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RAPID COMMUNICATION

METABOLIC CHIRAL INVERSION OF FLURBIPROFEN-CoA *IN VITRO*

David J. Porubek,[1] Susan M. Sanins,[2] Jeffrey R. Stephens,[2] Mark P. Grillo,[1] David G. Kaiser,[3] Gordon W. Halstead,[4] Wade J. Adams[3] and Thomas A. Baillie[1]*

Departments of [1]Medicinal Chemistry and [2]Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA 98195; and [3]Drug Metabolism Research and [4]Control Research and Development, The Upjohn Company, Kalamazoo, MI 49001, U.S.A.

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Flurbiprofen (*RS*-2-[2-fluoro-4-biphenylyl]propionic acid; Ansaid, Upjohn) is a 2-arylpropionic acid non-steroidal anti-inflammatory agent (NSAID) employed clinically for the treatment of a variety of arthritic disorders. By virtue of the asymmetric center at C-2, flurbiprofen and related 2-arylpropionic acids exist in enantiomeric forms, and although the pharmacological activity of this series is associated almost exclusively with the *S* isomers [1], only naproxen is marketed as the pure *S* enantiomer. Interestingly, however, many 2-arylpropionates undergo an unusual metabolic transformation which converts the inactive *R* isomers into the corresponding *S* antipodes [2]. As a result, the *R* enantiomers of these compounds effectively serve as pro-drugs for their *S* counterparts, and considerable interest has focused on the underlying mechanism of this metabolic chiral inversion reaction [3-8].

In a preliminary communication, Nakamura *et al.* [5] reported that inversion of *R*-ibuprofen in rat liver homogenates requires CoA and ATP as cofactors, and they proposed that the CoA thioester conjugate of ibuprofen served as an obligatory intermediate in the chiral inversion. Support for this view came from work with the long-chain acyl-CoA synthetase from rat liver microsomes, which was found to convert *R*-ibuprofen [9] and *R*-fenoprofen [10] into their respective thioester conjugates. The *S* enantiomers of these drugs, in contrast, were not converted to CoA derivatives *in vitro* and were not inverted *in vivo*. Moreover, it was found that ibuprofen [11] and fenoprofen [12] are incorporated into "hybrid triglycerides" (a process which is believed to require the intermediacy of a CoA derivative), and that such incorporation is stereoselective for the *R* isomers.

Despite the above findings which implicate CoA thioesters as key intermediates in the metabolic chiral inversion of 2-arylpropionic acids, much of the evidence is indirect and no work appears to have been carried out with authentic specimens of the CoA conjugates of these drugs. The present study, which was undertaken in an effort to address this deficiency, employed flurbiprofen as a model compound since it has been reported [13-15] that this NSAID does *not* undergo chiral inversion *in vivo* (presumably as a consequence of the failure of flurbiprofen to be activated to the corresponding CoA thioester intermediate [16]). The study set out to test the following three hypotheses: (i) the CoA conjugate (but not the free acid form) of *R*-flurbiprofen undergoes chiral inversion *in vitro*, (ii) the inversion of *R*-flurbiprofen-CoA is enzyme mediated, and (iii) the inversion process is bidirectional, leading to *racemization* of the profen in the form of its CoA thioester.

R-Flurbiprofen (96% optical purity), *S*-flurbiprofen (98% optical purity) and the racemic mixture were converted to their CoA derivatives according to a two-step procedure similar to that described by Becker and Harris [17]. Flurbiprofen (180 mg; 0.73 mmol) was treated with $SOCl_2$ (238 mg; 2.0 mmol) and the mixture was heated at 70° for 4 hr. Removal of excess reagent under reduced pressure

* Corresponding author.

