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Evaluation of loxoprofen and its alcohol metabolites for potency and selectivity of inhibition of cyclooxygenase-2

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Abstract—Loxoprofen, its *trans*-alcohol and *cis*-alcohol metabolites were evaluated for selectivity of inhibition of COX-2 over COX-1. The (2*S*,1′*R*,2′*S*)-*trans*-alcohol derivative was found to be the most active metabolite and to be a potent and nonselective inhibitor of COX-2 and COX-1 in both enzyme and human whole blood assays.

Loxoprofen is a 2-arylpropionic acid anti-inflammatory agent with analgesic and anti-pyretic properties. It has been shown to be an effective inhibitor of the production of inflammatory prostaglandins in vivo but only a weak inhibitor of cyclooxygenase (COX) activity when tested using enzyme and cell assays in vitro.[1–3] Its inhibitory effects on cyclooxygenase activity have been attributed to its (2*S*,1′*R*,2′*S*)-*trans*-alcohol reduction product (**5a**, see Scheme 1), the major metabolite of loxoprofen detected after administration.[1–6]

Assessing the selectivity of loxoprofen as a cyclooxygenase inhibitor is a difficult task since the compound is available as a mixture of 4 isomers, acts as pro-drug and has a rather complex profile of metabolites. Loxoprofen can be metabolized by stereoselective reduction, hydroxylation, chiral inversion, and conjugation to glucuronide derivatives.[4–8] As described for several other profens, the 2*R* propionic acid enantiomer of loxoprofen undergoes rapid chiral inversion to 2*S* after oral administration.[7,8] A major route of metabolism for loxoprofen involves the stereoselective reduction of its cyclopentanone moiety to generate the (2*S*,1′*R*,2′*S*)-*trans*-alcohol derivative (**5a**). In addition to the *trans*-alcohol metabolite, reduction of the cyclopentanone can also yield the corresponding *cis*-alcohol (**6**), which has been detected mainly with the 2*S* configuration at the 2-aryl propionic chiral centre.[7,8]

Previous studies have shown that the metabolite **5a** is more potent at inhibiting COX activity in ram seminal vesicle microsomes (a source of COX-1) than loxoprofen.[1] Limited data are available on selectivity. Inhibition of COX-2 mediated PGE_2 production by IL-1 stimulated human synovial cells ($IC_{50} = 0.12$ μM) showed a 3-fold selectivity *vs* a COX-1 platelet assay for **5a**, about 10-fold higher than indomethacin and 10-fold lower than diclofenac.[9] In the present study we have prepared the four *trans*-alcohols and a mixture of the *cis*-alcohol metabolites of loxoprofen and have evaluated them for their potency and selectivity of inhibition of human COX-2 over COX-1 using both enzyme and whole blood assays.

The four *trans*-cyclopentanol metabolites **5a–d** and the *cis*-cyclopentanol metabolite **6** were synthesized according to Scheme 1.[10] Loxoprofen **1** was derivatized with (*R*)-pantolactone to give the ester intermediate **2**, which was reduced with sodium cyanoborohydride and separated by flash column chromatography to yield the *trans*-cyclopentanol **3** and the *cis*-cyclopentanol **4** in a ~5:1 ratio. The four diastereomers of **3** were then separated by chiral HPLC and the corresponding diastereomeric intermediates were subsequently hydrolyzed to provide the four *trans*-cyclopentanol metabolites **5a–d**.[11] The *cis*-cyclopentanol **4** was hydrolyzed in a similar manner to give the *cis*-cyclopentanol metabolite **6** as a mixture of four diastereomers.

