Liquid Chromatographic Determination and Plasma Concentration Profile of Optical Isomers of Ibuprofen in Humans

EDMUND J. D. LEE *§x, KENNETH M. WILLIAMS *, GARRY G. GRAHAM ‡, RICHARD O. DAY *, and G. DAVID CHAMPION *

Received August 17, 1983, from the *Department of Clinical Pharmacology, St. Vincent's Hospital, Darlinghurst, 2010, Australia and ‡Department of Pharmacology and Physiology, University of New South Wales, Kensington, 2033, Australia. Accepted for publication December 12, 1983.
§Present address: Department of Pharmacology, National University of Singapore, Kent Ridge, Singapore 0511.

Abstract □ A sensitive and specific high-performance liquid chromatographic assay for the optical isomers of ibuprofen in plasma is reported. The isomers were converted to their diastereoisomeric *S*(+)-2-octyl esters and were resolved with two silica columns in series and a mobile phase containing 0.05% isopropyl alcohol in heptane. The lower limit of sensitivity was 0.5 μg/mL. The plasma concentrations of the optical isomers in a normal volunteer following administration of racemic, *S*(+)-, and *R*(−)-ibuprofen are reported. Following administration of *R*(−)-ibuprofen, there was stereoselective inversion of *R*(−)- to *S*(+)-ibuprofen.

Keyphrases □ Optical isomers—liquid chromatography, plasma concentration profiles, ibuprofen, humans □ Ibuprofen—liquid chromatography, plasma concentration profile, optical isomers, humans

The chromatographic resolution of optical isomers is difficult because they have the same physicochemical properties. Methods available for resolution include the use of an optically active stationary phase (1), an optically active mobile phase (2), or, more conventionally, conversion of the optical isomers to diastereoisomers by reaction with an optically active reagent. Separation of isomers of arylpropionic acid derivatives usually can be achieved by the formation of the diastereoisomeric amides with optically active reagents like *R*(+)-α-phenylethylamine (3), *S*(−)-α-methylbenzylamine (4, 5), (*R*)-4-(dimethylamino)-α-methyl-1-naphthalenemethyl-amine (6), and L-leucine (7). Johnson *et al.* (8) have reported a liquid chromatographic assay for the isomers of naproxen based on the formation of diastereoisomeric *S*(+)-2-octyl esters. In this paper is reported the successful modification of the latter method for the quantification of ibuprofen optical isomers in plasma.

Ibuprofen (I) is a nonsteroidal anti-inflammatory agent exhibiting optical isomerism because of a chiral carbon atom on the propionic acid side chain. It is administered clinically as the racemate. In early *in vitro* studies, it has been demonstrated that the *S*(+)-isomer (III) was responsible for the anti-inflammatory activity. However, the *R*(−)-isomer (II) was as active as III *in vivo* (9) because there was stereoselective inversion of II to III *in vivo* (10–13).

There have been few studies in which the pharmacokinetics of II and III in humans have been described because of the difficulty in resolving II and III. Although the inversion of II to III has been inferred from the examination of the urinary metabolites of II and III in humans (10, 11, 13), no data have been presented detailing the time course of the plasma concentrations of each isomer following the administration of II and III. A sensitive and specific high-performance liquid chromatographic (HPLC) assay is described for the determination of II and III in plasma. It was used to follow the concentrations of II and III after oral administration of I (800 mg), II (400 mg), and III (400 mg) and to document the inversion of II to III in humans.

EXPERIMENTAL SECTION

Apparatus—An HPLC pump[1] was attached to an autosampler[2] and a variable-wavelength UV detector[3] set at 220 nm. Two 5-μm silica columns[4] (25 × 0.46 cm) were used in series. The detector signal was recorded and reported as peak areas on a plotter recorder[5]. The whole operation was coordinated by means of a system controller[6].

Reagents—*n*-Hexane and *n*-heptane were HPLC grade[7]; isopropyl alcohol was analytical grade[7]; *S*(+)-2-octanol (>99% pure) was obtained commercially[8]. Hydrogen chloride was bubbled through the octanol. Dichloromethane[7] and methanol[7] were dried over sodium and anhydrous sodium sulfate, respectively.

