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Stereoselective disposition of ibuprofen enantiomers in man

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1 This study has examined the stereoselective disposition of the enantiomers of ibuprofen in four healthy male subjects following separate administration of racemic ibuprofen (800 mg) and of each enantiomer (400 mg).

2 A mean of 63 ± 6% of an administered dose of R(−) ibuprofen was stereospecifically inverted to the S(+) enantiomer. There was no measurable inversion of the S(+) to R(−) ibuprofen. The kinetics of the individual enantiomers were altered by concurrent administration of the respective optical antipode. It is likely that this change reflects an interaction between the enantiomers at plasma protein binding sites.

3 It was found that formation of ester glucuronide conjugates stereoselectively favoured the S(+) enantiomer.

4 The data have demonstrated that the pharmacokinetics of ibuprofen and other α-methylarylacetic acids cannot be interpreted adequately without studying the pharmacokinetics of the individual enantiomers.

Keywords ibuprofen stereoselective disposition

Introduction

Anti-inflammatory analogues of α-methylarylacetic acid exhibit optical isomerism. These compounds show clear stereospecificity in their pharmacodynamic properties in that only the S(+)-enantiomers inhibit prostaglandin synthesis (Adams *et al.*, 1976; Fried *et al.*, 1973; Gaut *et al.*, 1975; Kuzuna *et al.*, 1975; Shen & Winter, 1977). However, except for naproxen these drugs are utilised clinically as their racemates.

Where pharmacokinetic studies have been undertaken, these have generally been with measurements of total drug as distinct from measurement of individual enantiomers. This is unsatisfactory as total drug kinetics give no insight into the stereoselective mechanisms underlying the disposition of individual enantiomers. The situation is further complicated by the *in vivo* stereospecific inversion of the inactive R(−) enantiomers of a number of these compounds to their active optical antipode (Bopp *et al.*, 1979; Goto *et al.*, 1982; Kaiser *et al.*, 1976; Lan *et al.*, 1976; Tamura *et al.*, 1981). To date, few studies have examined either the differential kinetics of the individual enantiomers of these drugs or the magnitude of this inversion reaction.

Our study with ibuprofen was designed firstly to demonstrate and quantify the inversion of the R(−) to S(+) enantiomer in man, and, secondly, to examine the stereoselective characteristics of the disposition of both enantiomers in terms of their clearances and volumes of distribution.

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Methods

This study was performed in four healthy male volunteers. The age range was 31–45 years. Each subject was studied after single oral doses of 400 mg S(+) ibuprofen [S(+)I], 400 mg R(−) ibuprofen [R(−)I] and 800 mg racemic ibuprofen [Rac I]. The purity of the individual isomers had been established to be > 97% by h.p.l.c. assay (Lee *et al.*, 1984). The subjects were required to fast overnight and up to 4 h after each dose. All doses were in the form of dilute alkaline solutions and were followed by 250 ml of water. The order in which the doses were administered was randomised and a 1 week break was observed between any two doses.

Venous blood samples were collected via an indwelling canula into heparinised tubes at the following times: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 14 h. In all cases, blood samples were separated immediately and the extraction of the plasma component was carried out within 24 h of collection.

Urine samples were collected by spontaneous voiding after regular intervals up to 14 h after drug ingestion. Aliquots were stored at −20°C for later analysis.

Plasma samples were assayed for the individual enantiomers of ibuprofen by a previously reported method (Lee *et al.*, 1984) and required the esterification of the enantiomers with S(+)-2-octanol. The diastereomers were subsequently chromatographed by h.p.l.c. utilising two silica columns in series and 0.05% isopropanol in heptane as the mobile phase. Urine samples were assayed similarly after acid hydrolysis. Acid hydrolysis was carried out by incubating 1 ml urine with 1 ml 3N hydrochloric acid at 100°C for 60 min. The resultant mixture was then extracted and treated as for the plasma samples.

