

**Animals**

Male Sprague-Dawley rats were obtained from Japan SLC Inc. (Shizuoka, Japan) and used as adult rats after 5 or more days of acclimatization. Pregnant Sprague-Dawley rats (day 14 of gestation) were obtained also from...
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the same source. Male neonatal rats born from the pregnant rats were used as young rats. All rats were housed in a temperature-controlled room with 12-hr light/dark cycle. Water and food were available ad libitum throughout the study except as described below. All experimental animals were handled in accordance with the institutional and national guidelines for the care and use of laboratory animals.

**Drug administration and sample collection**

Single administration: For the pharmacokinetic study, 10 mg/kg rofecoxib as a solution in polyethylene glycol 400 (PEG 400) was administered orally to 3-and 12-week-old rats (n = 3), and subcutaneously to 3-week-old rats (n = 3) after overnight-fasting. Blood samples (0.15 to 0.2 ml) were collected from jugular vein with heparinized syringes under isoflurane anesthesia at 0 (pre-dose), 0.5, 1, 2, 6, 24 and 48 hr after drug administration, and were centrifuged (4°C, 3,000g, 10 min) to obtain plasma. The plasma samples were frozen at -80°C until the measurement of rofecoxib concentration.

Repeated administration: Young and adult rats were used for histopathological analysis and determination of the content of desmosine and isodesmosine as described below. Young rats (1 day old at the start of treatment, n = 4) were administered rofecoxib at a dose of 10 mg/kg/day for a total of 7 weeks; it was administered subcutaneously from postnatal day 1 to 20 and orally from postnatal day 21 to 49, continuously. As a control, the vehicle (PEG 400) was administered in the same manner. Twenty-four hours or more after the last dose, the thoracic aorta was isolated from the rats after euthanasia by exsanguination under isoflurane anesthesia at 0 (pre-dose), 0.5, 1, 2, 6, 24 and 48 hr after drug administration, and were centrifuged (4°C, 3,000g, 10 min) to obtain plasma. The area under the plasma concentration versus time curve (AUC) and the elimination half-life (t1/2) of rofecoxib were calculated by the computer program “MOMENT (EXCEL)” described by Tabata et al. (1999).

**Histopathological analyses of elastic lamellae**

Isolated aortas were sliced into about 5 mm lengths and fixed using 1/2-Karnovsky’s solution (2% paraformaldehyde and 2.5% glutaraldehyde) for more than 3 hr at room temperature. After the fixation, the samples were embedded in paraffin and sliced at 5 μm thickness. The sections were stained with the Elastica-van Gieson method to show the elastic lamellae, and were observed with a light microscope (200 ×).

**Determination of the content of desmosine and isodesmosine**

Isolated aortas were sliced into about 5 mm lengths, lyophilized completely and weighed. Five-hundred microliters of a 12 M HCl solution containing 4 μg/ml pyridylethyl-cysteine as an internal standard was added to each lyophilized sample. Then, the mixture was heated at 110°C for 24 hr for complete hydrolysis of the peptide bonds in elastin into desmosine, isodesmosine and its constituent amino acids, and was lyophilized again. The residue of the lyophilized sample was re-dissolved in 1 ml of the mobile phase solvent C (ultrapure water containing 7 mM heptfluorobutyric acid and 5 mM ammonium acetate). The contents of desmosine, isodesmosine...
and pyridylethyl-cysteine in these samples were measured by an API-3200 LC-MS/MS system. The analytes and internal standard were separated by HPLC using an Atlantis T3 column (2.1 × 150 mm, 3 μm, Waters, Milford, MA, USA). The mobile phase, consisting of solvent C and solvent D (80% acetonitrile solution containing 7 mM heptafluorobutyric acid and 5 mM ammonium acetate), was delivered at a flow rate of 0.35 ml/min. In each measurement, the proportion of solvent D in the mobile phase was increased linearly from 5 to 8% from the start to 12 min, decreased to 5% at 12.1 min and held at 5% until the next analysis. The eluent from the column was analyzed by tandem mass spectrometry using the electrospray ionization interface in the positive ion mode. The transitions m/z 526.3 for desmosine and isodesmosine, and m/z 227.1 → 106.1 for pyridylethyl-cysteine were detected in the multiple reaction monitoring mode. Desmosine and isodesmosine was measured in single mass-spectrometry mode.

