Biochemical Pharmacology, Vol. 37, No. 1, pp. 105–114, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

THE METABOLIC CHIRAL INVERSION AND DISPOSITIONAL ENANTIOSELECTIVITY OF THE 2-ARYLPROPIONIC ACIDS AND THEIR BIOLOGICAL CONSEQUENCES

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Abstract—The 2-arylpropionic acids are currently an important group of non-steroidal anti-inflammatory agents. They contain a chiral centre, and *in vitro* studies on inhibition of prostaglandin synthesis show that their activity resides almost exclusively in the *S*(+)-isomers. However, this stereoselectivity of action is not manifest *in vivo*, due to the thus-far-unique unidirectional metabolic inversion of the chiral centre from the inactive *R*(−)-isomers to the *S*(+)-antipodes. Available evidence strongly suggests that this reaction proceeds *via* the formation of the acyl CoA thioesters of the 2-arylpropionates, but the participation of enzyme(s) in the inversion process remains uncertain. Although the chiral inversion is seemingly a general feature of the fate of 2-arylpropionates, there do occur important combinations of acid and species where the reaction is not extant. The stereochemistry of the chiral centre of these acids also influences other aspects of their disposition, including the oxidative metabolism of the aryl/arylalkyl moiety, glucuronidation of the -COOH group and plasma protein binding, and the importance of certain of these becomes more evident when renal function is impaired. The biological consequences of the metabolic chiral inversion and enantioselective disposition of the 2-arylpropionates have been summarized in terms of their implications for the development and use of safer and more effective drugs of this class.

Inflammatory diseases of various types commonly occur in the population and non-steroidal drugs able to interfere with the inflammatory process are thus widely used. Currently, the 2-arylpropionic acids or "profens" are an important group of non-steroidal anti-inflammatory drugs (NSAIDs), being both widely prescribed and generally perceived as of considerable benefit in diseases such as arthritis and rheumatism. Regrettably, there has also been considerable emphasis upon the problem of adverse reactions to NSAIDs, and a number of these drugs have been subjected to close scrutiny [1]. Phenylbutazone has been withdrawn in many countries, and even the toxicity of aspirin has been re-evaluated. Of more recently introduced agents, zomepirac has been withdrawn due to allergenicity, while various profens have given rise to major problems, e.g. benoxaprofen, suprofen and indoprofen.

There is widespread recognition of the value of metabolic and pharmacokinetic information in aiding the discernment of mechanisms of drug action, the establishment of concentration–effect relationships and informing safety evaluation. Although conventional metabolic and pharmacokinetic studies of profens are in general unhelpful in understanding adverse reactions [2], it has recently become apparent that a consideration of the stereochemical aspects of their fate can provide notable insights [2–4].

The profens contain a chiral centre, and exhibit optical activity, and thus exist as pairs of (relatively) readily separable stereoisomers (Fig. 1). In general, enantiomers have very similar physiochemical properties, and it is hard to distinguish them in an achiral environment [5]. However, when they are allowed to interact with other chiral centres, it is frequently the case that such interactions exhibit a high degree of handedness [5, 6]. The body, of course, provides an intensely chiral environment in which the great majority of important processes exhibit stereospecificity [6]. Many critical features of the pharmacology and toxicology of organic compounds arise from their interaction with highly chiral endogenous molecules present in receptors, enzymes etc. [7]. It thus is not surprising that the biological actions of chiral pharmacologically active molecules reside partly or exclusively in one of the enantiomers [7]. The more active is often referred to as the eutomer

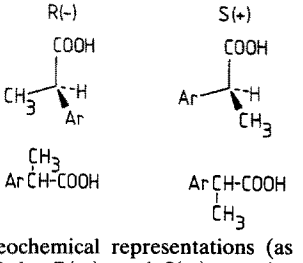

Fig. 1. Stereochemical representations (as flying wedge diagrams) of the *R*(−)- and *S*(+)-enantiomers of the 2-arylpropionic acids.

Table 1. Stereoselectivity of action of profen NSAIDs at putative sites of action

Drug	Eudismic (*S*/*R*) ratio	System examined	Reference
Ibuprofen	160	PG synthesis inhibition (bovine)	8
Flurbiprofen	878	SRS-A antagonism (guinea pig)	9
Indoprofen	*ca.*100	PG synthesis inhibition	10
Naproxen		PG synthesis inhibition	
	133	(sheep)	11
	70	(bovine)	12
Pirprofen	6.4	PG synthesis inhibition (sheep)	11
Carprofen	>16	PG synthesis inhibition (sheep)	13
	>23	Platelet aggregation	13
Fenoprofen	35	PG synthesis inhibition (human platelet)	14, 15

and the less active as the distomer, and the eudismic ratio (i.e. activity ratio) is used to show the magnitude of selectivity of action, which in some cases is so great as to make the compound's effects stereospecific.

The anti-inflammatory actions of the profens, like other NSAIDs, apparently originate in their ability to block one or more of the enzymes responsible for the activation of arachidonic acid to prostaglandins, leukotrienes and other mediators involved in the inflammatory process. When the profen enantiomers are tested for relevant inhibitory actions *in vitro*, it is seen that the activities of the profens reside almost exclusively in their *S*(+) isomers (Table 1).

It is only very rarely that the stereoselectivity of action of pairs of enantiomers is identical *in vivo* and *in vitro*. In the whole animal, it is well known that stereoisomers may differ in terms of their absorption, distribution, metabolism and excretion, particularly when these depend upon the interaction of the drug with chiral macromolecules [6]. These differences will cause corresponding differences in the concentrations of the two enantiomers at their sites of action, so that the eudismic ratio *in vivo* will differ from that seen *in vitro*.

Even in the light of the above, the profens are unprecedented in that the high degree of stereoselectivity of action seen *in vitro* is very markedly reduced, and in some cases entirely lost, when the activities of the enantiomers are compared in whole animal models of inflammatory disease. Available data are presented in Table 2.

