The stereoselective disposition of the enantiomers of ibuprofen in blood, blister and synovial fluid

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- 1 A sensitive, stereospecific assay using gas chromatography-mass spectrometry (GC/MS) was established to measure the concentrations of the enantiomers of ibuprofen in small volumes (50 µl) of blister fluid.
- 2 The concentrations of the enantiomers in blister fluid, assessed in eight patients, were similar to those in synovial fluid, both fluids behaving as peripheral compartments with respect to plasma.
- 3 The mean rate constants of transfer of R-ibuprofen into ($0.14 \pm 0.06 \text{ h}^{-1}$) and out of ($0.20 \pm 0.04 \text{ h}^{-1}$) blister fluid were not significantly different from those for synovial fluid ($0.19 \pm 0.12 \text{ h}^{-1}$, $0.34 \pm 0.11 \text{ h}^{-1}$, respectively). Similarly, the mean rate constants of transfer of S-ibuprofen into ($0.22 \pm 0.07 \text{ h}^{-1}$) and out of ($0.27 \pm 0.08 \text{ h}^{-1}$) blister fluid were not significantly different from those for synovial fluid (0.29 ± 0.10 , $0.36 \pm 0.11 \text{ h}^{-1}$). However, the correlations were poor between the transfer constants for each of the enantiomers between plasma, and both blister and synovial fluid ($P > 0.2$).
- 4 The complex rate constant of transfer of S-ibuprofen into blister fluid ($0.22 \pm 0.07 \text{ h}^{-1}$) was greater than that of R-ibuprofen ($0.14 \pm 0.07 \text{ h}^{-1}$), which may be explained by the lesser protein binding of the S-enantiomer.
- 5 The mean AUC of the S-enantiomer was greater than of the R-enantiomer in both blister ($116 \pm 43 \text{ mg l}^{-1} \text{ h}$, $73 \pm 32 \text{ mg l}^{-1} \text{ h}$, respectively; $P < 0.05$) and in synovial fluid ($110 \pm 28 \text{ mg l}^{-1} \text{ h}$, $56 \pm 8 \text{ mg l}^{-1} \text{ h}$, respectively; $P < 0.01$). The difference between the mean AUC values of the enantiomers in synovial fluid vs blister fluid was not significant.
- 6 Despite similar concentration-time profiles, and mean pharmacokinetic parameters, blister fluid was not a good model for predicting the kinetics of the enantiomers of ibuprofen in synovial fluid in *individual* patients. However, the blister as a peripheral compartment, better simulates the time course of concentrations in synovial fluid than does plasma, and consequently drug concentrations therein might better relate to clinical efficacy than do those in plasma.

Keywords stereoselectivity ibuprofen enantiomers

Introduction

The major site of action of the non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of rheumatic diseases is probably the synovium but this tissue is not easily sampled for pharmacokinetic and

pharmacodynamic studies. Drug concentrations in synovial fluid may better reflect those in the synovium than plasma concentrations. However, the collection of synovial fluid is not always possible and

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a suggested alternative is to measure drug in blister fluid. Suction-induced blisters have been used to assess the pharmacokinetics of antimicrobials in extravascular fluid [1–4] and a recent study demonstrated that the time courses of the concentrations of the enantiomers of ibuprofen in blister fluid resemble those in synovial fluid [5]. However, in this study the kinetics of ibuprofen were evaluated only in blister fluid and the findings compared with the kinetics in synovial fluid from other subjects in a separate study. By contrast, in the present study, the pharmacokinetics of ibuprofen were examined in both synovial and blister fluids in the sample patients to determine whether blister fluid is a suitable alternative to synovial fluid in investigations of the extravascular pharmacokinetics of this NSAID. Additionally, ibuprofen has a rather complex metabolic disposition, being racemic with the interesting feature that there is chiral inversion of the R-enantiomer to the S-enantiomer *in vivo* [6,7]. The latter is a much more potent inhibitor of prostanoid synthesis [8]. For these reasons, it was also important that concentrations of the individual enantiomers be monitored.