(0.5 mm Hg) afforded the desired acid chloride in 90% yield. A mixture of aqueous $NaHCO_3$ (0.1 M; 10 mL) and tetrahydrofuran (30 mL) was stirred in a sealed flask under a stream of N_2 for 5 hr. To this mixture was added CoA (100 mg; 0.14 mmol) followed, 5 min later, by the above acid chloride (100 mg; 0.38 mmol). After stirring for 15 min at ambient temperature, the reaction was quenched with 20% HCl (0.15 mL) and concentrated *in vacuo*. The residue was extracted with diethyl ether (3x20 mL) and the aqueous phase evaporated to dryness to afford flurbiprofen-CoA as a white solid in 60% yield. TLC (*n*-butanol / acetic acid / H_2O, 5:2:3): single spot, R_f = 0.58. ^1H NMR (300 MHz; ^2H_2O, DDS as reference): δ 0.68 (s,3H), 0.78 (s,3H), 1.43 (d,3H), 2.41, 2.51, 3.18 (all m,2H), 6.03 (d,1H), 7.1-7.7 (m,8H), 8.34 (s,1H) and 8.52 (s,1H). FAB/MS (matrix of thioglycerol / dimethyl sulfoxide / 0.1% HCl, 100:10:1): *m/z* 994 ($[M+H]^+$), 1016 ($[M+Na]^+$), 1038 ($[M-H+2Na]^+$), 1060 ($[M-2H+3Na]^+$), 1082 ($[M-3H+4Na]^+$) and 487 ([M+H-phosphoadenosine diphosphate]$^+$).

Metabolic experiments were carried out using freshly prepared liver mitochondria from adult male Sprague-Dawley rats (*ca.* 250 g) since it has been reported that ibuprofen [5,16] and fenoprofen [16] undergo metabolic chiral inversion in this subcellular organelle. Mitochondria were obtained by differential centrifugation of liver homogenates, according to standard procedures, and were solubilized by sonication with a micro-tip ultrasonic homogenizer (20 kHz; 3x10-sec bursts with 10-sec rests). Control incubations were performed either in the absence of mitochondria or in the presence of mitochondria which had been boiled for 5 min to denature protein. Incubation mixtures (total volume = 1.0 mL) contained substrate (0.1 μmol) and mitochondrial protein (1.0 mg) in 0.25 M phosphate buffer (pH 7.2). After incubation at 37° for 20 min, CoA esters were hydrolyzed by adding 1.0 M NaOH (0.25 mL) and allowed to stand at ambient temperature for 20 min. *RS*-[$^2H_8$]Flurbiprofen (0.1 μmol) was then added as internal standard, and the flurbiprofen enantiomers were extracted into toluene and converted to their respective 1-phenylethylamide diastereoisomers, as described in detail elsewhere [6]. Quantitative determination of *R*- and *S*-flurbiprofen was based on a stable isotope dilution assay, which involved selected ion monitoring of the $M^{+\cdot}$ ions (at *m/z* 347 for unlabeled drug and *m/z* 355 for internal standard). The ratios of peak areas (analyte : internal standard) in the resulting ion current chromatograms were used to calculate the concentrations of each flurbiprofen enantiomer by reference to appropriate standard curves. Incubations were carried out in quadruplicate, and the results are expressed as means ± SD. Statistical significance was assessed by Student's *t*-test.

Table 1. Chiral inversion of flurbiprofen enantiomers *in vitro*

Substrate	Additives	Opposite antipode present after 20 min (%)
R-Flurbiprofen (free acid)	Buffer only	4.8 ± 1.4
	Solubilized mitochondria	3.6 ± 0.5
	Boiled mitochondria	3.8 ± 0.7
R-Flurbiprofen-CoA	Buffer only	24.0 ± 3.5
	Solubilized mitochondria	32.2 ± 0.4*
	Boiled mitochondria	21.6 ± 1.6
S-Flurbiprofen (free acid)	Buffer only	1.6 ± 0.1
	Solubilized mitochondria	1.7 ± 0.2
	Boiled mitochondria	1.7 ± 0.1
S-Flurbiprofen-CoA	Buffer only	23.6 ± 0.6
	Solubilized mitochondria	50.0 ± 1.6*
	Boiled mitochondria	23.1 ± 1.3

*Significantly different from result obtained in corresponding "buffer only" and "boiled mitochondria" studies ($P < 0.001$).