The resolution of the contradictory findings shown in Table 2 is obtained by a study of the stereochemistry of metabolism of the 2-arylpropionic acids. These agents are now well known to undergo an entirely fortitutious and thus-far-unique metabolic inversion of the chiral centre with no other covalent change to the drug [3]. The reaction is apparently unidirectional, and transforms the *R*(−)enantiomers to their *S*(+)antipodes. The first example of this reaction is that of ibuprofen, reported first in 1973, and since then a considerable number of profens have been shown to undergo this chiral inversion. In other cases, where definitive experimentation is lacking, there exists considerable suspicion that inversion does occur, and it appears that the reaction is a general one for 2-arylpropionates. A list of those acids known, or suspected, to undergo this inversion is presented in Table 3.

Table 2. Differential stereoselectivity of action of profen NSAIDs *in vitro* and *in vivo*

Drug	Eudismic (*S*/*R*) ratio *in vitro**	*in vivo*	Reference†
Ibuprofen	160	1.3	8
Flurbiprofen	878	8	9
Indoprofen	*ca.* 100	25	10
Naproxen	133		16
	70	21	
Carprofen	>16		13
	>23	15	
Fenoprofen	35	*ca.* 1	4, 15

* Data from Table 1.
† References to *in vivo* data.

Table 3. Chiral inversion of 2-arylpropionic acids from *R*(−)- to *S*(+)-enantiomers

Shown with [reference]	
2-Phenylpropionic acid [17]	Clidanac [3]
Ibuprofen [3]	Naproxen [3]
Cicliprofen [3]	Loxoprofen [20]
Benoxaprofen [3]	Fenoprofen [15, 21]
2-[3-(2-Chlorophenoxyphenyl)]propionic acid [18]	2-(2-Isopropylindan-5-yl)-propionic acid [3]
Ketoprofen [19]	Thioxaprofene [22]
Suspected for	
2-(4-Phenylphenoxy)propionic acid [3]	Carprofen [3]
Indoprofen [3]	

MECHANISM OF CHIRAL INVERSION

The inversion of a chiral centre is, in chemical terms, an energetically demanding reaction, and a number of possible reaction sequences have been postulated for the inversion of the profens. On purely chemical grounds, likely routes [3] could involve hydroxylation either of the α-methyl group or at the α-methine proton, yielding in either case an alcohol which could then dehydrate to give a common α-methylene intermediate. This in turn could then undergo a stereoselective reduction to yield a single isomer of the 2-arylpropionate. It will be apparent that these various routes represent somewhat unlikely possibilities for the *in vivo* metabolism of a 2-arylpropionate, and we are fortunate in the case of 2-phenylpropionic acid to have data on the fate of all three of the putative intermediates [3]. The two hydroxylated compounds, atrolactic acid and tropic acid, are both very extensively excreted unchanged, while the putative α-methylene intermediate, atropic acid, is completely broken down and no 2-phenylpropionic acid is formed. These data present very strong evidence that the chiral inversion of the 2-arylpropionates does not involve oxidative reactions [3].

A far more plausible reaction mechanism for the inversion has been proposed by Nakamura *et al.* [23]. This involves the stereoselective conversion of the *R*-enantiomer of the acid to its acyl CoA thioester, a process which results in the activation of the α-methine proton, which is both benzylic and esteratic. The acyl CoA may then undergo one of three separate fates, as follows:

(i) racemization of the chiral centre, since the α-methine proton very rapidly exchanges with protons from aqueous media: this may or may not involve enzymic catalysis. The acyl CoA "racemate" so produced will then hydrolyse to give a mixture of the *R*- and *S*-enantiomers of the parent profen [3];

(ii) hydrolysis with retention of configuration to yield the original *R*-2-arylpropionate [3], or

(iii) acyl transfer of the profen moiety into a hybrid triglyceride, resulting in the retention of the profen in adipose tissue [24].

The exchange of the alpha-methine proton upon racemization was shown in the original studies by the use of specifically deuterated ibuprofen, but since our first review of the literature [3] no further work upon the mechanism of chiral inversion has appeared in the literature.

The stereochemical aspects of the disposition of ibuprofen into adipose tissue have been reported [24]. Male Wistar rats were treated chronically with either *RS*-, *R*- or *S*-ibuprofen (at the same dose level, 20 mg/kg i.p. for 7 days) and sacrificed at 20 or 116 hr following the final dose. Samples of fat were collected, the triglycerides isolated and examined for ibuprofen enantiomers. The lipid content of ibuprofen was greatest following administration of the *R*-enantiomer and the levels of both *R*- and *S*-ibuprofen were approximately twice those following administration of the racemate. Only trace quantities of ibuprofen were detected in lipids following the administration of the *S*-enantiomer [24].

The enantiomeric composition of the incorporated ibuprofen showed stereoselectivity for the *R*-enantiomer, which is reasonable if the formation of the CoA thioester is stereoselective/stereospecific for the *R*-enantiomer and the rate of inversion is slower than the rate of incorporation into triglyceride. Meffin and Sallustio (cited in ref. 21) have reported similar stereospecificity for the incorporation of fenoprofen into rat hepatocytes.

In association with the above, it is interesting to note that fenoprofen and ketoprofen, which together with ibuprofen, were shown by Fears *et al.* [25] to undergo incorporation into triglycerides, have since been reported to undergo metabolic chiral inversion. Of particular interest is the similarity between the ratio of the rate of incorporation of fenoprofen/ketoprofen into lipid (*ca.* 8) using rat liver preparations and the ratio of the clearance values for the inversion process in the rabbit of *ca.* 12.5 [19, 21]. This similarity may not be surprising because, as pointed out by Hayball and Meffin [21] the initial step is formation of the CoA thioester. However, any relationships between inversion and incorporation into lipid based on these figures should be treated with care due to species differences and *in vivo/in vitro* differences.

Until recently the chiral inversion was thought to be unidirectional, i.e. from *R* to *S*. However, Lee *et al.* [26] tentatively suggested that the reverse reaction may occur with ibuprofen in man. More recently Fournel and Caldwell [17] have shown that *S*-2-phenylpropionic acid yields small but significant quantities of the *R*-enantiomer in rats.

