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# The stereoselective uptake of ibuprofen enantiomers into adipose tissue

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Ibuprofen belongs to the chemical class of 2-arylpropionic acids which is a large and important group of non-steroidal anti-inflammatory drugs. These drugs are asymmetric and with the exception of naproxen, are administered as an equal mixture of the two enantiomers, i.e. the racemates. In *in vitro* tests, anti-prostaglandin synthesis activity of these drugs has been found to reside in the *S*-enantiomers. This contrasts with the observation that frequently, although not universally, the anti-inflammatory activities of the enantiomers were similar *in vivo* [1]. Consequently, an *in vivo* pathway of stereoselective inversion was invoked [2] and subsequently this hypothesis was confirmed for several of these drugs, including ibuprofen [3–6], benoxaprofen [7], cicloprofen [8, 9] and clidanac [10]. The mechanism of this inversion appears to be as follows: the *R*-enantiomer is stereospecifically converted to its coenzyme A thioester, while the *S*-enantiomer is not a substrate for this ligase. This thioester is racemized by a non-stereoselective racemase, and is then hydrolysed to release the *S*-enantiomer [11].

Coenzyme A thioesters of xenobiotic carboxylic acids, including 2-arylpropionic acids, can replace the natural fatty acids in triacylglycerols to form "hybrid" triglycerides [12, 13]. It is reasonable to assume that the xenobiotic must first be activated by formation of its coenzyme A thioester. We hypothesised that if the proposed mechanism of inversion was correct then uptake of 2-arylpropionic acids into adipose tissue should occur stereoselectively [14]. Additionally, as inversion is stereospecific for *R*-ibuprofen, administered *S*-ibuprofen should not form coenzyme A thioesters and consequently should be unavailable for incorporation into triglycerides [15]. The present study was undertaken to investigate this hypothesis.

## *Materials and methods*

*R*, *S* and *RS*-ibuprofen were supplied by Boots U.K. *S*-2-octanol (>99% optical purity) was purchased from Sigma.

Groups of 6 male Wistar rats were treated with either *RS*-ibuprofen, *R*-ibuprofen or *S*-ibuprofen (20 mg/kg, i.p.) twice daily for 7 days. Four rats from each group were sacrificed 20 hr after the last dose and perinephric fat collected. The remaining two rats were sacrificed at 116 hr and fat similarly collected. Aliquots (50 mg) of fat were fractionated by thin layer chromatography using silica gel plates (Kieselgel $60F_{254}$, Merck) and petroleum ether/diethyl ether/acetic acid (20:80:1) solvent system. The band corresponding to the triglycerides was scraped off the plate and the silica eluted with chloroform/methanol (2:1, 10 ml). This extract was taken to dryness under a stream of nitrogen and with gentle heating (40°). The triglycerides were hydrolysed with alcoholic KOH (1.0 ml, 50% MeOH/50% 10 N KOH, heated at 75° for 90 min) and, following acidification, extraction into hexane (2 × 10 ml) and evaporation to dryness, the residue was esterified with *S*-2-octanol as described previously [5]. Samples were assayed by methane chemical ionization mass spectrometry using single ion monitoring to detect each derivatised enantiomer of ibuprofen following gas chromatographic separation [17]. Results were calculated against similarly treated ibuprofen standards (2–100 μg/g). Results for standards demonstrated that under these conditions there was no racemization during sample preparation.

The optical purity of the administered enantiomers was checked by the HPLC procedure described previously [5].

## *Results*

The *R*-enantiomer was found to contain a maximum of 4.3% *S*-enantiomer and similarly, the *S*-enantiomer contained 4.3% *R*-enantiomer.

Following chronic treatment of rats with *R*-ibuprofen, both *R* and *S*-ibuprofen were incorporated into adipose tissue with uptake being stereoselective for the *R*-enantiomer. In contrast, there was very little drug detected in fat after similar treatment with the active *S*-enantiomer (Fig. 1). These concentrations following administration of the *S*-