**Measurement of blood pressure**

Blood pressure of rats during repeated administration of rofecoxib was measured once a week before the daily dosing from young rats at 4 to 6 weeks old and from adult rats at 12 to 18 weeks old as described below. In young rats at 7 weeks old and in adult rats at 19 and 22 weeks old, the blood pressure was only measured more than 48 hr after the last dose to avoid the direct effects of rofecoxib on blood pressure. The rats under pentobarbital anesthesia (40 mg/kg, i.p.) were placed on a heated surface at 40ºC. After at least 15 min on the heating pad, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a non-invasive tail-cuff/pulse transducer and a Lab Chart 6 recorder (ADInstruments Pty Ltd, Bella Vista, Australia). Sym pathetic stimulation was performed by subcutaneous administration of 10 μg/kg L-epinephrine. In addition, the effects of angiotensin II at a dose of 0.7 μg/kg as another vasopressor agent were measured only in adult rats after 10 weeks of treatment with rofecoxib; the angiotensin II challenge was done more than 48 hr after the last dose, in the same manner as with L-epinephrine. Ten minutes later, SBP and DBP were measured again in the same manner as described above. The change between SBP before and after administration of vasopressors (Δ-SBP) was calculated.

**Data analyses**

Statistical analyses were performed using the Student’s t-test. Differences were considered to be significant when p < 0.05.

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**RESULTS**

**Plasma concentration-time profile of rofecoxib**

To assure the exposure of rats to rofecoxib, plasma concentrations of the drug were measured after single doses of 10 mg/kg rofecoxib at different ages. In all cases, after the time of the maximum concentration (0.5 to 1 hr) the plasma concentration decreased gradually and rofecoxib was almost cleared from the plasma at 24 hr after administration (Fig. 2). These AUC<sub>0-48</sub> of rofecoxib were not so different in rats after 3 weeks old not only between subcutaneous and oral administrations at 3 weeks old but also between 3 and 12 weeks old after oral administration (Table 1). In the case of rats younger than 3 weeks old, plasma concentrations were not determined because a sufficient amount blood could not be collected for the measurements. These results indicated that similar exposures without marked accumulation could be expected in all the groups of rats at least after 3 weeks old during repeated administration at a dose of 10 mg/kg regardless of the route of dosing.

**Histopathological analysis**

The histopathological changes of the elastic lamellae of thoracic aorta were evaluated under an optical microscope after 7-weeks repeated administration to young rats and 7- and 10-weeks repeated administration to adult rats at a dose of 10 mg/kg of rofecoxib (Fig. 3, representative photomicrographs). In young control rats (7 weeks old),...

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the elastic lamellae of all rats were clearly observed as clear lines without any branching (Fig. 3 A). In the rofecoxib-treated rats (Fig. 3 B), disruption and swelling of the elastic lamellae were noted in all rats. In the adult rats after 7 weeks of treatment (Figs. 3 C and D) and 10 weeks of treatment with rofecoxib (Figs. 3 E and F), comparable disruption and swelling of the elastic lamellae were also observed. These histopathological changes by rofecoxib were consistent with the previous report (Oitate et al., 2007a).