It has been proposed that the enzyme system involved in the inversion process is, or is similar to, methylmalonyl Coenzyme A racemase (EC 5.1.99.1) [26]. Whilst the above is an interesting hypothesis, problems arise as this enzyme appears to be unidirectional with respect to *S*-methylmalonyl CoA, while the putative enzyme racemizes the acyl CoAs. Furthermore, this proposal implies that $\underline{R}$-C_6H_4— is bioisosteric with either —COOH or —COSCoA, for which there is no precedent. It is important to note that there is <u>no</u> experimental evidence for the involvement of this enzyme in the metabolism of profens.

ANALYTICAL CONSIDERATIONS

A variety of methods have been applied to the determination of the enantiomeric composition of profen NSAIDs in biological media. These involve either the separation of the enantiomers by HPLC on a chiral stationary phase, or the formation of diastereoisomers with one enantiomer of a chiral derivatizing agent followed by the chromatographic separation of the products. The great majority of methods reported in the literature involve the formation of diastereoisomeric derivatives (for compilations see Refs. 3 and 27) and chiral HPLC and GLC columns have not been widely applied to the profens. In our experience, diastereoisomeric derivatives have proved much more successful than chiral columns for this purpose [28, 29].

SPECIES OCCURRENCE OF THE CHIRAL INVERSION

Although the metabolic chiral inversion of 2-aryl-

Table 4. Species occurrence of the chiral inversion of profen NSAIDs

Profen	Species	References
Ibuprofen	Man, rat, mouse, guinea pig	3
Naproxen	Rat	3
Clidanac	Guinea pig, NOT rat, mouse	3
Benoxaprofen	Man, rat	3
Cicliprofen	Man, Rhesus monkey, rat, dog	3
2-(2-Isopropylindan-5-yl) propionic acid	Rat	3
Fenoprofen	Man, rabbit	15, 21
2-[3-(2-Chlorophenoxy) phenyl]propionic acid	Rat	18
Ketoprofen	Rabbit	19
Loxoprofen	Rat	20
2-Phenylpropionic acid	Rat, rabbit, NOT mouse	17, 31, 32
Thioxaprofene	Man, Rhesus monkey, dog, rat	22
Indoprofen	Rat, mouse, NOT man?	3, 10, 33
Tiaprofenic acid	NOT man	34

propionates is to be regarded as a general feature of the fate of these agents, it must be appreciated that there are notable instances of the failure of a species to express this reaction. Table 4 lists the species occurrence of the chiral inversion of a number of profens: the failure of inversion of clidanac in the rat and mouse, of 2-phenylpropionic acid in the mouse and of indoprofen in man are noteworthy.

In addition, there can occur considerable differences between species in the rate of inversion, presumably reflecting the activities of other competing metabolic and dispositional options for the molecule in question. In the case of benoxaprofen, the half-life for the inversion reaction in the rat is 2.5 hr compared with 108 hr in man [3].

STEREOSELECTIVE METABOLISM OF THE PROFENS

Functionalization reactions

In addition to the chiral inversion reaction the stereochemistry of the "profens" can influence the routes of metabolism of these drugs. However, this may be difficult to discern due to the chiral inversion, as is seen by an examination of the available data on ibuprofen and its two major oxidative products, 2-[4-(2-hydroxy-2-methylpropyl) phenyl]propionic acid (I) and 2-[4-(2-carboxypropyl)phenyl]propionic acid (II). Both of these are dextrorotatory irrespective of the enantiomer of ibuprofen administered [35, 36]. The formation of metabolite II introduces a second chiral centre, which could account for the observed change in optical rotation from that of the administered drug. However, this would not account for the change in optical rotation of metabolite I. More recent studies by Wechter *et al.* [37] and Kaiser *et al.* [38] indicated an enantiomeric excess of the *S*(+)-isomer of metabolite I regardless of the isomer of ibuprofen administered, but from the results presented it is difficult to establish whether this is due to stereoselective oxidation or a result of the chiral inversion reaction. Indeed it is not beyond the realms of possibility that the metabolite itself may undergo chiral inversion, particularly in view of the structural diversity of the compounds known to undergo this process (see above).

The situation with metabolite II is made more complex by the second chiral centre, as it can exist as 4 isomers, enantiomeric in pairs. Interpretation of the data is hindered by methodological problems: neither the original packed column GLC assay of Kaiser *et al.* [38] or the more recent capillary GC–MS procedure of Young *et al.* [39], allow the resolution of all four chiral species. The enantiomeric composition of the metabolite arising from the administration of *R*-, *S*- and *RS*-ibuprofen is shown in Table 5.

Table 5. Isomeric composition of the major metabolite of ibuprofen (2-[4-(2-carboxypropyl)phenyl]propionic acid) excreted in urine of volunteers given different optical forms of ibuprofen

Form administered	% Metabolite as that isomer	
S	*S,S*	51
	R,S/*S,R*	47
	R,R	2
R	*S,S*	33
	R,S/*S,R*	42
	R,R	25
RS	*S,S*	43
	R,S/*S,R*	44
	R,R	13

Drawn from Kaiser *et al.* [38].

The results are difficult to interpret due to the lack of resolution of the *R,S-* and *S,R*-isoisomers. However, it seems reasonable to assume that the *R,S*-enantiomer is present in vast excess after administration of the *S*-enantiomer, compared to its *S,R*-antipode. This indicates that the oxidation of *S*-ibuprofen to metabolite II is essentially non-stereoselective, as similar quantities of the *S,S-* and *R,S*-diastereoisomers are produced. Similar deductions are not possible following the administration of *R*- or *RS*-ibuprofen, due to the chiral inversion and the failure to resolve two of the forms of II.

Fenoprofen undergoes aromatic oxidation yielding the 4′-hydroxy derivative, which is excreted in urine as a glucuronide. The enantiomeric composition of the metabolite in human urine, following administration of the racemic drug, has been investigated by Rubin *et al.* [15]. The great majority of the excreted material was found to have the *S*-configuration: indeed GC–MS was required to detect the *R*-enantiomer in some urine samples. A total of 78–86% of the administered dose was excreted as *S*-fenoprofen and *S*-4′-hydroxyfenoprofen. The significance of the excess *S*-enantiomer of the metabolite, in terms of stereoselective oxidation, is again difficult to determine as the chiral inversion of *R*-fenoprofen is extremely rapid in man [15] and the rabbit [21].

