Covalent Binding of Rofecoxib, but Not Other Cyclooxygenase-2 Inhibitors, to Allysine Aldehyde in Elastin of Human Aorta

Masataka Oitate, Takashi Hirota, Takahiro Murai, Shin-ichi Miura, and Toshihiko Ikeda

Drug Metabolism & Pharmacokinetics Research Laboratories (M.O., T.M., S.M., T.I.), and Global Project Management Department (T.H.), Daiichi Sankyo Co., Ltd., Tokyo, Japan

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ABSTRACT:

In rats, it has been reported that rofecoxib, a cyclooxygenase-2 (COX-2) inhibitor, reacts with the aldehyde group of allysine in elastin to give a condensation covalent adduct, thereby preventing the formation of cross-linkages in the elastin and causing degradation of the elastic fibers in aortas in vivo. Acid, organic solvent, and proteolytic enzyme treatments of human aortic homogenate after incubation with [14C]rofecoxib demonstrated that most of the radioactivity is covalently bound to elastin. The in vitro covalent binding was inhibited in the presence of β-aminopropionitrile, o-phenylenediamine, and hydralazine, which suggested that the aldehyde group of allysine in human elastin was relevant to the covalent binding. The in vitro covalent binding of [14C]rofecoxib was significantly decreased by the addition of only nonradiolabeled rofecoxib but not the other COX-2 inhibitors, celecoxib, valdecoxib, etoricoxib, and CS-706 [2-(4-ethoxyphenyl)-4-methyl 1-(4-sulfamoylphenyl)-1H-pyrrrole], a novel selective COX-2 inhibitor. All the above COX-2 inhibitors except for rofecoxib had no reactivity with the aldehyde group of benzaldehyde used as a model compound of allysine elastin under a physiological pH condition. On the other hand, no retention of the radioactivity of [14C]rofecoxib was observed in human aortic endothelial cells in vitro, suggesting that rofecoxib is not retained in aortic endothelial cells in vivo. These results suggest that rofecoxib, but not other COX-2 inhibitors, is capable of covalently binding to the aldehyde group of allysine in human elastin. This might be one of the main causes of cardiovascular events by rofecoxib in clinical situations.

In 2004, rofecoxib (Vioxx; Merck & Co. Inc., Whitehouse Station, NJ), a potent and highly selective cyclooxygenase-2 (COX-2) inhibitor, was withdrawn from the worldwide market based on the results of some clinical studies indicating its association with increased risk of cardiovascular (CV) events, such as heart attack and stroke (Merck, Merck announces voluntary worldwide withdrawal of Vioxx. (http://www.vioxx.com/vioxx/documents/english/vioxx_press_release.pdf)). In the last few years, it has been reported that other selective COX-2 inhibitors, e.g., etoricoxib, valdecoxib (and its prodrug, parecoxib), and even nonselective nonsteroidal anti-inflammatory drugs, e.g., naproxen, may also have a potential for increased CV risk (National Institutes of Health, Use of non-steroidal anti-inflammatory drugs, e.g., naproxen, may also have a potential for increased cardiovascular events by rofecoxib in clinical situations. (Fredy et al., 2005; Meier et al., 2005). However, rofecoxib has a distinctly higher potential for CV risk in comparison with these drugs (Fredy et al., 2005; Graham et al., 2005). Although some hypotheses regarding the mechanism have been proposed so far, such as prostacyclin (PGI2)/thromboxane A2 (TXA2) imbalance in arteries (McAdam et al., 1999), most of these hypotheses have not been verified yet.

In our previous study using rat aortas (Oitate et al., 2007), it was suggested that rofecoxib itself reacts with the aldehyde group of allysine (α-aminoadipic-δ-semialdehyde) in elastin to give a condensation covalent adduct (Fig. 1B). This was also strongly supported by an in vitro reaction using benzaldehyde, a model compound of allysine, in which rofecoxib reacted with the aldehyde group of benzaldehyde in a kind of condensation reaction under a physiological pH condition. Similar to collagen, elastin is a key extracellular matrix protein which provides CV tissues, e.g., arteries and heart valves, with tensile strength and elasticity (Vrhovski and Weiss, 1998). The elastic property is due to the existence of covalent cross-linkages, such as desmosine and isodesmosine, and allysine is the most important precursor of physiologically essential cross-linkage formation in elastin and collagen and is formed from the lysine residue by lysyl oxidase (LOX) (Fig. 1A). It has been reported that the prevention of these cross-linkages in elastin or collagen causes serious lesions in the connective tissues in animals and humans (Herd and Orbison, 1966; Andrews et al., 1975; Hashimoto et al., 1981; Junker et al., 1982; Light et al., 1986; Yoshikawa et al., 2001). In fact, multiple oral administration of rofecoxib to rats caused a marked degradation of the elastic fibers in the aorta in vivo, conceivably by covalently binding to allysine elastin and by the inhibition of the normal cross-linking process of elastin (Oitate et al., 2007).