Contents of the cross-linkages of elastin in the aorta

The contents of the cross-linkages in aorta were measured after repeated administration of rofecoxib. In young rats administered rofecoxib for 7 weeks, the contents of desmosine and isodesmosine were 1.28 ± 0.07 and 1.78 ± 0.1 μg/mg dry weight of the thoracic aorta, respectively. Meanwhile in control young rats the contents were 1.38 ± 0.08 and 1.65 ± 0.07 μg/mg dry weight, respectively. The contents of these cross-linkages were not significantly different between the groups (Figs. 4 A and B). On the other hand, in adult rats, the content of desmosine was a little lower than in young rats regardless of whether rofecoxib was administered. However, the contents of the cross-linkages, both desmosine and isodesmosine, were not significantly different between the treated and control groups, not only for 7 weeks of treatment (Figs. 4 C and D) but also for 10 weeks of treatment (data not shown).

Blood pressure and its regulation

SBP and DBP were measured with and without sympathethic stimulation by L-epinephrine during the repeated administration period, and also with and without the vasopressors, angiotensin II and L-epinephrine, more than 48 hr after the last dose after 10-weeks repeated administration. In both young and adult rats, the SBP and DBP without sympathetic stimulation (in normal condition) were not significantly different between the rofecoxib treated and control groups (Fig. 5).

The Δ-SBP, the change between SBP before and after administration of the vasopressor, was calculated as an index of the ability to regulate the temporary increase in blood pressure induced by the vasopressor. In young rats after 4 weeks of treatment, the Δ-SBP induced by L-epinephrine was 27.1 ± 4.9 mmHg with rofecoxib, which was about 3-fold larger than the control value of 8.5 ± 2.9 mmHg. The significant enhancement of Δ-SBP in the treated group compared to that in the control group was observed after 4 weeks of treatment (Fig. 6A).

In adult rats, after 4 weeks of treatment with rofecoxib, the Δ-SBP was not significantly different from that in the control group yet. However, the Δ-SBP in rats after 7 weeks of treatment with rofecoxib was 39.4 ± 4.4 mmHg, which was about 3-fold larger than the control value of 13.9 ± 3.7 mmHg (Fig. 6 B). At 48 hr or more after the last dose of 10 weeks of treatment, by stimulation with L-epinephrine the Δ-SBP was about 2.5-fold larger in the treated rats than that in the control rats (35.7 ± 9.8 mmHg and 14.2 ± 3.9 mmHg). And by stimulation with angiotensin II, it was also about 2.5-fold larger than the control (27.0 ± 2.3 mmHg versus 11.7 ± 3.1 mmHg) (Fig. 7).

DISCUSSION

Elastin is a major component in the extracellular matrices of connective tissues such as ligament and cartilage and of connective tissues in organs such as aorta, which need elasticity to keep their function. Elastin has a lot of

Table 1. Pharmacokinetic parameters after single administration of rofecoxib by various routes and at various ages.

<table>
<thead>
<tr>
<th>Route</th>
<th>Age (weeks)</th>
<th>t½ (hr)</th>
<th>AUC₀→∞ (μg·hr/ml)</th>
<th>Cmax (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s. c.</td>
<td>3</td>
<td>8.2 ± 2.8</td>
<td>16 ± 1.1</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>p. o.</td>
<td>3</td>
<td>-</td>
<td>9.2 ± 2.1</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.4 ± 0.2</td>
<td>13 ± 2.1</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

Cmax, maximum plasma concentration (observed), t½, elimination half-life, AUC₀→∞, area under the plasma concentration curve (0 to infinity).
s.c.; subcutaneous administration, p.o.; peroral administration
v; not calculated, because there were not more than 3 measurement points in the elimination phase.
b; AUC₀→∞, the concentration at 24 h after administration was regarded as 0.001 μg/ml (lower limit of detection) because it was below the detection limit.
Data are shown as mean ± S.D. (n = 3).
inter- and intra-molecular cross-linkages, desmosine and isodesmosine, which play an important role to give elasticity to the tissue (Vrhovski and Weiss, 1998). Oitate et al. hypothesized that one of the major reasons for the increased risk of CV events by rofecoxib was the disruption of elastic lamellae in arterial walls, which was caused by inhibition of the formation of desmosine and isodesmosine. They demonstrated that rofecoxib covalent-
ly bound to elastin in the aorta after oral administration of 14C-labelled compound, and speculated that rofecoxib was bound to allysine (α-aminoacidic-δ-semialdehyde) (Oitate et al., 2007a), which is a reactive aldehyde-type intermediate generated from a lysine residue in elastin. Desmosine or isodesmosine is formed by aldol condensation with four molecules of allysine.