The metabolism of clidanac, an indan-1-carboxylic acid derivative, has been investigated in the Rhesus monkey [40]. Its metabolism involves the oxidation of the cyclohexyl moiety, mainly in the 3 and 4 positions, to yield alcohol and ketone derivatives. The 4-hydroxy derivatives give rise to geometrical isomers, i.e. both *cis* and *trans* metabolites were identified. The two 3-hydroxy derivatives identified were both *cis* and were diastereoisomers, due to formation of a new chiral centre in the molecule [40]. In addition, the formation of a mixture of dihydroxy derivatives was reported, but the stereochemistry of these was not investigated.

The metabolism of loxoprofen (2-[4-(2-oxocyclopentylmethyl)phenyl]propionate) has been investigated in the rat, mouse, dog, crab-eating macaque (*Macaca fascicularis*) and man [20, 30, 41]. The stereochemical aspects of the metabolism of this compound are of significance, as in addition to the chiral inversion in the rat [20], the drug undergoes reduction of the ketone function to yield the pharmacologically active metabolite 2-(4-[*trans*-(1*R*,2*S*)-2-hydroxycyclopentylmethyl]phenyl) propionic acid [41, 42]. In addition to the latter, reduction of the ketone moiety yields the corresponding *cis*-alcohol, which is known to have the *S*-configuration at the 2-arylpropionic acid chiral centre and to be "racemic" at the two chiral centres in the hydroxycyclopentyl moiety; and a series of three 2,4-diol and two α-ketol metabolites [41].

Following administration of racemic loxoprofen to rats, the enantiomeric composition of "unchanged" drug and the *trans-* and *cis*-alcohols in plasma increased to yield *ca.* 100% *S*-enantiomers after 6 hr, 3 hr and 5 min respectively [20]. The rapid attainment of plasma enantiomeric "purity" of the *cis*-alcohol indicates stereoselective reduction of 2*S*-loxoprofen, particularly as this metabolite was not detected following administration of 2*R*-loxoprofen. The 2*S-trans*-alcohol is a major plasma metabolite following administration of the racemate or either enantiomer to rats. Examination of dose-normalized AUCs indicated no significant differences in the formation of the active metabolite following the three forms [20]. An indication of the very rapid chiral inversion of loxoprofen is given by the detection of the 2*S*-parent acid and 2*S-trans*-alcohol in plasma within 5 min of the oral administration of the 2*R*-enantiomer [20].

An examination of the human urinary metabolites of racemic loxoprofen indicated the presence of "unchanged" drug and the *cis-* and *trans*-alcohols [43]. The enantiomeric composition of both the parent drug and its metabolites indicated a progressive increase in the proportion with the 2*S*-configuration, being *ca.* 100% in a 4–8 hr urine sample [43], the results indicating the possible occurrence of the chiral inversion in man.

Several other profen NSAIDs undergo oxidative metabolism, e.g. flurbiprofen, indoprofen, pirprofen and cicliprofen [44], the metabolism of the latter introducing a second chiral centre into the molecule, but the stereochemical aspects of these transformations have not been investigated.

As stated previously, the significance of stereoselectivity in the oxidative or reductive metabolism of the profens is difficult to discern due to the metabolic chiral inversion, particularly if the inversion is rapid, e.g. fenoprofen and loxoprofen, or where analytical problems arise, e.g. the dicarboxy metabolite of ibuprofen. Further complications may arise if the products of oxidative metabolism also undergo inversion. The combined effects of the chiral inversion and stereoselective metabolism result, in the majority of cases examined, in the more rapid clearance of the *R*-enantiomers [3] except in the case of tiaprofenic acid [34].

Stereoselective glucuronidation

In general, the principal urinary metabolites of the profens are their acyl glucuronides and stereoselectivity in this reaction has been reported (Table 6). Since glucuronic acid itself is chiral, and has the β-D-configuration, the conjugates of profens will be diastereoisomers. Selectivity in the excretion of glucuronides appears to be both structure and species dependent, thus excretion of the *S*-conjugates is greater than the *R* in the cases of benoxaprofen and ibuprofen in man, 2-phenylpropionic acid in the mouse, while $R > S$ for fenoprofen and ketoprofen in the rabbit and $R \simeq S$ for tiaprofenic acid in man and 2-phenylpropionic acid in the rabbit.

A study [46] of the enantioselectivity of the glucuronic acid conjugation of 2-phenylpropionic acid in rat liver microsomal preparations has yielded some interesting results, which are summarized in Table 7. Although the V_{max} values for the two enantiomers are different ($R > S$), their K_m values are essentially identical, indicating that the isomers do not differ in affinity for the conjugating enzyme(s). It seems likely that the differential orientation of the two enantiomers in the active site of UDPGT determines the ease with which glucuronic acid may be transferred from UDPGA, and this is reflected in the different V_{max} values.

Table 6. Summary of the literature on the stereoselective glucuronidation of 2-arylpropionic acids *in vivo*

Drug (form administered)	Species	Findings	Reference
Benoxaprofen (racemate)	Man	20–25% dose recovered in urine over 96 hr as glucs, ratio $S/R = 2.2$	45
Fenoprofen (R, S and RS)	Rabbit	CL_{gluc} R/S 2.12 Pretreatment with phenobarbitone decreased the ratio to 1.49	21
Ibuprofen (R and S)	Man	% dose excreted as $R(-)$ 1.5; $S(+)$ 12.5. CL_{gluc} R 1.1 ml/min/S 9.1 ml/min. Ratio $S/R = 8.3$ Log-linear relationship between fraction inverted and the ratio of isomeric clearance as glucs	26
Ketoprofen (R and S)	Rabbit	CL_{gluc} $R/S = 1.43$	19
2-Phenylpropionic acid (hydratropic acid)			
(R,S and RS)	Mouse, rat	Stereoselective $S(+)$	17
	rabbit	Not stereoselective	17
(RS)	Rat	$S(+)$ gluc excess in bile	31
		$R(-)$ gluc excess in urine	31
Tiaprofenic acid (RS)	Man	Urinary recovery of NaOH sensitive material indicates non-stereoselective glucuronidation	34

gluc = glucuronide; CL_{gluc} = clearance of glucuronide.