The structures of the various metabolites are provided in Scheme 1 and were identified using the conventional

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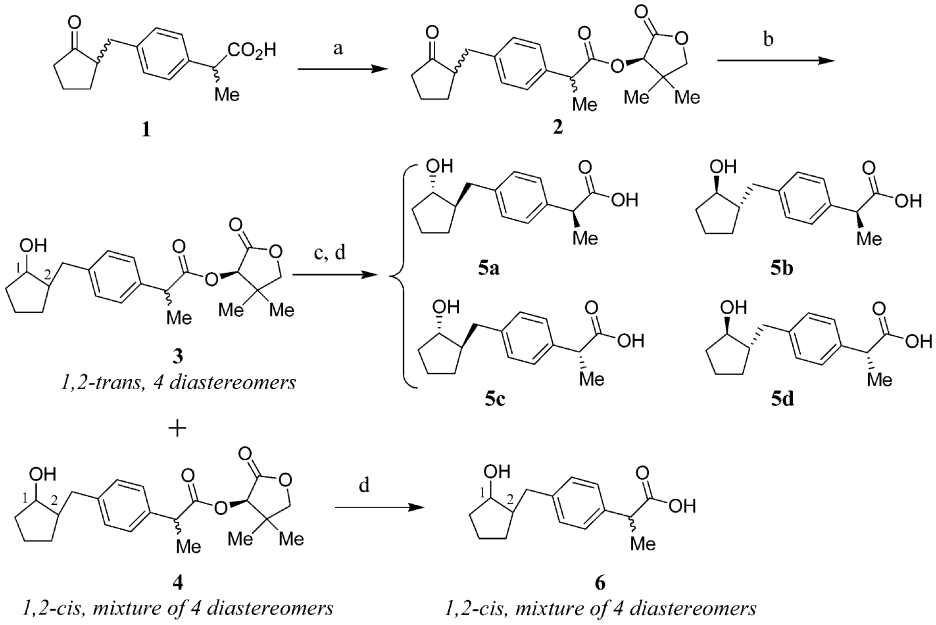

Scheme 1. (a) (*R*)-Pantolactone, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfate (CMC), DMAP; (b) $NaBH_3CN$, MeOH; (c) HPLC separation on Prep Chiral Pak AD column using 30% EtOH in hexane; (d) 0.4 M Na_2CO_3 in 50% aq MeOH, then 2 M HCl in HOAc; or 2 M HCl in 50% aq 1,4-dioxane.

names described by Takasaki and Tanaka.[8] The IC_{50} values for the inhibition of recombinant COX-1 and COX-2 [12] by loxoprofen and its alcohol metabolites are summarized in the Table 1. Indomethacin and MF-tricyclic (a close analogue of rofecoxib[13]) were used as comparators for nonselective and selective COX-2 inhibitors. The (2*S*,1′*R*,2′*S*)-*trans*-alcohol derivative **5a** was found to be the most potent inhibitor and was equipotent at inhibiting the enzymatic activities of COX-2 ($IC_{50}=0.39$ μM) and of COX-1 ($IC_{50}=0.5$ μM) assayed under identical conditions. In contrast, the other *trans*-alcohols (**5a–d**), or loxoprofen itself, were much weaker or inactive as inhibitors of COX-2 with $IC_{50}>100$ μM. Similar data were obtained for COX-1, with a very weak inhibition detected for the (2*S*,1′*S*,2′*R*)-*trans*-alcohol (**5b**) ($IC_{50}=51$ μM). The *cis*-alcohol isomers (**6**) were not potent but gave an incomplete inhibition (~50%) of COX-1 and COX-2 at high doses (<25 μM).

The selectivity of the (2*S*,1′*R*,2′*S*)-*trans*-alcohol derivative **5a**, the most potent metabolite, was further characterized using whole blood assays.[14] In agreement with the enzyme data, **5a** was a potent inhibitor of COX-2 ($IC_{50}=0.3$ μM) and showed no selectivity for the inhibition of COX-2 over COX-1 ($IC_{50}=0.28$ μM) (Table 2). As a comparison, the values for rofecoxib in these assay are $IC_{50}=0.53\pm0.02$ μM for COX-2 and $IC_{50}=18.8\pm0.9$ μM for COX-1.[15]

Table 1. Inhibitory effects of loxoprofen and its alcohol metabolites on the activities of recombinant human COX-1 and COX-2 (mean ± SE)