Standard Solutions—Stock solutions of I[9] were prepared by using dilute sodium hydroxide (solution A, 50 μg/mL; solution B, 500 μg/mL). A stock solution of the internal standard, 4-*n*-pentylphenylacetic acid (IV)[9], was prepared (80 μg/mL). Plasma standard curves for each isomer were constructed (at each run) over the concentration ranges of 0.5–5 and 5–60 μg/mL. In each case, the peak area ratios of each isomer to internal standard were plotted against the concentration of the isomers.

Sample Preparation—Venous blood (10 mL) was collected *via* an indwelling catheter in heparinized tubes[10]. Duplicate plasma samples (1 mL) were then transferred to screw-capped glass tubes (12 mL). Internal standard (100 μL) was added, and the mixture was acidified with 3 M hydrochloric acid (200 μL). Isopropyl alcohol (0.1% v/v) in hexane (10 mL) was added, and the tube was capped and shaken for 5 min. The organic phase was then transferred to a glass tube (20 mL). The extraction was repeated, and the second volume of the organic phase was added to the first volume before being blown to dryness under air at 40°C. The walls of the tube were washed with chloroform (2 mL) which was then transferred to a reaction vial (2 mL). Acidified *S*(+)-2-octanol (25 μL) was added, and the vial was capped and incubated for 1 h at 150°C. On cooling, 0.1% isopropyl alcohol in hexane (2 mL) and saturated sodium bicarbonate (150 μL) were added. The vials were recapped and the mixture vortexed for 30 s and then centrifuged for 5 min. The organic phase was transferred to conical tubes and the solvent evaporated under a stream of air at 40°C. The residue was then reconstituted in mobile phase (100 μL) and transferred to 200-μL sampling vials. An aliquot (4–40 μL) was then injected *via* the autosampler.

To obtain standard curves, either solution A (20–200 μL) or B (20–240 μL) was transferred into screw-capped glass tubes (12 mL). The volume was made up to 1 mL by the addition of blank plasma, and the resultant mixture was treated in a manner similar to that for the unknown samples.

Chromatography—Chromatography was performed at ambient temperature, and resolution of the octyl esters of II, III, and IV was achieved by using two 5-μm silica columns connected in series. The mobile phase was 0.05% v/v isopropyl alcohol in heptane and was pumped at a flow rate of 1 mL/min for 30 min followed by a 20-min washout period at 2 mL/min. The pressure at 1 mL/min was ~800 psi.

[1] Model 6000A; Waters Associates.
[2] WISP 710 B; Waters Associates.
[3] UV-50; Varian Instruments.
[4] Ultrasphere Si 5 μm; Altex.
[5] Data module; Waters Associates.
[6] System controller; Waters Associates.
[7] Ajax Chemicals.
[8] Fluka AG Chemishe Fabrik.
[9] The Boots Co., Nottingham, United Kingdom.
[10] Vacutainer 6484; Becton, Dickinson and Co.

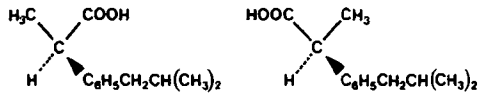
0022-3549/84/1100-1542$01.00/0

After every 12 injections there was a 30-min washout period at 2 mL/min. At regular intervals (about 150 injections), the columns were generated by pumping 20 column volumes of dry dichloromethane, 20 column volumes of dry methanol, and then another 20 column volumes of dry dichloromethane through the columns. The columns were reequilibrated with at least 50 column volumes of 0.05% isopropyl alcohol in heptane before use. No attempt was made to dry the isopropyl alcohol–heptane mixture.

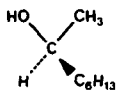
The individual peaks were identified by HPLC of the octyl esters of pure II, III, and IV. The eluting fractions representing the octyl esters of II and III were subjected to GC–MS.[11]

To exclude possible interference by other nonsteroidal anti-inflammatory agents, standard solutions of aspirin, carprofen, diflunisal, flurbiprofen, indomethacin, naproxen, and salicylic acid were esterified and chromatographed as described above for I.

Volunteer Study—A normal subject was given I (800 mg)[9], II (400 mg)[9], and III (400 mg)[9] orally on three separate occasions, 1 week apart. Compounds I, II, and III were administered orally in solution. These test solutions were prepared by first dissolving appropriate amounts of pure I, II, or III in dilute sodium hydroxide (0.1 M, 10–20 mL). The volume was then made up to 250 mL with distilled water. Prior to each study, the subject fasted overnight and until 4 h after administration of the drug. Frequent blood samples were collected up to 14 h after administration of the dose.