Although the administration of hepatic enzyme inducers causes an up-to-three-fold increase in the overall rate of conjugation of 2-phenylpropionic acid, the enantioselectivity of glucuronidation observed in untreated animals is unaltered. This provides further evidence that the enantioselectivity arises from the two isomers binding in different ways to a single UDPGT rather than the two enantiomers being glucuronidated by different isozymes.

The observed proportions of the diastereoisomeric glucuronic acid conjugates of a profen in urine may thus be due to stereoselectivity in their synthesis, renal clearance and/or hydrolysis [26]. Additionally, metabolic selectivity in conjugation may influence the inversion reaction.

It is thus not surprising that there occur discrepancies (often marked) between the enantioselectivity of the glucuronidation of profens *in vitro*, where the reaction may be examined in isolation, and *in vivo*, where a variety of competing metabolic and dispositional mechanisms, often exhibiting their own enantioselectivities, are operational.

STEREOSELECTIVE PROTEIN BINDING OF PROFENS

The profens, like other carboxylic acid NSAIDs are highly (> 99%) bound to plasma proteins [44]. However, only relatively recently has the stereoselectivity of this binding and its implications for the therapeutic use of these drugs been examined.

Early studies to demonstrate stereoselectivity of protein binding used circular dichroism spectra [47, 48]. Using this technique Rendic *et al.* [48] found a stronger interaction of S-ketoprofen with human serum albumin than its R-antipode, but Perrin [47] was unable to demonstrate stereoselectivity of fenoprofen binding using a similar technique. Hayball and Meffin [21] believe that binding of fenoprofen to rabbit plasma is likely to be stereoselective, and the drug–albumin ratios of 14 used by Perrin [47] are irrelevant to those found in rabbit plasma (*ca.* 1 or less).

In vitro studies on the binding of ibuprofen enantiomers to albumin indicated a mean enantiomeric ratio (S/R) of 1.70 for the free fraction, suggesting stereoselectivity of binding for the R-isomer, and

Table 7. Enantioselectivity of the glucuronidation of 2-phenylpropionic acid by rat liver microsomal preparations

Pretreatment	S/R (V_{max})	Extent of induction
Control	0.64 ± 0.05*	1.00
Phenobarbitone	0.63 ± 0.02	2.99
3-Methylcholanthrene	0.60 ± 0.09	1.32
Clofibrate	0.70 ± 0.15	0.82

* Kinetic constants for glucuronidation in control microsomes were: $R(-)$ K_m 3.90 mM; V_{max} 7.24 nmol/min/mg protein; $S(+)$ K_m 3.63 mM; V_{max} 4.72 nmol/min/mg protein. Mean ± S.D., n = 5.
Drawn from Fournel-Gigleux *et al.* [46].

this may be an important factor in the intra-individual differences in the kinetics of the drug [49]. The same group proposed that differences in the pharmacokinetics of ibuprofen enantiomers, following their individual administration and as the racemate, could be due to enantiomer–enantiomer interactions in protein binding [26].

A similar enantiomeric ratio (*S/R ca.* 2) for free 2-phenylpropionic acid in rabbit plasma at steady state has been reported by Meffin *et al.* [32]. In a more detailed study, the same group examined the binding of 2-phenylpropionic acid to rabbit albumin stripped of fatty acids, albumin stripped of fatty acids to which oleic acid was added and rabbit plasma [50]. The results obtained indicated the presence of two possible specific binding sites per albumin molecule, one of which could be blocked by free fatty acids, in addition to non-specific binding. Binding of 2-phenylpropionic acid enantiomers at the remaining specific site was found to be stereoselective for the *R*-enantiomer, as was the non-specific binding [50]. The complexity of the situation is increased by the fact that the binding of either enantiomer reduces the binding of the other, at least for 2-phenylpropionic acid and possibly for other profens, e.g. fenoprofen [21]. The binding of individual enantiomer is further dependent on its plasma concentration, which is constantly changing due to chiral inversion and other stereoselective metabolism and disposition processes.

STEREOSELECTIVE DISPOSITION

The foregoing summaries have documented both the metabolic chiral inversion of the profens and the dependence of other routes of metabolism, including oxidations, reduction and glucuronidation, and plasma protein binding, upon the configuration of the chiral centre. These various stereoselectivities, possibly together with those of other processes not so far discerned, combine together to result in the observed stereoselectivity of disposition, with the inactive *R*(−)antipodes being more rapidly eliminated from the body, with smaller plasma AUCs and shorter plasma elimination half-lives. This has been shown to occur with naproxen, carprofen, indoprofen and ibuprofen [3].

INFLUENCE OF RENAL IMPAIRMENT UPON THE CHIRAL INVERSION REACTION

In the normal situation, the final step in the elimination of the majority of profen NSAIDs is the clearance into the urine of their acyl glucuronides. The overall enantiomeric composition of these excretion products will be the result of the enantioselective glucuronidation of the profen, whose enantiomeric composition will in turn be influenced by the chiral inversion process. In situations where renal function is impaired, there will be a reduced clearance of the glucuronides into the urine, and a phenomenon termed "futile cycling" is observed. Acyl glucuronides are (relatively) unstable, and can be hydrolysed in the body both by β-glucuronidases present in various tissues and spontaneously at the pH of plasma. The retention and subsequent cleavage of the acyl glucuronides of profen NSAIDs serves to throw greater emphasis upon the chiral inversion reaction, the activity of which is unaffected by renal impairment. In human subjects, this has been shown by the co-administration of probenecid, an inhibitor of the renal tubular transport of organic acids, and benoxaprofen [45]. Probenecid caused a reduction in the % dose excreted in the urine as the acyl glucuronides, and changed the enantiomeric ratio of the excreted acid in favour of the *S*-isomer, i.e. the interaction resulted in enhanced chiral inversion. Meffin and his collaborators [32] have studied the chiral inversion of the model compound 2-phenylpropionic acid in rabbits in which renal impairment was induced by treatment with uranyl nitrate. In these animals, there was a 3.5-fold increase in the fraction of the dose undergoing chiral inversion, as a consequence of the "futile cycling" of the acyl glucuronide, which in turn resulted in a 5.7-fold increase in the unbound plasma concentration of the free S-enantiomer. Although data on the stereochemical aspects of the disposition of profen NSAIDs in patients with renal failure are scant at present, it is of some importance to note that the incidence of adverse reactions to a variety of these drugs is far higher in this situation. In this context, it is perhaps unfortunate that it was not possible to study the chiral inversion of benoxaprofen in such patients, in view of their susceptibility to this drug [51].