The terminal half-lives were calculated after non linear regression of all data points after 8 h. The area under the concentration time curves (AUC) for each profile obtained was calculated by the trapezoidal rule with extrapolation to infinity from the last data point. In general, the extrapolated area was less than 2% of the total AUC. Apparent oral clearances of each isomer were calculated by dividing the administered dose of that enantiomer by its respective AUC. In each case availability was assumed to be complete. The volume of distribution at steady state (V_{ss}) was calculated by moment analysis (Perrier & Mayersohn, 1982), with the assumption that the rates of oral absorption were sufficiently large so as to negate the end term in the expression:

$$V_{ss} = \frac{F.\text{Dose}.\text{AUMC}}{\text{AUC}^2} - \frac{F.\text{Dose}}{k_a.\text{AUC}}$$

where F = availability, AUMC = area under the moment curve, k_a = rate constant for absorption.

The fraction of an administered dose of R(−)I that was inverted to S(+)I was estimated following the principles discussed by Pang & Kwan (1982). The expression used was:

$$\text{Fraction inverted } (f_I) = \frac{\text{AUC}^{R(-)I}_{S(+)I}.\text{Dose S(+)I}}{\text{AUC}^{S(+)I}_{S(+)I}.\text{Dose R(−)I}}$$

where $\text{AUC}^{R(-)I}_{S(+)I}$ and $\text{AUC}^{S(+)I}_{S(+)I}$ are the AUCs of S(+)I after administration of R(−)I and S(+)I respectively. $\text{Dose}_{R(-)I}$ and $\text{Dose}_{S(+)I}$ are the oral doses of R(−)I and S(+)I respectively.

The amount of each enantiomer measured in the urine after acid hydrolysis was summed over the entire collection period and was subsequently expressed as the percentage of administered dose excreted as conjugates. When the fraction of the administered dose for each enantiomer was multiplied by its respective apparent oral clearance, a value was obtained that was taken to represent the clearance by glucuronidation.

Results

Typical plasma concentration profiles of the individual enantiomers following administration of R(−)I, S(+)I and Rac I, respectively are reproduced in Figure 1. In all instances, absorption was rapid with peak drug concentrations occurring at either the first (15 min) or second (30 min) sample. Following administration of Rac I, S(+)I concentrations were observed to decline more slowly than that for R(−)I. Substantial amounts of S(+)I were detectable following administration of R(−)I, whereas only negligible amounts of R(−)I were found after administration of S(+)I.

The pharmacokinetic parameters of the individual enantiomers following administration of each enantiomer and Rac I are shown in Table 1.

The mean AUC for R(−)I and S(+)I following administration of Rac I was 82.3 and 128.3 μg ml^{-1} h, respectively and are consistent with values obtained in other studies measuring total ibuprofen levels (Gillespie *et al.*, 1982; Juhl *et al.*, 1982; Lockwood *et al.*, 1983). In all four subjects, the AUC of R(−)I obtained after administration of Rac I was less than that obtained when the same amount of R(−)I was given individually (paired t-test; $P < 0.05$). Consequently, apparent oral clearance of R(−)I was greater in all subjects when R(−)I was administered as part of the racemate. Similarly the AUC of S(+)I (128.3 ± 28.5 μg ml^{-1} h) obtained after administration of Rac I was less than predicted by summing the AUC of S(+)I