Methods

Materials

The enantiomers and racemate of ibuprofen were supplied by the Boots Company PLC, U.K. who also supplied the internal standard, (R)-flurbiprofen, for the h.p.l.c. assay. The internal standard for GC/MS analysis, S-[²H₃]-ibuprofen, was synthesised in-house and was shown to be 94% isotopically pure (6% undeuterated, 94% trideuterated). Data were corrected for the isotopic impurity.

The Sigma Chemical Company (St Louis, MO, USA) supplied 1,1-carbonyldiimidazole (CDI) and Aldrich Chemical Co. (Milwaukee, Wis, USA) (S)- α -methylbenzylamine (S-MBA; >98% optically pure).

A Hewlett-Packard 5890 Series II gas chromatograph directly interfaced with a Hewlett-Packard 5971A mass selective detector was used for all quantitative GC/MS analyses. Gas chromatography was performed using a fused silica capillary column (12 m $\times$ 0.22 mm i.d., cross-linked methylsilicone stationary phase BP1, 0.33 μ m film thickness; SGE Scientific, Australia). Helium was the carrier gas (inlet pressure 34 kPa). The oven temperature was held at 80° C for 1 min and then raised to 280° C at 30° C/min. A split/splitless injector operated in the splitless mode was used (split flow 50 ml min⁻¹, septum purge approx. 1.0 ml min⁻¹). A Hewlett-Packard 7673A autoinjector was used to deliver samples (1 μ l) to the column. The injection port and GC/MS interface were maintained at 250° C and 280° C, respectively. For quantitation, masses of m/z 309 and m/z 312 were scanned (dwell-time 50 ms).

H.p.l.c. analyses were performed using a Varian

5000 h.p.l.c., a Waters Wisp 710B autosampler and a Hitachi F1000 fluorescence detector (excitation 225 nm, emission 290 nm). A Beckman Ultrasphere C-18 reversed phase column (5 μ , 4.6 mm $\times$ 25 cm) was used. The mobile phase was acetonitrile (58% v/v), water (42% v/v), triethylamine (0.0002% v/v), and acetic acid (0.001% v/v) at a flow rate of 1.6 ml min⁻¹.

Study protocol

The study was conducted in patients with rheumatoid arthritis and chronic effusions of the knee. Written informed consent from the patients and approval from the ethics committee of the Karolinska Hospital were obtained. Blisters were made by suction [9] immediately prior to the study. Ibuprofen tablets (Boots, 2 $\times$ 600 mg) were taken after the patients had fasted overnight. Blood, blister (5) and synovial (10) fluids were collected aseptically at 1, 2, 4, 6, 8, 12 and 24 h after dosage. The blood was collected into Venoject® tubes and the plasma separated and stored at -20° C until assay.

Analytical method—assay of ibuprofen enantiomers

The enantiomers of ibuprofen were assayed by h.p.l.c. (plasma) and by GC/MS (blister fluid and synovial fluid). The stereospecific assay was based on the preparation of the diastereomeric amides produced by reaction with S- α -methylbenzylamine described previously for GC/MS analyses by Baillie *et al.* [11], but was further developed to allow the sensitivity required to assay the small sample volumes of blister fluid (30–50 μ l).

Plasma Plasma (0.8 ml) was extracted with hexane-ether (70:30; 10 ml) after addition of internal standard (5 μ g) and acidification with HCl solution (0.5 ml, 3 N). The organic layer was evaporated under nitrogen and to the residue was added a freshly prepared solution of CDI in toluene (0.5 ml, 5.0 mg ml⁻¹). After 10 min at room temperature, glacial acetic acid (10 μ l) was added followed by S-MBA (50 μ l). Samples were allowed to stand for 1 h and, after acidification (5 ml, 0.2 N HCl), they were extracted into toluene (0.5 ml). The toluene was evaporated under nitrogen and the residue resuspended in mobile phase (100 μ l).