In the present study, to investigate whether the above observations seen in rats also occur in clinical situations, that is, whether rofecoxib

ABBREVIATIONS: COX-2, cyclooxygenase-2; CV, cardiovascular; PGI2, prostacyclin; TXA2, thromboxane A2; LOX, lysyl oxidase; CS-706, 2-(4-ethoxyphenyl)-4-methyl 1-(4-sulfamoylphenyl)-1H-pyrrrole; BAPN, β-aminopropionitrile; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; TCA, trichloroacetic acid; HBSS, Hanks’ balanced salt solution; HAOEC, human aortic endothelial cell(s); ANOVA, analysis of variance.
can bind with allysine aldehyde in human elastin, we conducted in vitro binding studies using a human aorta sample. In addition, the reactivity of other COX-2 inhibitors, celecoxib (Celebrex; Pfizer, New York, NY), valdecoxib (Bextra; Pfizer), etoricoxib (Arcoxia; Merck & Co. Inc.), and CS-706 [2-(4-ethoxyphenyl)-4-methyl 1-(4-sulfamoylphenyl)-1H-pyrrole], a novel COX-2 inhibitor, with allysine aldehyde was estimated using benzaldehyde, and their inhibitory potencies in the cross-linking process of human elastin were evaluated.

**Materials and Methods**

**Chemicals and Reagents.** \[^{14}C\]Rofecoxib (17 mCi/mmol) and \[^{14}C\]celecoxib (13 mCi/mmol) were synthesized at GE Healthcare Biosciences (Little Chalfont, Buckinghamshire, UK; radiochemical purities: >98%), and nonradio-labeled rofecoxib, celecoxib, valdecoxib, etoricoxib, and CS-706 were synthesized at Daiichi Sankyo Co., Ltd. (Tokyo, Japan) (Fig. 2). \(\beta\)-Aminopropionitrile (BAPN), d-penicillamine, hydralazine, and benzaldehyde were purchased from Sigma-Aldrich Inc. (St. Louis, MO). All other reagents and solvents used were commercially available and were of extra pure, guaranteed or high-performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) grade.

**Animals and Sample Collection.** Male Sprague-Dawley rats (6 weeks of age) were obtained from Charles River Japan, Inc. (Yokohama, Japan) and were used after 1 week of acclimatization. The rats were housed in a temperature-controlled room with a 12-h light/dark cycle and were allowed free access to water and a diet (FR-2; Funabashi Farm Co., Ltd., Chiba, Japan) throughout the study. The thoracic aortas were collected from the rats weighing 210 to 230 g after being euthanized by exsanguination under diethyl ether anesthesia (\(n = 10\)).

**Procurement of Human Aorta Sample.** A human abdominal aorta (Caucasian, female, 69 years old) was obtained from the Human Animal Bridging Research Organization in Chiba, Japan. The sample was legally procured from the National Disease Researcher Interchange in Philadelphia, PA, with permission to use the aorta for research purposes only, based on the international partnership between the National Disease Researcher Interchange and Human Animal Bridging Research Organization.

**Cell Culture.** Human aortic endothelial cells (HAOEC) were obtained from
Cell Applications, Inc. (San Diego, CA). The cells were cultured in endothelial cell medium (HAOEC Total Kit; Cell Applications, Inc.) according to the supplier’s instructions. For the uptake and efflux experiments, HAOEC were seeded at 9.0 × 10^4 cells/cm² in 24-well dishes (type I collagen-coated plate; Asahi Techno Glass, Tokyo, Japan) and used at 90% confluence.