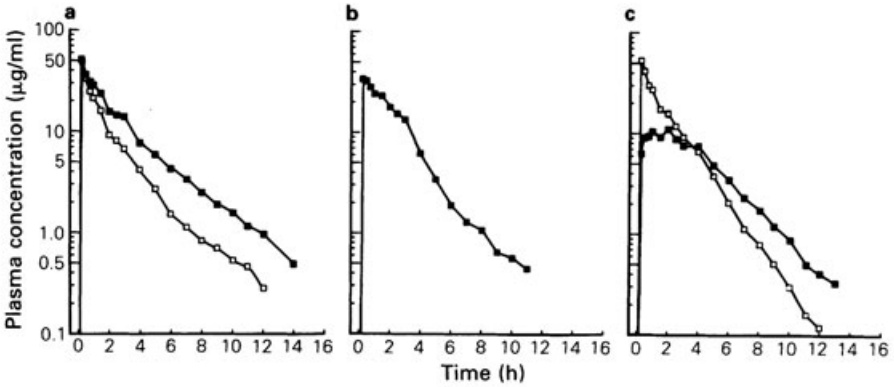

Figure 1 Plasma profiles of R(−)I (□) and S(+)I (■) after oral administration of (a) 800 mg Rac I (b) 400 mg S(+)I and (C) 400 mg R(−)I for subject 2.

Table 1 Pharmacokinetic parameters for R(−)I and S(+)I

	Subjects					
	1	*2*	*3*	*4*	*Mean*	*s.d.*
AUC (μg ml^{-1} h)						
a. R(−)I after R(−)I	99.3	86.9	131.2	86.6	101.0	20.9
b. S(+)I after R(−)I	55.9	54.8	84.0	42.5	59.3	17.5
c. R(−)I after Rac I	82.8	69.7	120.9	55.9	82.3	27.9
d. S(+)I after Rac I	141.7	109.2	161.8	100.7	128.3	28.5
e. S(+)I after S(+)I	97.4	86.8	118.6	69.4	93.1	20.6
Clearance (ml/min)						
f. R(−)I after R(−)I	67.1	76.7	50.8	77.0	67.9	12.3
g. S(+)I after S(+)I	68.4	76.8	56.2	96.0	74.4	16.7
h. R(−)I after Rac I	80.5	95.6	55.2	119.3	87.6	26.9
V_{ss}(I)						
i. R(−)I after R(−)I	9.6	9.1	10.2	10.2	9.9	0.62
j. S(+)I after S(+)I	9.4	10.7	9.4	12.6	10.5	1.49
k. R(−)I after Rac I	11.0	11.3	9.2	14.5	11.5	2.25
Terminal half-life (h)						
a. R(−)I after R(−)I	2.22	1.35	2.60	1.93	2.03	0.53
b. S(+)I after S(+)I	2.04	1.4	1.91	1.48	1.71	0.31
c. R(−)I after Rac I	2.13	1.6	1.69	1.27	1.67	0.35
d. S(+)I after Rac I	2.23	2.7	2.25	2.57	2.50	0.20
Fraction inverted (f_I)	0.57	0.63	0.71	0.61	0.63	0.06

(152.4 ± 38.1 μg ml^{-1} h) obtained after the separate administration of R(−)I and S(+)I ($P < 0.05$, paired *t*-test). It was not possible to derive valid estimates of the apparent oral clearance of S(+)I after administration of Rac I because of the contribution of the inversion reaction.

In three of the four subjects, V_{ss} for R(−)I was larger when R(−)I was administered as the racemate, than when it was administered separately. This change was not statistically significant. There was, also no significant difference between the V_{ss} for R(−)I and S(+)I.

The f_I calculated for the four subjects ranged from 0.57 to 0.71 (Table 1).

Studies, whether measuring total (Lockwood & Wagner, 1982) or enantiomeric (Lee, unpublished data) concentrations of ibuprofen, have indicated that only negligible amounts of ibuprofen are excreted unchanged in the urine. We have taken the amounts of ibuprofen enantiomers measured after acid hydrolysis as representing ester glucuronides.