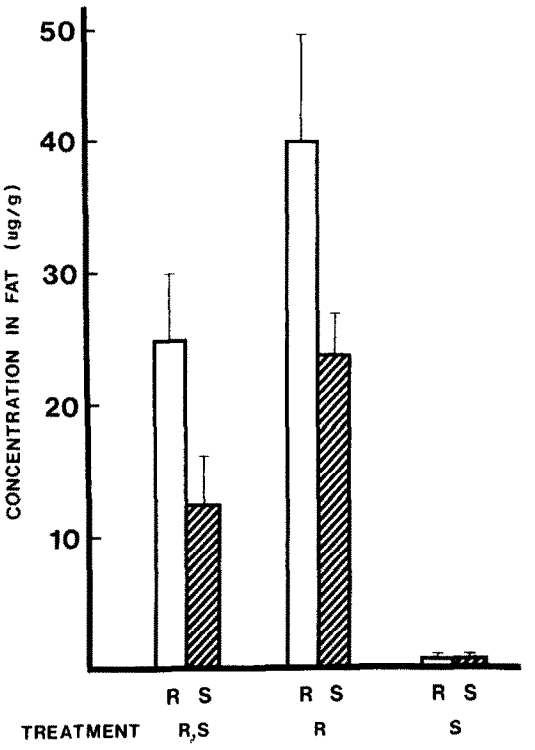


Fig. 1. Concentrations of the enantiomers of ibuprofen in adipose tissue following chronic treatment of rats with either racemic (*RS*) drug or with each of the individual *R* or *S*-enantiomers.

enantiomer were less than the lowest standard (2 μg/g) and approached the limits of assay sensitivity. Treatment of rats with *RS*-ibuprofen gave concentrations of the enantiomers in fat approximately half those observed for the *R*-enantiomer alone as expected (see Discussion).

Ibuprofen enantiomers were eliminated very slowly from adipose tissue (Fig. 2). This data should not be overinterpreted with respect to the elimination half-life because of the small numbers of rats involved. In fact, the mean concentrations of the enantiomers in fat for the *R*-ibuprofen treated rats were greater at 116 hr (*R*-ibuprofen, 65 μg/g; *S*-ibuprofen, 21 μg/g) than the mean data at 20 hr (*R*-ibuprofen, 40 μg/g; *S*-ibuprofen, 24 μg/g). This was because one of the two rats sacrificed at 116 hr had the highest concentrations of enantiomers in fat of all rats studied (*R*-ibuprofen, 94 μg/g; *S*-ibuprofen, 28 μg/g).

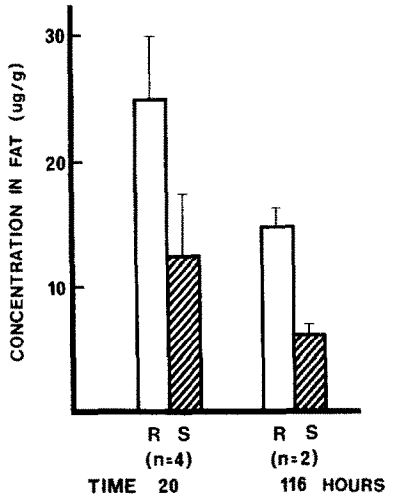


Fig. 2. Time course of elimination of the enantiomers of ibuprofen from adipose tissue following chronic dosing of rats with *RS*-ibuprofen.

It was further observed that the *S*/*R* ratio in the rats treated with *RS* and *R*-ibuprofen was 0.56 ± 0.06 (mean ± SEM) at 20 hr after cessation of dosing. Rats killed 116 hr after treatment had a smaller ratio of 0.37 ± 0.06. However, this difference did not reach statistical significance ($P = 0.09$).

*Discussion*

The data demonstrated that *S*-ibuprofen was not significantly incorporated into adipose tissue following chronic treatment with this enantiomer, while there was stereoselective incorporation of the enantiomers into adipose tissue in rats treated chronically with *RS* and *R*-ibuprofen, respectively. The small uptake of enantiomers into adipose tissue after treatment with *S*-ibuprofen deserves further comment. Frequently, in comparing the disposition and relative activities of enantiomers, the possible contamination of one enantiomer by its antipode is overlooked. The *S*-enantiomer used in our studies contained 4–5% *R*-ibuprofen, i.e. a 20 mg dose of *S*-ibuprofen is effectively, 19 mg *S*-ibuprofen and 1 mg *R*-ibuprofen. This level of impurity is sufficient to account for all the drug detected in adipose tissue following treatment with the *S*-enantiomer. It is our conclusion that administered *S*-ibuprofen was stereospecifically excluded from fat. These findings are consistent with the stereospecific role of coenzyme A thioester formation in inversion of *R*-ibuprofen and in the incorporation of ibuprofen into triglycerides.

The slow elimination of the enantiomers from fat agrees with published data. Adams *et al.* [16] reported that following chronic treatment of rats with racemic radio-labelled ibuprofen, drug accumulated in adipose tissue and was eliminated with a half-life of approximately 148 hr. The present data also suggest that this elimination was stereoselective, favouring removal of the *S*-enantiomer. This may be attributable to the stereoselective action of hydrolases or esterases on the hybrid triglycerides.