**Acid, Organic Solvent, and Proteolytic Enzyme Treatments of Human Aorta.** After the removal of the adhering tissues, the human aortic sample was weighed and homogenized [5% (w/v)] in isotonic saline using a motor-driven homogenizer. The homogenate in final volume of 10 ml was incubated with 0.6 M of each nonradiolabeled COX-2 inhibitors rofecoxib, celecoxib, valdecoxib, etoricoxib, or CS-706 (solvent B), was delivered at a flow rate of 1 ml/min, starting at 20% solvent B in solvent A and increasing the proportion of solvent B to 60% linearly for 20 min, and holding for 10 min at 60% solvent B. The effluent was monitored at 254 nm.

**Uptake and Efflux Experiments with HAOEC.** Immediately before the uptake experiment, the culture medium was removed and the cells were washed three times with 1 ml of Hanks’ balanced salt solution (HBSS; pH 7.4, 37°C; Invitrogen Corp., Carlsbad, CA). Uptake was initiated by adding 1 ml of endothelial cell medium containing 10 μM [14C]rofecoxib or [14C]celecoxib (final concentration of dimethyl sulfoxide, 0.1%), and the cells were incubated at 37°C up to 360 min. After the incubation, the cells were washed three times with 1 ml of ice-cold HBSS to stop the uptake and to remove any extracellular [14C]-compounds. For the quantification of the compounds taken up, the reaction mixtures were frozen to stop the reaction and stored at −80°C until analysis by LC/MS.

**Radiodeetection HPLC Analyses.** After the incubation of [14C]rofecoxib with aortic homogenates, the supernatants were analyzed by radiodetection HPLC as previously reported (Oitate et al., 2007). The chromatographic system included a LC-10A system (Shimadzu Corp., Kyoto, Japan) equipped with an Xterra MS C18 column (4.6 × 150 mm, 5 μm; Waters Corp., Milford, MA) heated to 40°C. The mobile phase, consisting of distilled water containing 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B), was delivered at a flow rate of 1 ml/min, starting at 20% solvent B in solvent A and increasing the proportion of solvent B to 60% linearly for 20 min, and holding for 10 min at 60% solvent B. The effluent was monitored using a SPD-10A UV detector (280 nm; Shimadzu Corp.) and a Radiomatic 525TR radiochemical detector (PerkinElmer Life and Analytical Sciences,
COVALENT BINDING OF ROFECOXIB TO ALLYSINE IN HUMAN ELASTIN

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Collagenase</th>
<th>Elastase</th>
<th>Pronase</th>
<th>Residue</th>
</tr>
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<tr>
<td>Radioactivity recovery (% of total covalent binding)</td>
<td>40.6</td>
<td>51.9</td>
<td>4.4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### Results

**Acid, Organic Solvent, and Proteolytic Enzyme Treatments of Human Aorta**

After incubation with [14C]rofecoxib, the human abdominal aortic homogenate was treated by successive extraction and washing with 0.6 M TCA, 80% methanol, and 100% methanol, and then the resultant precipitated fraction was enzymatically treated successively with collagenase, elastase, and pronase (n = 1). As shown in Table 1, the largest amount of the radioactivity from [14C]rofecoxib was recovered in the elastolytic fraction (51.9% of the total covalent binding). The second largest amount of radioactivity was recovered in the collagenolytic fraction (40.6% of the total). The radioactivity recovered in the fraction after pronase treatment was 4.4%, and the radioactivity in the residue after the treatments with the proteolytic enzymes was 3.0%.

**Effect of Protein Modifiers on in Vitro Covalent Binding of [14C]Rofecoxib to Human Aorta Homogenate**

Homogenate of human aorta, which had been pretreated with 10 mM of protein modifiers (BAPN, d-penicillamine, or hydralazine) under physiological pH condition (pH 7.4), and then the resultant precipitated fraction was enzymatically treated with collagenase, elastase, and pronase in series as described under Materials and Methods. In each step after the enzyme treatment, the removable radioactivity from the tissue was measured.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Collagenase</th>
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**Effect of Several COX-2 Inhibitors on in Vitro Covalent Binding of [14C]Rofecoxib to Rat and Human Aorta Homogenates**

Homogenate of rat aorta was incubated with [14C]rofecoxib (50 μM) under a physiological pH condition (pH 7.4) in the presence or absence of nonradiolabeled COX-2 inhibitors (0.5 mM), and the amount of the radioactivity that bound covalently to the proteins was measured (Table 2). The covalently bound aortic radioactivity from [14C]rofecoxib in the control was 1.66 ± 0.07 μg Eq/g. The amount was significantly decreased in the presence only of nonradiolabeled rofecoxib to 53% of the control, but not of the other COX-2 inhibitors at all. On the other hand, the binding amount of 50 μM [14C]celecoxib (0.19 ± 0.17 μg Eq/g) was significantly lower than that of [14C]rofeczoxib (control).