Blister and synovial fluid

Extraction and derivatisation were as for plasma samples but only 50 μ l of sample was taken, and 25 ng internal standard was added. Derivatisation was accomplished with 250 μ l CDI and 25 μ l S-MBA. Samples were resuspended in toluene (20 μ l) for GC/MS analysis.

GCMS analysis Peak area ratios were used to establish standard curves and for sample analysis. Day-to-day variability in the GC/MS assay was assessed at

drug concentrations of 0.25, 1.0, 5.0, 10.0 and 20.0 $\mu\text{g ml}^{-1}$. A full set of standards and a quality control specimen were analysed with each batch of samples. Standard curves were generated by unweighted least-squares linear regression analysis using the ratio of the 309/312 ion pair.

Pharmacokinetic modelling

Plasma concentrations of the ibuprofen enantiomers (C) were fitted by two or three exponential functions of the general form:

$$C = \sum C_i e^{-\lambda_i t} \quad (1)$$

where C_i and λ_i are constants. If it is assumed that the ibuprofen enantiomers are transferred between the central compartment of distribution and synovial or blister fluids by first order processes and that the amounts so transferred do not alter the concentrations in the central compartment significantly [12,13], then the concentrations in these fluids are defined by equations 2 and 3.

$$C_s = k_{ps} \cdot V_c / V_s [\sum C_i \cdot (e^{-\lambda_i t} - e^{-k_{sp} t}) / (k_{sp} - \lambda_i)] \quad (2)$$

$$C_b = k_{pb} \cdot V_c / V_b [\sum C_i \cdot (e^{-\lambda_i t} - e^{-k_{bp} t}) / (k_{bp} - \lambda_i)] \quad (3)$$

where k_{ps} and k_{pb} are the first order rate constants of transfer from the central compartment to synovial and blister fluids, while V_c , V_s and V_b are the volumes of the central compartment, synovial fluid and blister fluid, respectively. The rate constants of loss from synovial and blister fluids are k_{sp} and k_{bp} , respectively. The constants C_i , λ_i , k_{sp} , k_{bp} and the complex constants $k_{ps} \cdot V_c / V_s$ and $k_{pb} \cdot V_c / V_b$ were determined by simultaneous logarithmic least squares analysis using the Minim program (R.D. Purves, University of Otago, New Zealand). The ratios of these kinetic and volume constants were also determined. These were $k_{ps} \cdot V_c / k_{sp} \cdot V_s$ and $k_{pb} \cdot V_c / k_{bp} \cdot V_b$. These ratios represent the ratios of the clearances of the drugs into and out of synovial and blister fluids, respectively [14,15]. The areas under the time-course of drug concentrations in plasma, synovial and blister fluids (AUC , AUC_s and AUC_b) were determined by trapezoidal integration.

Statistical analysis

Data were analysed using the paired Student's t -test.

Results

Assay validation

Day-to-day variability in the GC/MS assay ($n = 7$) of the enantiomers of ibuprofen in synovial fluid was less than 8.6% at all concentrations. Similarly, for blister fluid, the coefficients of variation were less than 7.2% for all concentrations for each enantiomer, except for R-ibuprofen at 0.25 $\mu\text{g ml}^{-1}$ (12.0%). The mean measured value of each standard for each enantiomer was within 5% of the spiked value.

H.p.l.c. retention times were: 12.0 min and 13.5 min for S-ibuprofen and R-ibuprofen, respectively, and 8.5 min for S-flurbiprofen. The coefficients of variation were less than 5.0% for each enantiomer at 2.0 $\mu\text{g ml}^{-1}$ and 5.0 $\mu\text{g ml}^{-1}$ for analysis of plasma samples ($n = 4$).