A much greater proportion of an administered dose of S(+)I was conjugated as S(+)I-glucuronide (12.5%) as compared to the formation of R(−)I glucuronide (1.5%) after administration of R(−)I (Table 2). Simple stoichiometry

Table 2 Glucuronidation of ibuprofen enantiomers

Subject	*R(−) ibuprofen* CL_{gluc}*	*R(−) ibuprofen* % Dose+	*S(+) ibuprofen* CL_{gluc}*	*S(+) ibuprofen* % Dose+
1	1.2	1.8	7.9	11.6
2.	0.8	1.1	7.2	9.4
3.	0.7	1.3	9.6	17.1
4.	1.5	1.9	11.5	12.0
Mean	1.1	1.5	9.1	12.5
s.d.	0.37	0.39	1.90	3.26

+ % of dose as acid hydrolysable conjugates
* Clearance (ml/min) by conjugation (predominantly glucuronides)

suggested that this difference was not accounted for by the inversion of R(−)I to S(+)I. As shown in Table 2, clearance by glucuronidation for S(+)I was 9.1 ml/min as compared to 1.1 ml/min for R(−)I.

Discussion

Following administration of Rac I to four normal male volunteers, both enantiomers had AUCs that were lower than predicted by the separate administration of the individual enantiomers. The concurrent increases in V_{ss} and apparent oral clearance observed in the case of R(−)I suggests that the underlying mechanism is perturbation of protein binding.

While in the case of S(+)I this change in kinetics may be attributed to the higher concentration of S(+)I achieved following administration of Rac I, it is very likely that these changes may in fact be due to the concurrent presence of the opposite enantiomer. In other words, there is likely to be an enantiomer-enantiomer interaction with regards protein binding. Further studies are in progress to clarify this proposition.

Non linearity in the kinetics of ibuprofen has only recently been reported (Lockwood & Wagner, 1982; Grennan *et al.*, 1983). This has been attributed mainly to non-linearity in protein binding. However, as ibuprofen was utilised as a racemate in these studies, any comment regarding non-linearity should be guarded especially since the kinetic estimations were based on total ibuprofen levels.

This study has demonstrated that an average of 63% of an administered dose of R(−)I is inverted to S(+)I. There are, of course, limitations in any attempt to generalise from such an estimate, the most obvious of which is the very small number of subjects studied. The other limitation lies in the assumption that the clearance of S(+)I is unchanged between administration of R(−)I and S(+)I. As discussed above, R(−)I may compete for binding on plasma proteins with S(+)I, and consequently, the clearance of S(+)I after dosage with R(−)I may be different to that after administration of S(+)I.

An assumption inherent in all the kinetic analyses has been that the absorption of ibuprofen is complete. This is a valid assumption, considering firstly that 80% of an oral dose (tablets or solutions) has been recovered in the urine as known metabolites (Lockwood & Wagner, 1982) and secondly that 95% of a dose of radiolabelled ibuprofen has been recovered in the urine after administration of a suspension of ibuprofen (Glass, data on file, The Boots Company, Nottingham, U.K.). Furthermore, the rapidity of absorption seen in the present study is consistent with the assumption that ibuprofen is completely absorbed.

Although the inversion reaction is probably universal for all α-methylarylacetic acid analogues, it is likely that f_I varies from drug to drug as well as from individual to individual.

In a study with benoxaprofen, f_I was estimated to be approximately 50% (Bopp *et al.*, 1979). However, that estimate was based to a large extent on extrapolated urinary data. No other attempts have so far been made to quantify this reaction for similar compounds. Simmonds *et al.* (1981), however, have reported that the inversion for benoxaprofen was biphasic with a terminal half-life of 108 h.

A number of studies have considered the mechanism of the inversion reaction for ibuprofen (Hutt & Caldwell, 1983; Nakamura *et al.*, 1980; Wechter *et al.*, 1974). The prerequisite for inversion appears to be the highly stereoselective esterification of R(−)I to its coenzyme A thioester (Figure 2). Once the ester of R(−)I is formed, racemisation occurs by means of an enzyme which in our view is likely to be either the same or very similar to methylmalonyl coenzyme A racemase. Since S(+)I is not a substrate for the coenzyme A ligase, inversion is seen to be stereoselective for R(−)I.