Homogenate of human aorta was treated in the same way as described above, and the effect of nonlabeled COX-2 inhibitors (0.5 mM) on the covalent binding of [14C]rofecoxib (50 μM) was investigated (Table 2). In the control, the covalent binding of [14C]rofecoxib was 0.46 ± 0.02 μg Eq/g. The addition of nonradiolabeled rofecoxib to the aortic homogenate significantly decreased it to 22% of the control, whereas the other COX-2 inhibitors had no such effect at all. The binding of 50 μM [14C]celecoxib (0.09 ± 0.09 μg Eq/g) was significantly lower than that of [14C]rofeczoxib (control). The binding of 25 and 100 μM [14C]rofeczoxib was 0.24 ± 0.01 and 0.88 ± 0.05 μg Eq/g, respectively.

Radiodetection HPLC analysis of the reaction mixtures of rat and human samples demonstrated that the major component in the reaction mixture supernatant after the incubation demonstrated that the major component is the unchanged rofecoxib (>95%, data not shown). In a separate experiment, BAPN and hydralazine significantly decreased the LOX activity to 41 and 0% of the control, respectively. Radiodetection HPLC analysis of the reaction mixture supernatant after the incubation demonstrated that the major component is the unchanged rofecoxib (>95%, data not shown).
tion of BAPN, a specific inhibitor of LOX (Tang et al., 1983), conducted in the presence of some protein modifiers (Fig. 3). Pretreatment in vitro binding studies using human aortic homogenate were conducted in the presence of some protein modifiers (Fig. 3). Pretreatment of BAPN, a specific inhibitor of LOX (Tang et al., 1983), was consistent with that obtained in rat aorta (Oitate et al., 2006). This result was confirmed not to be peaks derived from the adducts with benzaldehyde.

**Fig. 4.** Reconstructed ion chromatograms of m/z 488 (A), 421 (B), 465 (C), 463 (D), and 421 (E), corresponding to the estimated protonated molecules of the adducts formed from each COX-2 inhibitor with benzaldehyde. Each COX-2 inhibitor (100 μM) was incubated with benzaldehyde (1 mM) in potassium phosphate buffer (pH 7.4) at 37°C for 24 h. Positive ion mode results are representatively shown. Nominal mass: celecoxib (381), valdecoxib (314), etoricoxib (358), CS-706 (356), rofecoxib (314), and benzaldehyde (106). Peaks shown as asterisks were confirmed not to be peaks derived from the adducts with benzaldehyde. **Fig. 5.** Uptake and efflux of [14C]rofecoxib (circle) and [14C]celecoxib (triangle) in HAOEC for 120 min, and then the cells were washed and incubated at 37°C with [14C]rofecoxib (only for humans), 100 μM [14C]rofecoxib, or 50 μM [14C]celecoxib at 37°C for 2 h (n = 3). The amount of covalent binding was measured as described under Materials and Methods.

![Table 2](image)

**TABLE 2**

Effect of several COX-2 inhibitors on the covalent binding of [14C]rofecoxib to aorta homogenate of rat and human (mean ± S.D., n = 3)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Covalent binding (μg Eq/g aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Rofecoxib (50 μM)</td>
<td>—</td>
<td>1.66 ± 0.07</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>—</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>—</td>
<td>0.88 ± 0.47***</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>—</td>
<td>1.90 ± 0.15</td>
</tr>
<tr>
<td>Etoricoxib</td>
<td>—</td>
<td>1.66 ± 0.11</td>
</tr>
<tr>
<td>CS-706</td>
<td>—</td>
<td>1.74 ± 0.17</td>
</tr>
<tr>
<td>[14C]Rofecoxib (25 μM)</td>
<td>—</td>
<td>N.D.</td>
</tr>
<tr>
<td>[14C]Rofecoxib (100 μM)</td>
<td>—</td>
<td>3.75 ± 0.12***</td>
</tr>
<tr>
<td>[14C]Celecoxib (50 μM)</td>
<td>—</td>
<td>0.19 ± 0.17***</td>
</tr>
</tbody>
</table>

**Discussion**

After incubation with [14C]rofecoxib, human abdominal aortic homogenate was washed and extracted using TCA/methanol, and the residue was successively enzymatically treated with collagenase, elastase, and pronase and then the radioactivity recovered in each fraction was measured. As shown in Table 1, the largest amount of the radioactivity from [14C]rofecoxib that was covalently bound to the aorta was recovered in the elastolytic fraction, suggesting that rofecoxib is covalently bound to elastin in the human aorta. This result was consistent with that obtained in rat aorta (Oitate et al., 2006).