BIOLOGICAL CONSEQUENCES OF THE METABOLIC CHIRAL INVERSION AND ENANTIOSELECTIVE DISPOSITION OF PROFENS

It is well established that the anti-inflammatory actions of the profen NSAIDs reside in their *S*-enantiomers, and thus the metabolic inversion of their chiral centre from *R*- to *S*- and the enantioselectivity of other aspects of their disposition acquire a particular significance. The enantiomeric composition of the drug present in the body and able to exert its effects will be constantly changing under the influence of these processes. The pharmacological, toxicological and therapeutic implications of the chiral inversion and enantioselective disposition may conveniently be considered under five headings:

1. Since the major biological activity of these agents, inhibition of the metabolism of arachidonic acid to a range of mediators of inflammation, which underlies their pharmacological activity, resides in the *S*-enantiomers. The unidirectional chiral inversion of the *R*-isomers to their *S*-antipodes is a form of metabolic activation of the racemic drug.

2. As well as being responsible for the therapeutic actions of profen NSAIDs, inhibition of arachidonic acid metabolism is the cause of certain of the well-documented adverse effects of this class of drug: this is especially the case for gastro-intestinal ulceration. In such cases, chiral inversion will also enhance toxicity.

3. Although the chiral inversion reaction is apparently a general feature of the metabolism of the 2-arylpropionates, there are considerable inter-species differences in its occurrence and rate. Since this reaction results in the activation of these acids, these

species differences in the relative proportions of *R*- and *S*-enantiomers present at any given time are expected to be associated with comparable inter-species differences in pharmacological and toxic effects. The occurrence of these species variations represents a major hindrance to the extrapolation of biological data from species to species.

4. There is generally a far better correlation between drug effect and plasma concentration than between effect and dose. The discernment of plasma concentration–effect relationships is thus important both during the development of a drug and for its optimal therapeutic use. However, in such studies it is essential that the compound responsible for the activity is assayed, and in the case of chiral drugs which exhibit enantioselectivity of action this means that the active enantiomer must be determined. In the case of the profens, the occurrence of the chiral inversion and enantioselective disposition processes mean that the enantiomeric composition of the drug is constantly changing, with a progressive enrichment with the active *S*-isomer. Unless stereospecific assays are used for the determination of the plasma concentrations of profens, the information obtained cannot be relied upon. Analytical methods which do not discriminate between active and inactive isomers, but merely measure the total quantity present, are at best of strictly limited value and can give highly misleading information. Although this matter has been aired in the literature a number of times in recent years, with respect to the profens by us [2–4] and more generally by Ariens [52, 53], it is disappointing to note that flawed studies continue to be peformed and, more worryingly, accepted for publication.

5. The various points above combine to suggest strongly that only the *S*-enantiomers of the profen NSAIDs should be used in therapy. This would overcome problems arising from differences in the rate and extent of the chiral inversion when the racemic drugs are used, both in terms of the origins of adverse drug reactions and differences in therapeutic response. The use of the active *S*-enantiomers alone would allow dose reduction, at least in terms of the total quantity of drug given to the patient, although the amount of active drug isomer would remain the same. At best, the *R*-isomers function as pro-drugs for the therapeutically active *S*-forms, which will be produced in an unpredictable manner. At worst, the *R*-enantiomers are undesirable impurities in the active drug, and may cause difficulties due to non-stereoselective toxicity, perhaps involving metabolism remote from the chiral centre, and/or by undesirable interactions with the *S*-antipodes.

The use of single enantiomers only would require the resolution or stereospecific synthesis of profen NSAIDs, which would represent a not-inconsiderable extra expense in the drug development process. However, it seems reasonable to suggest that the use of the *S*-isomers of the profens could allow the safer and more effective use of this class of drug. In view of the notoriety of NSAIDs of all types from the viewpoint of adverse drug reactions, the extra expense involved in the production of single enantiomers would be worthwhile if it enhanced their safety. At the present time, in the U.K. at least, the only 2-arylpropionate NSAID available as the *S*-enantiomer is naproxen. It is likely that the next few years will see the addition of a considerable number of single profen enantiomers to the therapeutic armamentarium.

Note added in proof. The attention of interested readers is drawn to two recent and highly relevant papers from Y. Nakamura and T. Yamaguchi, "Stereoselective metabolism of 2-phenylpropionic acid in rat. I. *In vitro* studies on the stereoselective isomerization and glucuronidation of 2-phenylpropionic acid", *Drug Metab Dispos* **15**: 529–534, 1987, and "Stereoselective metabolism of 2-phenylpropionic acid in rat. II. Studies on the organs responsible for the optical isomerization of 2-phenylpropionic acid *in vivo*", *Drug Metab Dispos* **15**: 535–539, 1987.

Acknowledgements—We are grateful to Professor R. L. Smith and Professor G. Siest for their encouragement of our work in this area and to Dr. Y. Tanaka for helpful discussion. The assistance of Alice Stolinska with the preparation of the manuscript is gratefully acknowledged.