R(–) IBUPROFEN (II) S(+) IBUPROFEN (III)



S(+)-2-OCTANOL

RESULTS AND DISCUSSION

Chromatography—The *S*(+)-2-octyl esters of II and III can be separated chromatographically with retention times of ~20 and ~21 min, respectively (Fig. 1). The retention time for the ester of IV was ~26 min. Presence of other anti-inflammatory agents like aspirin, carprofen, diflunisal, flurbiprofen, indomethacin, naproxen, and salicylic acid did not interfere with the assay.

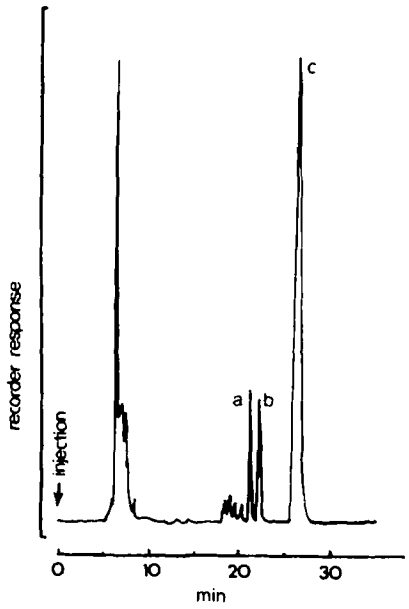


Figure 1—*Typical chromatogram of the S(+)-2-octyl esters of II (0.5 μg/mL of plasma) (a), III (0.5 μg/mL of plasma) (b), and IV (c).*

[11] Model 3200; Finnigan CI-GCMS.

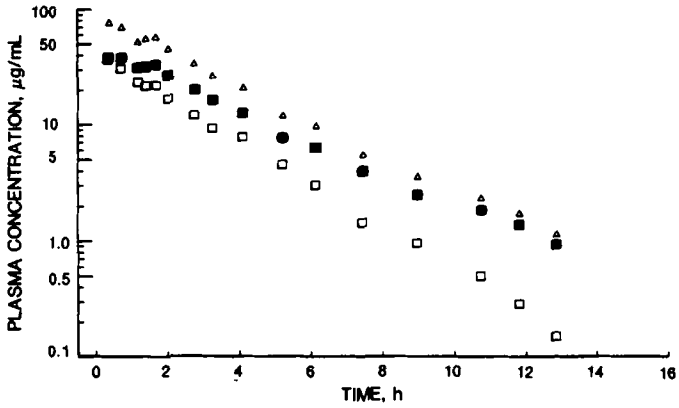


Figure 2—*Plasma levels of I (△), II (□), and III (■) following oral administration of I (800 mg).*

When a sample of II which showed one peak on HPLC was esterified and chromatographed, some III was detected. The amount of III thus detected was small and represented only ~2% of the total amount of II. A similar amount of II was found to be present when a sample of III which showed one peak on HPLC was esterified and chromatographed. These results can be attributed to impurity of the sample, impurity of the octanol, or the possibility of a fractional amount of racemization occurring during sample preparation.

Chromatographic conditions were selected to maintain the retention times described above at which resolution and peak shapes were optimal.

The methane chemical ionization mass spectrum for the esters of II and III were similar and gave the following mass ions: $(M - H)^+$ m/z 317 (28%); $(M - CH_3)^+$ m/z 303 (10%); $(M - C_6H_{11})$ m/z 235 (16%); $(M - C_8H_{15})^+$ m/z (207 (100%); $(M - C_8H_{17}O)$ m/z 189 (8%); $(M - C_8H_{17}OCO)$ m/z 161 (50%).

During development of the assay, four to five peaks, possibly due to contaminants extracted from plasma, were found to elute just prior to that of the octyl ester of II. Significant interference from these peaks occurred at low concentrations of II (<0.5 μg/mL). However, interference could be minimized by maintaining the retention time of II beyond 20 min.

Sample Preparation—Various extraction methods were assessed. In general, more polar solvents extracted greater amounts of plasma contaminants. Extraction with *n*-hexane was found to produce the least interference by contaminants with reasonable recovery of II and III. By using 0.1% isopropyl alcohol in hexane, recovery of II and III was found to be ~64 and 84% after single and double extractions, respectively. Recovering of IV was 74% after two extractions. (Recovery was defined by the ratio of peak areas obtained after treatment of spiked plasma extracts to that obtained after direct esterification of equivalent amounts of II, III, or IV.) Esterification of II and III was complete after 0.5 h at 150°C.