As shown in Table1, neither *R*- nor *S*-flurbiprofen underwent appreciable chiral inversion when incubated as their free acids, since the percentage of the opposite antipode recovered after incubation was essentially the same as that present in each substrate at the outset. In contrast, the CoA thioester derivatives of *R*- and *S*-flurbiprofen underwent substantial inversion when incubated with solubilized mitochondria and, in the case of the *S* enantiomer, complete racemization occurred within 20 min (Fig. 1). There were no significant differences in the degrees of inversion between the "buffer only" and "boiled mitochondria" controls for either *R*- or *S*-flurbiprofen-CoA, and the observed values of *ca.* 23% for the opposite enantiomer recovered after such incubations may be attributed largely to chemical racemization which accompanied the synthesis or basic hydrolysis of the CoA thioester substrates. The step(s) at which this partial chemical racemization takes place is presently under investigation.

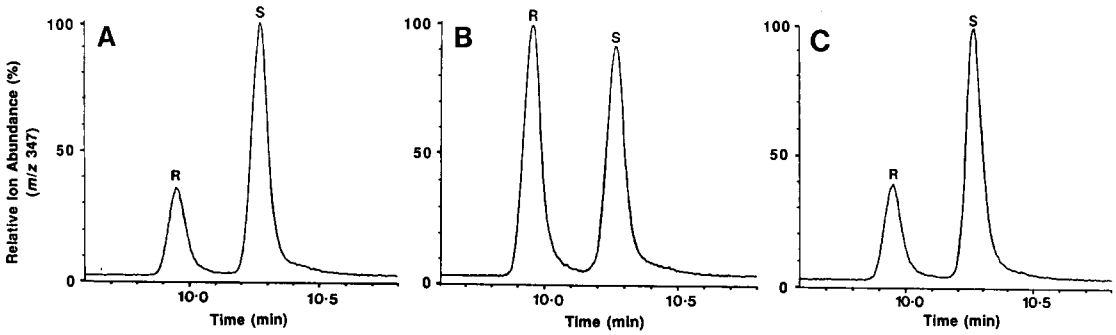

Fig. 1. Ion current chromatograms (*m/z* 347) from the GC-MS analysis of products formed during the incubation of *S*-flurbiprofen-CoA with (A) buffer alone, (B) solubilized mitochondria, and (C) boiled mitochondria. Derivatized *R*- and *S*-flurbiprofen enantiomers eluted at 9.96 and 10.27 min, respectively.

The results of this *in vitro* study provide convincing evidence that the CoA thioesters of 2-arylpropionic acid NSAIDs play a key role in the metabolic chiral inversion of this structural class. Furthermore, the process appears to be largely enzyme-mediated, as opposed to being chemical in nature [3,7], and occurs in the mitochondrial fraction of rat liver. At present, the identity of the "acyl-CoA epimerase" which catalyzes this transformation remains unknown. It is also evident from Table 1 that the inversion reaction is bidirectional, in that the CoA derivatives of both *R*- and *S*-flurbiprofen were transformed readily into one another. In view of these findings, it may be concluded that the failure of flurbiprofen to undergo metabolic chiral inversion in rats and humans *in vivo* [13-15] and its lack of incorporation into hybrid triglycerides *in vitro* [18] most likely reflect its inability to form a CoA derivative.

Collectively, these results underscore the pivotal role of acyl-CoA synthetase(s) in mediating the chiral inversion of 2-arylpropionic acids. Those members of the series which are substrates for this conjugation pathway may be expected to exhibit metabolic chiral inversion, whereas those which are not will fail to do so. Similarly, the degree to which a particular 2-arylpropionate inhibits the β-oxidation of fatty acids may be governed by the efficiency with which it is transformed to its CoA thioester [19], and interspecies variations in the chiral inversion of a particular 2-arylpropionate [12,20] probably reflect species differences in the activity of the respective acyl-CoA synthetases (in addition to that of the "acyl-CoA epimerase[s]"). The controversial issue of interindividual variability in the clinical response to 2-arylpropionates also may prove to be linked to interindividual differences in the activity of these two enzymes in the human population [21]. Further studies on the identities and substrate requirements of these enzymes should lead to a clearer understanding of the factors which determine both the therapeutic effects and adverse properties of this important group of anti-inflammatory drugs.

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