To investigate the reactivity of rofecoxib with the allysine aldehyde in human elastin compared with that in rat elastin (Oitate et al., 2007), in vitro binding studies using human aortic homogenate were conducted in the presence of some protein modifiers (Fig. 3). Pretreatment of BAPN, a specific inhibitor of LOX (Tang et al., 1983), significantly decreased the covalent binding of [14C]rofecoxib, probably via the prevention of allysine production from lysine. The covalent binding of [14C]rofecoxib was also significantly decreased by pretreatment with D-penicillamine and hydralazine, which have been reported to react with protein aldehydes in connective tissues and to form thiazolidine or hydrazone analog (hydralazine can also inhibit LOX activity) (Pinnell et al., 1968; Deshmukh and Nimni, 1969; Gallop and Paz, 1975; Numata et al., 1981; Howard-Lock et al., 1986). These results strongly suggested that the aldehydic functional...
the rat aorta (Oitate et al., 2006). The covalent binding of \([^{14}C]\)rofecoxib to human aorta was concentration-proportional in the range between 25 to 100 \(\mu M\) (Table 2), suggesting that the binding capacity is fairly high.

In the enzymatic fractionation of the human aorta (Table 1), the rank order of recovery of radioactivity was similar to that in rat aortas after oral administration of \([^{14}C]\)rofecoxib in vivo (Oitate et al., 2006).

However, it differed in one way: in the rat aorta almost all of the radioactivity (>90%) was recovered in the elastolytic fraction with a small percentage in the collagenolytic fraction, whereas in the human aorta approximately 41% of the total binding radioactivity was recovered in the collagenolytic fraction as well as in the elastolytic fraction. This difference might come from dissimilarities in the parts of the aortas used (human, abdominal; rat, thoracic). It has been reported that the content of elastin in the aortic wall decreases progressively, whereas in contrast, the content of collagen increases, since the aorta is traversed longitudinally from the arch to abdominal (Grant, 1967).

In collagen, allysine is also formed from the lysine residue by LOX and contributes importantly to the elasticity of CV tissues, as well as elastin (Eyre et al., 1984). Pinnell and Martin (1968) have reported that, in the case of elastin, 5 to 16 lysyl residues per \(10^2\) amino acids are converted to allysine residues, compared with one lysyl residue per \(10^3\) amino acids in collagen. From the above results in the human abdominal aorta, it was suggested that rofecoxib is capable of covalently binding to the allysine aldehyde in elastin and partially binding to that in collagen.

After the incubation of \([^{14}C]\)rofecoxib with rat aortic homogenate in vitro in the presence or absence of nonradiolabeled rofecoxib, celecoxib, valdecoxib, etoricoxib, and CS-706, the amount of covalent binding was measured (Table 2). The binding of \([^{14}C]\)celecoxib was significantly lower than that of \([^{14}C]\)rofecoxib, supporting our previous result; that is, there was no retention of radioactivity in the aorta after oral administration of \([^{14}C]\)celecoxib to rats in vivo, whereas the radioactivity from \([^{14}C]\)rofecoxib was retained by and accumulated in the rat aorta (Oitate et al., 2006). The covalent binding of \([^{14}C]\)rofecoxib to rat aortic homogenate in vitro was significantly decreased by the addition of only rofecoxib, whereas the other COX-2 inhibitors had no such effect. As well as the above results in rats, only the addition of nonradiolabeled rofecoxib to human aortic homogenate could significantly reduce the covalent binding of \([^{14}C]\)rofecoxib, and the binding of \([^{14}C]\)celecoxib was much less than that of \([^{14}C]\)rofecoxib (Table 2). In the cases of both rats and humans, rofecoxib did not affect the LOX activity at all. Therefore, the decrease of \([^{14}C]\)rofecoxib binding by nonradiolabeled rofecoxib was thought to be due to a competitive inhibition of allysine aldehyde. This suggested that other COX-2 inhibitors except for rofecoxib have no reactivity with the allysine aldehyde in human elastin, the same as in rats and collagen.