It was reported that elastin biosynthesis is more active in the juvenile period, such as a few weeks after birth in rats (Looker and Berry, 1972). Thus, if the disruption of elastic lamellae were initiated by binding of rofecoxib to allysine followed by inhibition of the cross-linkage formation, the disruption might be more severe and the content of the cross-linkages might be lower in young rats than in adult rats. Unexpectedly, however, the disruption of elastic lamellae in the aorta was similar in young and adult rats after the same exposure to rofecoxib. Furthermore, the contents of desmosine and isodesmosine in the aorta were not different in the two groups of rofecoxib-treated and vehicle-treated rats at the time when the disruption was observed (Figs. 3 and 4). These results suggest that the disruption by rofecoxib is not due to the inhibition of the formation of the desmosine and isodesmosine cross-links, but is due to the harmful influence on other targets.

Fig. 4. Contents of desmosine and isodesmosine in the thoracic aorta after repeated administration of rofecoxib at a dose of 10 mg/kg/day to young and adult rats. A and B, treatment for 7 weeks in young rats (0 to 7 weeks old, n = 4); C and D, treatment for 7 weeks in adult rats (12 to 19 weeks old, n = 3). Data are shown as mean ± S.D.
that form the elastic lamellae.

It is important for elastin bio-synthesis not only to form the inter- and intra-molecular cross-linkages in tropoelastin but also to assemble various other proteins such as fibrillins and fibulin-5 to form insoluble mature elastin (Kielty et al., 2002; Nakamura et al., 1999, 2002; Hirai et al., 2007). Thus, these proteins might be one of the targets where rofecoxib binds and causes the disruption in elastin. Furthermore, it is possible that rofecoxib binds to elastin and forms abnormal inter- and intra-molecular cross-linkages by some mechanism, leading subsequently to stiffening of arterial walls. Hence, further study is needed to clarify the mechanism of the disruption of elastic lamellae by rofecoxib.

Blood pressure is mainly determined by the blood volume and the vessel diameter, and is regulated, in other words buffered, by the elasticity in the aorta to avoid excessive increase in blood pressure and to keep a moderate pulse pressure (difference between SBP and DBP). In particular, it was reported that elastin in the thoracic aorta has an important role for buffering the pressure in the heart (Belz, 1995; London and Guerin, 1999). Thus, it was expected that the disruption of elastic lamellae in the thoracic aorta would interfere with the buffering action and induce a rise in SBP, which would be a risk factor of CV events. In the absence of stimulation, however, the SBP and DBP of both young and adult rats were not significantly altered during the treatment and were not significantly different between the rofecoxib treated and control groups (Fig. 5). It was thought that the disruption in elastic lamellae by rofecoxib progressed slowly for several weeks, so that in the normal condition the blood volume and the vessel diameter adapted well with the progression. These results suggested that the disruption of elastic lamellae was not serious enough to affect blood pressure in the normal condition.

On the other hand, the Δ-SBP after sympathetic stimulation with L-epinephrine was significantly larger in the rofecoxib treated rats than in the control rats (Fig. 6). This enhancement of Δ-SBP might occur because the rapid increase in blood pressure by L-epinephrine overwhelmed the adaptation capacity. That is, the deteriorated elasticity in the aorta leads to a loss of the buffering function to regulate the blood pressure under sympathetic conditions. Furthermore, similar results were obtained in the experiment using angiotensin II instead of L-epinephrine, which means that the enhancement of Δ-SBP was a response to a rapid rise in blood pressure regardless of the type of receptors (Fig. 7).