	COX-2 IC_{50} (μM)	COX-1 IC_{50} (μM)
MF-tricyclic	0.23 ± 0.04	>100
Indomethacin	0.73 ± 0.07	0.11 ± 0.02
Loxoprofen (**1**)	>100	>100
(2*S*,1′*R*,2′*S*)-*trans*-alcohol (**5a**)	0.39 ± 0.05	0.5 ± 0.08
(2*S*,1′*S*,2′*R*)-*trans*-alcohol (**5b**)	>100 (30% Inh.)	51 ± 23
(2*R*,1′*R*,2′*S*)-*trans*-alcohol (**5c**)	>100	>100
(2*R*,1′*S*,2′*R*)-*trans*-alcohol (**5d**)	>100	>100
cis-alcohols (mixture of 4 isomers) (**6**)	>25 (50% Inh.)	>25 (44% Inh.)

Loxoprofen and the other metabolites were also characterized for their effect on COX-1 or COX-2 in human whole blood assays. Although it is expected that the enantiomeric composition of the tested compounds might change during incubations in vitro,[4,7,8,16] these assays were performed to detect potential inhibitory effects of loxoprofen and its metabolites in a more physiologically relevant milieu with cells using endogenously generated arachidonic acid substrate for the COX reaction. This assay would also detect alternative mechanisms of inhibition (such as inhibition of PLA_2). In the whole blood assay, loxoprofen was a very weak inhibitor of COX-2 (IC_{50} of 13.5 ± 4.6 μM) and about 2-fold more potent against COX-1. Inhibitory effects on COX-2 and COX-1 were also detected with other metabolites (Table 2) but with a much lower potency than **5a**, possibly due to some isomerization to the most active **5a** metabolite during incubations with blood. The chirality of the 2-aryl propionic moiety is clearly important for the inhibitory effects on COX-2 or COX-1, (as reported for other 2-arylpropionyl acids [16]), with a large decrease in potency for the corresponding 2*R* enantiomers (**5c**,**d**). Considering their lower potency and the fact that they represent more minor metabolites than **5a** in vivo, the other metabolites would not be expected to contribute significantly to COX inhibition at normal effective doses.

In conclusion, the (2*S*,1′*R*,2′*S*)-*trans*-alcohol reduction product (**5a**) of loxoprofen is the most active major

Table 2. Potency of loxoprofen and its alcohol metabolites on COX-1 and COX-2 in human whole blood assays (mean ± SE)

	COX-2 IC_{50} (μM)	COX-1 IC_{50} (μM)
MF-tricyclic	0.48 ± 0.07 (n = 16)	15.3 ± 2.2 (n = 22)
Loxoprofen (**1**)	13.5 ± 4.6 (n = 8)	6.5 ± 1.3 (n = 9)
(2*S*,1′*R*,2′*S*)-*trans*-alcohol (**5a**)	0.30 ± 0.07 (n = 7)	0.28 ± 0.14 (n = 5)
(2*S*,1′*S*,2′*R*)-*trans*-alcohol (**5b**)	2.0 ± 1.3 (n = 5)	4.7 ± 1.4 (n = 9)
(2*R*,1′*R*,2′*S*)-*trans*-alcohol (**5c**)	49% at 30 μM	20.2 ± 4.3 (n = 5)
(2*R*,1′*S*,2′*R*)-*trans*-alcohol (**5d**)	> 33	> 25
cis-alcohols (mixture of 4 isomers) (**6**)	22 ± 10 (n = 6)	1.9 ± 0.3 (n = 5)
Indomethacin[15]	0.44 ± 0.07	0.19 ± 0.02
Rofecoxib[15]	0.53 ± 0.02	18.8 ± 0.9

metabolite of loxoprofen with a potency of inhibition of COX-2 comparable to that of indomethacin or of potent selective COX-2 inhibitors. This result is consistent with the high efficacy of loxoprofen as an anti-inflammatory agent.[1–3] However, **5a** showed no selectivity for the inhibition of COX-2 as compared to COX-1 as assessed in both enzyme and whole blood assays. Although the gastrointestinal tolerability of loxoprofen has been shown to be superior to indomethacin,[3,17] the data on the nonselective inhibition are in agreement with the ability of loxoprofen to cause gastric lesions in animals at lower doses than selective COX-2 inhibitors.[18,19]

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