To estimate the reactivity of COX-2 inhibitors with the allysine aldehyde in human elastin and collagen with certainty, they were incubated with benzaldehyde, a model compound of allysine, under a physiological pH condition, as previously reported (Oitate et al., 2007). In the case of rofecoxib (as a positive control), the two peaks \(P_a\) and \(P_b\) were detected as reported previously (Fig. 4). By LC/MS and LC-NMR analyses, they had been identified as the covalent adducts of rofecoxib with benzaldehyde and diastereomers (Oitate et al., 2007). On the other hand, in the cases of other COX-2 inhibitors, no reaction products that were considered to be adducts with benzaldehyde were detected at all by LC/MS analyses operated in both the positive and negative ion modes. The C5 position in rofecoxib was considered to be sufficiently nucleophilic to react with aldehyde (Oitate et al., 2007). The other COX-2 inhibitors tested structurally do not contain a carbon atom carrying hydrogen \(\alpha\) or allylic to the carbonyl group (Fig. 2), and therefore it is reasonable to conclude that these compounds did not react with benzaldehyde.

In clinical situations, the \(C_{max}\) of rofecoxib at a general therapeutic dose (25 mg) was reported to be \(\sim 1 \mu M\) (Davies et al., 2003). On the other hand, the in vitro binding of \([^{14}C]\)rofecoxib to the human aorta was linear, at least in the concentration range of 25 to 100 \(\mu M\) (Table 2), and the binding capacity was high, as mentioned above. Considering that rofecoxib binds to allysine in a covalent manner, rofecoxib has often been chronically dosed in clinical situations, and because both elastin and collagen have very slow turnover rates (Maroudas et al., 1992; Petersen et al., 2002), rofecoxib would be increasingly accumulated in the human aorta depending on the dose frequency. From the above results, it is presumed that in clinical situations rofecoxib, but not other COX-2 inhibitors, might prevent the cross-linking process of elastin and collagen, leading to the degradation of elastin and collagen, the dysfunction of arteries, and finally CV events.

It has been generally accepted that CV events caused by selective COX-2 inhibitors might be partially due to an imbalance of the concentration ratio of two prostanooids with major CV actions: PGI\(_2\), a vasodilator and inhibitor of platelet aggregation, and TxA\(_2\), a vasoconstrictor and promoter of platelet aggregation. That is, selective COX-2 inhibitors diminish the production of PGI\(_2\) in the endothelium, but not TxA\(_2\) in the platelets, so that the relative concentration of TxA\(_2\) increases around the affected area, which might increase the CV risks (McAdam et al., 1999). Both \([^{14}C]\)rofecoxib and \([^{14}C]\)celecoxib were taken up by HAOEC, and the intracellular concentration of \([^{14}C]\)celecoxib at 120 min was about 3-fold higher than that of \([^{14}C]\)rofecoxib (Fig. 5). On the other hand, the efflux of both compounds was very rapid without any retention of radioactivity in the cells, suggesting that both compounds might not be retained by and accumulated in human aortic endothelial cells in vivo. When the concentrations in the plasma are high immediately after the administrations, it is thought that the concentrations in the endothelial cells and platelets are also high and that all selective COX-2 inhibitors, including rofecoxib and celecoxib, would theoretically cause a PGI\(_2\)/TxA\(_2\) imbalance. However, it is quite difficult to postulate that rofecoxib has an especially strong effect in causing an imbalance compared with other compounds.

In conclusion, it was suggested that rofecoxib, but not the other COX-2 inhibitors, can covalently bind to the allysine aldehyde in human elastin as well as that in rats (Fig. 1B) and that it can bind partially in human collagen. This would lead to the prevention of the normal cross-linking processes and dysfunction of the arteries. This might be one of the main causes of CV events by rofecoxib in clinical situations. In this view, other COX-2 inhibitors could be less toxic in terms of CV risks than rofecoxib.

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**References**


Address correspondence to: Masataka Oitate, Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58, Hiroamachi, Shinagawa-ku, Tokyo 140-8710, Japan. E-mail: oitate.masataka.3@daiichisankyo.co.jp