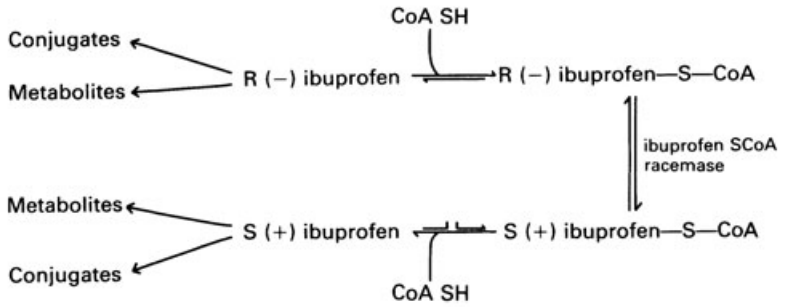

Figure 2 Proposed mechanism of the inversion reaction of R(−)I to S(+)I.

No information is available so far on what factors may possibly perturb this reaction. However, it appears reasonable that inversion could be encouraged if S(+)I was removed preferentially from the system. It was therefore interesting when an apparent log-linear relationship ($r > 0.99$) was observed between f_I and the ratio of clearances by glucuronidation for S(+)I and R(−)I (Figure 3). This relationship if far from conclusive because of the limited number of data points and, clearly, many more subjects need to be studied. However, the implication remains that differences in the extent of inversion between compounds in this class may be due, at least in part, to the stereoselectivity of processes involved in removal of the isomers.

Stereoselectivity of the glucuronidation process in this study appears to account for the large difference in fractional recovery of R(−) and S(+) ester glucuronides from the urine. However, in view of recent studies (Meffin *et al.*, 1983) on the enzymatic hydrolysis of clofibric acid ester glucuronides, it is possible that this apparent stereoselectivity in glucuronidation may in fact be stereoselectivity of enzymatic hydrolysis. Nonetheless, our data shows clearly that whether stereoselectivity resides in glucuronyl transferase or esterase enzymes, the net balance of the two reactions is more strongly in favour of glucuronidation for the S(+)I enantiomers.

Finally, our data have demonstrated that the kinetics of ibuprofen are complex and cannot be defined satisfactorily in terms of total ibuprofen

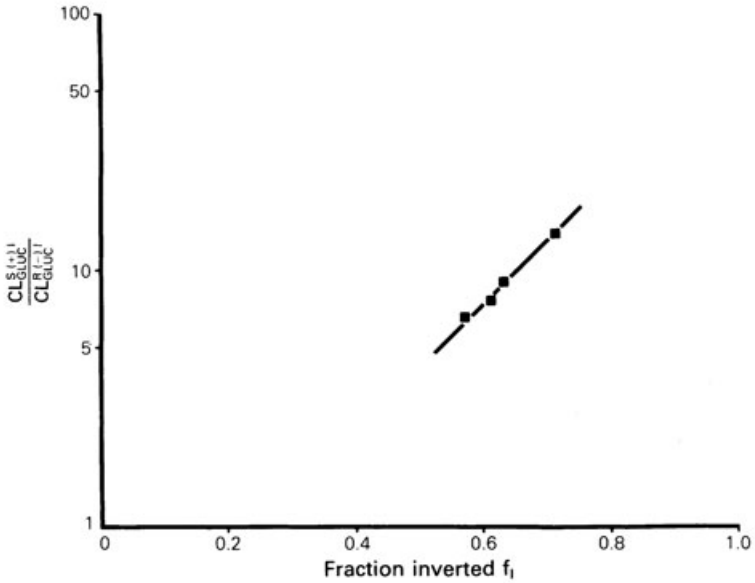

Figure 3 Apparent log-linear relationship ($r > 0.99$) between the fraction of inversion, (f_I) and the ratio of clearance by glucuronidation for S(+)I ($CL^{S(+)I}_{gluc}$)to that for R(−)I ($CL^{R(-)I}_{gluc}$).

concentrations. The inconvenience of assaying the enantiomers individually is more than justified by the greater insight into stereoselective mechanisms, which are important for a proper understanding of the disposition of ibuprofen and other α-methylarylacetic acids.

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