REFERENCES

1. Rainsford KD and Velo GP (Eds), *Side-effects of Anti-inflammatory Drugs*. MTP Press, Lancaster, 1987.
2. Caldwell J and Hutt AJ, Biological implications of the metabolic chiral inversion of 2-arylpropionic acid non-steroidal anti-inflammatory drugs. In: *Side-effects of Anti-inflammatory Drugs* (Eds. Rainsford KD and Velo GP), Part 1, pp. 217–226. MTP Press, Lancaster, 1987.
3. Hutt AJ and Caldwell J, The metabolic chiral inversion of 2-arylpropionic acids. A novel route with pharmacological consequences. *J Pharm Pharmacol* **35**: 693–704, 1983.
4. Hutt AJ and Caldwell J, The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. *Clin Pharmacokin* **9**: 371–373, 1984.
5. Testa B, The chromatographic analysis of enantiomers in drug metabolism studies. *Xenobiotica* **16**: 265–280, 1986.
6. Caldwell J, Winter SM and Hutt AJ, The pharmacological and toxicological significance of the stereochemistry of drug disposition. *Xenobiotica*, in the press.
7. Ariens EJ, Stereoselectivity of bioactive agents: general aspects. In: *Stereochemistry and Biological Activity of Drugs* (Eds. Ariens EJ, Soudijn W and Timmermans PBMWM), pp. 11–32. Blackwell, Oxford, 1983.
8. Adams SS, Bressloff P and Mason CG, Pharmacological differences between the optical isomers of ibuprofen: evidence for the metabolic inversion of the (−)-isomer. *J Pharm Pharmacol* **28**: 256–257, 1976.
9. Greig ME and Griffin RL, Antagonism of slow reacting substances in anaphylaxis (SRS-A9) and other spasmogens on the guinea pig tracheal chain by hydratropic acids and their effect on anaphylaxis. *J Med Chem* **18**: 112–116, 1975.
10. Buttinoni A, Ferrari M, Colombo M and Cesarini R, Biological activity of indoprofen and its optical isomers. *J Pharm. Pharmacol* **35**: 603–604, 1983.
11. Ku EC and Wasvary JM, Inhibition of prostaglandin synthetase by pirprofen, studies with sheep seminal vesicle enzyme. *Biochem Biophys Acta* **384**: 360–368, 1975.
12. Tomlinson RV, Ringold HJ, Qureshi MC and Forchielli E, Relationship between inhibition of prostaglandin synthesis and drug efficacy: support for the current theory on mode of action of aspirin-like drugs. *Biochem Biophys Res Commun* **46**: 552–559, 1972.