Assay Reproducibility and Sensitivity—It was necessary to construct a standard curve for each of the plasma concentration ranges, 0–10 and 10–60 μg/mL. Typical intercepts and slopes were, respectively, 0.2 and 9.26 for the former and −0.66 and 8.76 for the latter standard curves. The within-run coefficient of variation (*CV*) at plasma concentrations of 2.5 and 50 μg of the octyl ester of II/mL were 4.0 and 5.0%, respectively (n = 5). The corre-

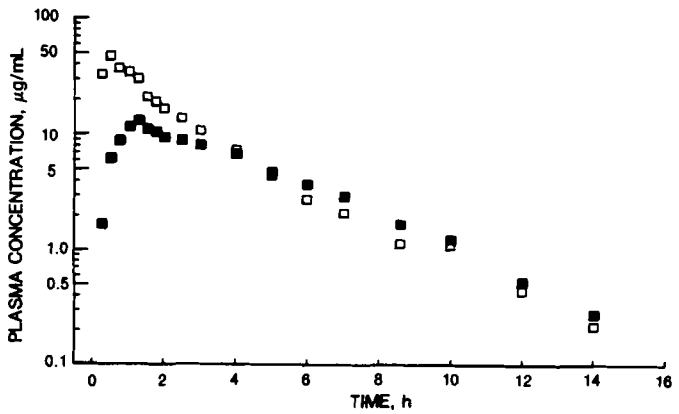


Figure 3—*Plasma levels of II (□) and III (■) following oral administration of II (400 mg).*

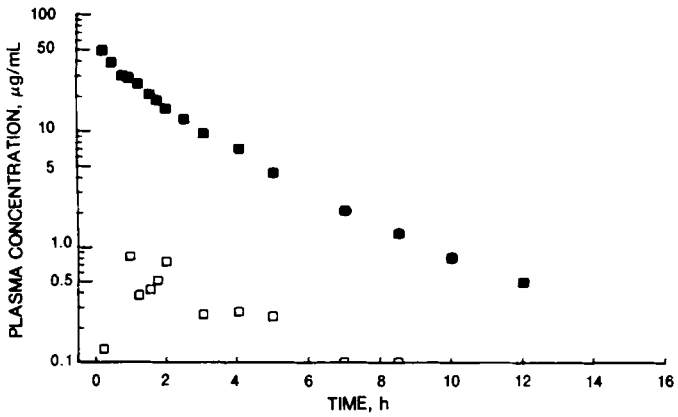


Figure 4—*Plasma levels of II (□) and III (■) following oral administration of III (400 mg).*

sponding *CV* values for the octyl ester of IV were 3.4 and 5.1%. Concentrations of II and III down to 0.5 μg/mL could be measured confidently. Values below this were rejected because of significant interference from the contaminating peaks mentioned above.

Volunteer Study—The applicability of the assay was demonstrated by a preliminary study of the plasma concentration profile of II and III in a normal subject. Absorption after administration of I, II, or III was rapid. Peak levels were attained within 0.5 h (Figs. 2–4). In most of the profiles, a definite biphasic decline could be discerned. Following administration of I, III was apparently eliminated more slowly than II (Fig. 2). This was presumably due to the contribution of the inversion of II to III. Following administration of II, plasma concentrations of III gradually increased and peaked at 1.3 h (Fig. 3). The concentration of III then declined more slowly than that of II. The concentration of III equaled that of II at ~4–5 h and subsequently remained higher than that of II. The peak concentration of III achieved after administrations of II was ~28% of that attained by II.

Only a small amount of II was detected after administration of III (Fig. 4). The low plasma concentrations of II that were observed may be attributed to incomplete optical purity of the *S*(+)-2-octanol and also possibly to some racemization of III during derivatization. However, as the rate of increase of II was slower than that observed for III, it is likely that some inversion of III to II occurs *in vivo*, although this is clearly insignificant compared with the inversion of II to III observed in these studies.