This deterioration of the buffering function in blood pressure was observed in young rats after 4 weeks administration of rofecoxib, while it was observed in adult rats after 7 weeks of administration (Fig. 6), indicating that young rats were more sensitive to disruption of elastic lamellae than adult rats the functional deterioration of the vaso-regulation occurred also earlier in young rats than in
adult rats. These results suggested that such deterioration could occur either before or after maturation, although the sensitivity to rofecoxib was higher during the juvenile period. This fact is consistent with the observation that the risk of CV events increased after long-term rofecoxib treatment in adults (Bresalier et al., 2005).

Based on the results of the above experiments, it might be suggested that the disruption of elastic lamellae was one of the reasons for the enhancement of Δ-SBP. Of course, the enhancement could be caused by other mechanisms, such as TXA2 in the “PGI2/TXA2 imbalance” hypothesis or radicals in the “pro-oxidant effect” hypothesis (McAdam et al., 1999; Walter et al., 2004; Mason et al., 2006). However, the former hypothesis addresses the question of why rofecoxib has the specific toxicity compared with other COX-2 inhibitors, and the latter hypothesis has been demonstrated only in vitro, but has not yet been verified in vivo. Moreover, the plasma concentration of 20-hydroxyeicosatetraenoic acid, one of the arachidonic acid metabolites, was reported to increase more than 100-fold in mice after rofecoxib intake for 2 months, and accordingly to raise their blood pressure (Liu et al., 2010). In any case, it was suggested that the vaso-regulating ability is reduced by rofecoxib. Future work should be conducted to confirm the main mechanism.

In a clinical study, the AUC0-∞ of rofecoxib was reported to be 4.2 μg·h/ml (Schwartz et al., 2000). In comparison, in the present study with rats, the AUC0-∞ of rofecoxib after single oral administration in 3- and 12-week-old rats and after single subcutaneous administration in 3-week-old rats was in the range of 9 to 16 μg·h/ml (Table 1), which is a few times higher than in humans (Fig. 2, Fig. 6. Changes of systolic blood pressure in response to L-epinephrine (10 μg/kg, s.c.) during repeated administration of rofecoxib at a dose of 10 mg/kg/day to young and adult rats. Blood pressure during repeated administration was measured before the daily dosing from young rats at 4 to 6 weeks old and from adult rats at 12 to 18 weeks old. In young rats at 7 weeks old and in adult rats at 19 weeks old, the blood pressure was only measured more than 48 h after the last dose. [Change in systolic blood pressure (Δ-SBP)] = (SBP after L-epinephrine administration) – (SBP before the administration). A, during 4 to 7 weeks administration in young rats (n = 4); B, before dosing and during 1 to 7 weeks administration in adult rats (n = 3). Data are shown as mean ± S.D. *p < 0.05 and **p < 0.01 vs. control group analyzed by t-test.

Fig. 7. Changes of systolic blood pressure in response to L-epinephrine (10 μg/kg, s.c.) and angiotensin II (0.7 μg/kg, s.c.) at 48 h or more after the last dose in adult rats administered rofecoxib for 10 weeks at a dose of 10 mg/kg/day (n = 3). [Change in systolic blood pressure (Δ-SBP)] = (SBP after L-epinephrine or angiotensin II administration) – (SBP before the administration). Data are shown as mean ± S.D. *p < 0.05 and **p < 0.01 vs. control group analyzed by t-test.
In conclusion, rofecoxib did not directly inhibit the formation of the cross-linkages, desmosine and isodesmosine, in the elastin of rat aorta, but promoted the disruption of elastic lamellae via another mechanism. On the other hand, it is suggested that long-term treatment with rofecoxib causes reduction of the capacity for vaso-regulation of elastic lamellae via another mechanism. On the other hand, it is suggested that long-term treatment with rofecoxib causes reduction of the capacity for vaso-regulation, which is a risk factor for CV events. To minimize this disruption of elastic lamellae by drugs, future studies are needed to clarify the target and the detailed mechanism of this disruption.

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