13. Gaut ZN, Baruth H, Randall LO, Ashley C and Paulsrud JR, Stereoisomeric relationships among anti-inflammatory activity, inhibition of platelet aggregation and inhibition of prostaglandin synthetase. *Prostaglandins* **10**: 59–66, 1975.
14. Nickander RC, Kroay RJ and Marshall WS, Anti-inflammatory and analgesic effects of fenoprofen. *Fed Proc* **30**: 563, 1971.
15. Rubin A, Knadler MP, Ho PPK, Bechtol LD and Wolen RL, Stereoselective inversion of (*R*)-fenoprofen to (*S*)-fenoprofen in humans. *J Pharm Sci* **74**: 82–84, 1985.
16. Harrison IT, Lewis, B, Nelson P, Rooks W, Roszkowski A, Tomolouis A and Fried JH, Non-steroidal anti-inflammatory agents. I. 6-Substituted 2-naphthylacetic acids. *J Med Chem* **13**: 203–205, 1970.
17. Fournel S and Caldwell J, The metabolic chiral inversion of 2-phenylpropionic acid in rat, mouse and rabbit. *Biochem Pharmacol* **35**: 4153–4159, 1986.
18. Tamegai T, Tanaka T, Kaneko T, Ozaki S, Ohmae M and Kawabe K, The determination of optical isomers of 2-[3-(2-chlorophenoxyphenyl)]-propionic acid in rat plasma by high performance liquid chromatography. *J Liq Chromatogr* **2**: 551–564, 1979.
19. Abas A and Meffin PJ, Enantioselective disposition of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs IV. Ketoprofen disposition. *J Pharmacol Exp Ther* **240**: 637–641, 1987.
20. Nagashima H, Tanaka Y, Watanabe H, Hayashi R and Kawada K, Optical inversion of (2*R*)- to (2*S*)-isomers of 2-[4-(2-oxocyclopentylmethyl)-phenyl]propionic acid (Loxoprofen), a new anti-inflammatory agent, and its monohydroxy metabolites in the rat. *Chem Pharm Bull* **32**: 251–257, 1984.
21. Hayball PJ and Meffin PJ, Enantioselective disposition of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs III. Fenoprofen disposition. *J Pharmacol Exp Ther* **240**: 631–636, 1987.
22. Diekmann H, Garbe A, Steiner K, Buhring KU, Faro HP and Nowak H, Disposition of thioxaprofene: kinetics of the stereoisomers. *Arch Pharmacol* **307**: Suppl R3, Abstr 10, 1979.
23. Nakamura Y, Yamaguchi T, Takahashi S, Hashimoto S, Iwatani K and Nakagawa Y, Optical isomerization mechanism of *R*(−)-hydratropic acid derivatives. *J Pharmacobiol Dynamics* **4**: S-1, 1981.
24. Williams K, Day R, Knihinicki R and Duffield A, The stereoselective uptake of ibuprofen enantiomers into adipose tissue. *Biochem Pharmacol* **35**: 3403–3405, 1986.
25. Fears R, Baggaley KH, Alexander R, Morgan B and Hindley RM, The participation of ethyl 4-benzyloxybenzoate (BRL 10894) and other aryl-substituted acids in glycerolipid metabolism. *J Lipid Res* **19**: 3–11, 1978.
26. Lee EJD, Williams K, Day R, Graham G and Champion D, Stereoselective disposition of ibuprofen enantiomers in man. *Br J Clin Pharmacol* **19**: 669–674, 1985.
27. Hutt AJ, Fournel S and Caldwell J, Application of a radial compression column to the high-performance liquid chromatographic separation of the enantiomers of some 2-arylpropionic acids as their diastereoisomeric *S*-1-(naphthen-1-yl)ethylamides. *J Chromatogr Biomed Appl* **378**: 409–418, 1986.
28. Hutt AJ and Caldwell J, Stereospecific analytical methodology. In: *Metabolism Foreign Compound* (Eds. Gorrod JW, Oelschlager H and Caldwell J), Taylor & Francis, London, in press.
29. Hutt AJ and Caldwell J, Enantiomeric analysis of 2-phenylpropionic acid NSAIDs in biological fluids by high-performance liquid chromatography. In: *Proceedings of the 7th Bioanalytical Forum, Guildford 1987* (Ed Reid E). Plenum Press, New York, in press.
30. Tanaka Y, Nishikawa Y and Hayashi R, Species differences in metabolism of sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (Loxoprofen sodium), a new anti-inflammatory agent. *Chem Pharm Bull* **31**: 3656–3664, 1983.
31. Yamaguchi T and Nakamura Y, Stereoselective disposition of hydratropic acid in rat. *Drug Metab Dispos* **13**: 614–619, 1985.
32. Meffin PJ, Sallustio BC, Purdie YJ and Jones ME, Enantioselective disposition of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs I. 2-Phenylpropionic acid disposition. *J Pharmacol Exp Ther* **238**: 280–287, 1986.
33. Tamassia V, Jannuzzo MG, Moro E, Stegnjaich S, Groppi W and Nicolis FB, Pharmacokinetics of the enantiomers of indoprofen in man. *Int J Clin Pharm Res* **4**: 223–230, 1984.
34. Singh NN, Jamali F, Pasutto FM, Russell AS, Coutts RT and Drader KS, Pharmacokinetics of the enantiomers of tiaprofenic acid in humans. *J Pharm Sci* **75**: 439–442, 1986.
35. Adams SS, Cliffe EE, Lessel B and Nicholson JS, Some biological properties of 2-(4-isobutylphenyl)propionic acid. *J Pharm Sci* **56**: 1686 (1 page only), 1967.
36. Mills RFN, Adams SS, Cliffe EE, Dickinson W and Nicholson JS, The metabolism of ibuprofen. *Xenobiotica* **3**: 589–598, 1973.
37. Wechter WJ, Loughhead DG, Reischer RJ, Van Giessen GJ and Kaiser DG, Enzymatic inversion at saturated carbon: Nature and mechanism of the inversion of *R*(−)p-isobutylhydratropic acid. *Biochem Biophys Res Comm* **61**: 833–837, 1974.
38. Kaiser DG, Van Giessen GJ, Reischer RJ and Wechter WJ, Isomeric inversion of ibuprofen (*R*)-enantiomer in humans. *J Pharm Sci* **65**: 269–273, 1976.
39. Young MA, Aarons L, Davidson EM and Toon S, Stereospecific assay of ibuprofen and its metabolites. *J Pharm Pharmacol* **38**: 60P, 1986.
40. Tanayama S and Kanai Y, Metabolic disposition of 6-chloro-5-cyclohexyl-1-indancarboxylic acid (TA1-284) a new non-steroidal anti-inflammatory agent in Rhesus monkeys. *Xenobiotica* **7**: 145–151, 1977.
41. Naruto S, Tanaka Y, Hayashi R and Terada A, Structural determination of rat urinary metabolites of sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (Loxoprofen sodium) a new anti-inflammatory agent. *Chem Pharm Bull* **32**: 258–267, 1984.
42. Matsuda K, Tanaka Y, Ushiyama S, Ohnishi K and Yamazaki M, Inhibition of prostaglandin synthesis by sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (CS-600), a new anti-inflammatory drug, and its active metabolite *in vitro* and *in vivo*. *Biochem. Pharmacol.* **33**: 2473–2478, 1984.
43. Nagashima H, Tanaka Y and Hayashi R, Column liquid chromatography for the simultaneous determination of the enantiomers of loxoprofen sodium and its metabolites in human urine. *J Chromatogr Biomed Appl* **345**: 373–379, 1985.
44. Hucker HB, Kwan KC and Duggan DE, Pharmacokinetics and metabolism of non-steroidal anti-inflammatory drugs. In: *Progress in Drug Metabolism*, Vol. 5 (Eds. Bridges JW and Chasseaud LF), pp. 165–253. John Wiley, Chichester, 1980.
45. Spahn H, Iwakawa S, Suganuma T, Benet LZ and Lin ET, Direct stereospecific determination of benoxaprofen glucuronides: influence of probenecid on the urinary excretion rates of the diastereoisomeric glucuronides. ISSX 2nd European Symposium on Foreign Compound Metabolism, Frankfurt. 1987 Abstr. P17.
46. Fournel-Gigleux S, Hamar-Hansen C, Motassim N, Antoine B, Mothe O, Decolin D, Caldwell J and Siest G, Substrate specificity and enantioselectivity of

arylcarboxylic acid glucuronidation. *Drug Metab Dispos*, in press.
47. Perrin JH, A circular dichroic investigation of the binding of fenoprofen, 2-(3-phenoxyphenyl)propionic acid to human serum albumin. *J Pharm Pharmacol* **25**: 208–212, 1973.
48. Rendic S, Sunjie V, Kajfez F, Blazevie N and Alehie-Kolbah T, Resolution of (±)−α−(3-benzoylphenyl)propionic acid (Ketoprofen) and diastereomeric interaction of its enantiomers with some biological systems. *Chimia* **29**: 170–172, 1975.
49. Hansen T, Day R, Williams K, Lee E, Knihinicki R and Duffield A, The assay and *in vitro* binding of the enantiomers of ibuprofen. *Clin Exp Pharmacol Physiol Suppl* **9**: 82–83, 1985.
50. Jones ME, Sallustio BC, Purdie YJ and Meffin PJ, Enantioselective disposition of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs II. 2-Phenylpropionic acid protein binding. *J Pharmacol Exp Ther* **238**: 288–294, 1986.
51. Rainsford KD, Mechanisms of gastric contrasted with intestinal damage by non-steroidal anti-inflammatory drugs. In: *Side-effects of Anti-inflammatory drugs* (Eds. Rainsford KD and Velo GP), Part 2, pp. 3–26. MTP Press, Lancaster, 1987.
52. Ariens EJ, Stereochemistry—a basis for sophisticated nonsense in clinical pharmacokinetics. *Eur J Clin Pharmacol* **26**: 663–668, 1984.
53. Ariens EJ, Wuis EW and Veringa EJ, Stereoselectivity of bioactive xenobiotics. A pre-Pasteur attitude in medicinal chemistry, pharmacokinetics and clinical pharmacology. *Biochem Pharmacol* **37**: 9–18, 1988.