The effects of accumulation of xenobiotics in adipose tissue as slowly eliminated stores of drug is unknown. It has been suggested that the hybrid triglycerides may have toxic effects as they could disrupt normal lipid metabolism and membrane function [13]. For example, perhaps some of the central nervous system toxicities of this class of non-steroidal anti-inflammatory drug are mediated by abnormal lipid products which accumulate in the brain. It is clear from the present data that at least for ibuprofen, accumulation via incorporation into hybrid triglycerides can be avoided by use of the active *S*-enantiomer rather than the racemic drug. The data are further evidence of the importance of understanding the enantiomeric disposition of asymmetric drugs, particularly those which are administered as their racemates [14].

In conclusion, the data support a mechanism of inversion of ibuprofen (and other 2-arylpropionic acids) mediated via stereospecific formation of coenzyme A thioesters of the *R*-enantiomer. The work confirms the proposed hypothesis that administered active *S*-enantiomer of ibuprofen is stereospecifically excluded from adipose tissue, while *R*-ibuprofen and *S*-ibuprofen formed by inversion *in vivo*, form hybrid triglycerides resulting in depots of slowly eliminated drug. The data have potential therapeutic and toxiological implications which suggest that the therapeutic index for this class of drug might be increased by use of the active *S*-enantiomers alone rather than presently administered racemic drug.

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# Characterization of melphalan-glutathione adducts whose formation is catalyzed by glutathione transferases

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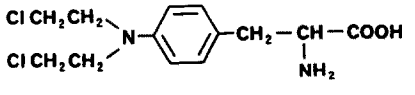
A number of authors have described an association between elevated sulfhydryl levels and resistance to alkylating agents. Current explanations for the development of resistance to antineoplastic nitrogen mustards, such as melphalan, include evidence of impaired drug intake [1] and increased levels of intracellular glutathione [2–4]. Recently, Vistica and coworkers [5] reported that murine L1210 cells resistant to melphalan contain elevated levels of both reduced glutathione and glutathione-*S*-transferase. However, none of these studies has suggested the direct formation of a melphalan-glutathione adduct(s) as being a potentially important consideration in this phenomenon. The objective of this study was to characterize the conjugation products between melphalan (**1**) and glutathione whose formation is catalyzed by both cytosolic and microsomal glutathione-*S*-transferases. The reaction products have been identified as the diglutathione conjugate formed by displacing both chloride groups in the mustard moiety, and a monoglutathione conjugate formed by displacing one chloride group.




**1**

*Materials and methods*

Melphalan (L-phenylalanine mustard, L-PAM) was supplied by the Division of Cancer Treatment, NCI, (Bethesda, MD). Reduced glutathione (GSH) was obtained from the Sigma Chemical Co. (St. Louis, MO). [$^{14}$C]Melphalan (sp. act. 10 mCi/mmole) was obtained from the Drug Development Branch of the National Cancer Institute. The radiolabeled compound was purified by high pressure liquid chromatography (HPLC) using a $C_{18}$ reversed-phase column (Bondapak, Waters Associates, Milford, MA) and a methanol-1% aqueous acetic acid gradient as the mobile phase. The purified drug was stored as a solution in 100 mM HCl at −70°.

Immobilized microsomal glutathione-*S*-transferases were prepared from rabbit and human livers by a modification of the method of Lehman *et al.* [6] and Pallante *et al.* [7]. Male New Zealand white rabbits (5–8 lb) were obtained from Bunnyville and given a 0.05% solution of phenobarbital *ad lib.* for 2 weeks. Food was withheld 24 hr prior to killing the animals with $CO_2$; hepatic microsomal protein was immobilized immediately. Histologically normal human liver was obtained from surgical resection samples, frozen immediately on dry ice, and stored at −80° before immobilization. Specific activity of the bound glutathione-*S*-transferase was determined spectrophotometrically using 1-chloro-2,4-dinithrobenzene as the assay standard [8]. Activity in nmoles/min/mg protein was: rabbit, 171; human, 108.

*Incubations with immobilized microsomal glutathione transferases.* The incubation mixture contained: melphalan (1.0 mM, 10.3 mg), GSH (3.0 mM, 27.6 mg), and packed immobilized Sepharose beads from rabbit or human liver (28 ml) in aqueous phosphate buffer (0.1 M, pH 7.4). The reaction was run at 37° for 1 hr, followed by filtration. The aqueous filtrate was evaporated to a volume of 1–2 ml and analyzed by thin-layer chromatography (TLC). A control reaction was carried out in the absence of immobilized enzyme. Products of the incubation were purified by HPLC using the method described below. Purified conjugates