Ibuprofen is cleared almost entirely by metabolism (13). Disposition of the isomers is complicated by the stereoselective inversion of II to III. This metabolic pathway may be common to all the arylpropionic acids, although this area has received little attention (6, 7, 14). The inversion apparently proceeds *via* stereoselective formation of the coenzyme A (CoA) ester of the *R*(−)-arylpropionic acid, with subsequent racemization and release of the *R*(−)- and *S*(+)-enantiomers (15). This mechanism suggests that the "racemase" may be methylmalonyl CoA epimerase. Furthermore, as CoA esters of ibuprofen, fenoprofen, and ketoprofen have been shown to be incorporated into "hybrid triglycerides" (16), we are currently investigating our hypothesis that arylpropionic acids may be stereoselectively sequestered into adipose tissue.

Direct evidence of the stereoselective inversion of II has not been previously demonstrated in humans, and inference of its existence has so far been based on the predominance of dextrorotatory metabolites in urine (10, 11, 13).

This HPLC assay was found to be reproducible and convenient for routine use and has been used to demonstrate unequivocally the *in vivo* inversion of II to III in humans. The method suffers from the drawback of having a long assay time. However, this disadvantage can be overcome by automation of sampling and peak integration. The method was also found to be suitable for analysis of urine and synovial fluid samples and was utilized in formal pharmacokinetic studies. The details of these studies and of the stereospecific uptake of *R*(−)-ibuprofen into adipose tissue will be the subject of later reports.

REFERENCES

(1) W. H. Pirkle, D. W. Honse, and J. M. Finn, *J. Chromatogr.*, **192**, 143 (1980).

(2) J. N. LePage, W. Lindner, G. Davies, D. E. Seitz, and B. L. Karger, *Anal. Chem.*, **51**, 433 (1979).

(3) S. Rendic, V. Sunjic, F. Kajfez, N. Blazevic, and T. Alebic-Kolbah, *Chimia*, **29**, 170 (1975).

(4) G. J. Vangiessen and D. G. Kaiser, *J. Pharm. Sci.*, **64**, 798 (1975).

(5) J. K. Stoltenborg, C. V. Puglisi, F. Rubin, and F. M. Vane, *J. Pharm. Sci.* **70**, 1207 (1981).

(6) J. Goto, N. Goto, and P. Nambara, *J. Chromatogr.*, **239**, 559 (1982).

(7) S. J. Lan, K. J. Kripalani, A. V. Dean, P. Egli, L. T. Difazio, and E. C. Schreiber, *Drug Metab. Dispos.*, **4**, 330 (1976).

(8) D. M. Johnson, A. Reuter, J. M. Collins, and G. F. Thompson, *J. Pharm. Sci.*, **68**, 112 (1979).

(9) S. S. Adams, P. Bresloff, and C. G. Mason, *J. Pharm. Pharmacol.*, **28**, 256 (1976).

(10) W. J. Wechter, D. G. Loughhead, R. J. Reischer, G. J. Vangiesen, and D. G. Kaiser, *Biochem. Biophys. Res. Commun.*, **61**, 833 (1974).

(11) D. G. Kaiser, G. J. Vangiessen, R. J. Reischer, and W. J. Wechter, *J. Pharm. Sci.*, **65**, 269 (1976).

(12) C. A. M. Van Genneken, Ph.D. Thesis, University of Mijmegen, Mijmegen, The Netherlands (1975).

(13) R. F. N. Mills, S. S. Adams, E. E. Cliffe, W. Dickinson, and J. S. Nicholson, *Xenobiotica*, **3**, 589 (1973).

(14) R. J. Bopp, J. F. Nash, A. S. Ridolfo, and E. R. Shepard, *Drug Metab. Dispos.*, **7**, 356 (1979).

(15) Y. Nakamura, T. Yaamaguchi, S. Hashimoto, S. Takahashi, K. Iwatani, and Y. Nakagama, Abstracts of the 12th Symposium on Drug Metabolism and Action, Kanazawa, Japan, 1980.

(16) R. Fears, K. H. Baggaley, R. Alexander, B. Morgan, and R. M. Hindley, *J. Lipids Res.*, **19**, 3 (1978).

ACKNOWLEDGMENTS

The authors are grateful to Dr. A. Duffield for performing the mass spectrometry. This study was supported by an N.H. & M.R.C. grant to K. M. Williams and R. O. Day. In addition, the authors wish to acknowledge the generous assistance from the Boots Co., Australia, and Nottingham, United Kingdom.