Pharmacokinetics of enantiomers in blood, blister and synovial fluid

The concentrations of the enantiomers in both fluids were initially lower than those in plasma but exceeded the plasma concentrations after about 4 to 8 h. In two patients, the concentrations of both enantiomers were initially higher in blister fluid than synovial fluid, while in two other patients the concentrations were higher in synovial fluid than in blister fluid for about 2 h after dosage. As shown by the greater values of AUC_s and AUC_b (Table 1), the concentrations of the S-enantiomer were, on average, greater than those of the R-enantiomer in both synovial and blister fluids. There was no corresponding difference between the enantiomers with respect to the plasma AUC values of the R- and S-enantiomers, although plasma AUC was considerably greater for the S-enantiomer in four of the seven patients.

The main aim of the study was to compare the kinetics of the enantiomers in blister fluid with those

Table 1 Mean ($\pm$ 95% confidence limits) constants describing the transfer of R-ibuprofen and S-ibuprofen into and out of synovial and blister fluids

	S-ibuprofen	R-ibuprofen	P	Difference S vs R
$k_{ps} \cdot V_c / V_s$ (h^{-1})	0.29 $\pm$ 0.10	0.19 $\pm$ 0.12	< 0.01	0.10 $\pm$ 0.06
k_{sp} (h^{-1})	0.36 $\pm$ 0.11	0.34 $\pm$ 0.11	NS	0.03 $\pm$ 0.09
$k_{pb} \cdot V_c / V_b$ (h^{-1})	0.22 $\pm$ 0.07	0.14 $\pm$ 0.06	< 0.01	0.08 $\pm$ 0.05
k_{bp} (h^{-1})	0.27 $\pm$ 0.08	0.20 $\pm$ 0.04	< 0.05	0.07 $\pm$ 0.05
AUC ($\text{mg l}^{-1} \text{ h}$)	134 $\pm$ 47	94 $\pm$ 21	NS	40 $\pm$ 47
AUC_s ($\text{mg l}^{-1} \text{ h}$)	116 $\pm$ 29	58 $\pm$ 8	< 0.01	58 $\pm$ 34
AUC_b ($\text{mg l}^{-1} \text{ h}$)	120 $\pm$ 48	78 $\pm$ 34	< 0.05	42 $\pm$ 38

in synovial fluid with the greater interest in the pharmacokinetics of S-ibuprofen because of its more marked pharmacological activity. There was no difference between AUC_s and AUC_b for either enantiomer in synovial fluid and blister fluid. There was a significant, although weak correlation between the values of AUC_s and AUC_b for S-ibuprofen ($r^2 = 0.62$, $P < 0.05$). No such correlation was observed for the R-enantiomer.

The data were well described by the model equations (Figure 1) and both the synovial and blister fluids behaved pharmacokinetically as peripheral compartments of distribution of the ibuprofen enantiomers. In four of the seven patients, the absorption phase was not well defined and the rate constant of absorption was set at 2.1 h^{-1} , corresponding to a half life of absorption of 20 min. However, changing the half-life of absorption to 10 or to 30 min changed the half-lives of transfer into or out of the synovial or blister fluids by less than 20% and did not alter the general conclusions of the study. In most patients, a lag time had to be introduced to the time variable

in equation 3 to provide a satisfactory fit to the experimental data.

There was a trend for the rate constants describing the kinetics of transfer into and out of blister fluid to be smaller than the corresponding kinetic constants for synovial fluid (Table 1, Figure 2) but most contrasts were not significant. The rate constant for diffusion of R-ibuprofen from blister fluid was smaller than that from synovial fluid ($P = 0.05$). The mean rate constants of loss of S-ibuprofen from synovial and blister fluids were 0.36 and 0.27 h^{-1} , corresponding to half-lives of 1.9 and 2.6 h. These half-lives are of the order of the values of the initial half-lives of the equations. The delayed times at which peaks were produced is due to the relatively slow loss of the enantiomers from these compartments.

The correlations between the kinetic constants of transfer of the enantiomers into or out of synovial fluid and the corresponding constants in blister fluid were poor and not statistically significant ($r^2 < 0.3$, $P > 0.20$). In most patients, the rate constants of

Figure 1 Concentrations of R-ibuprofen (left panel) and S-ibuprofen (right panel) in plasma (P; ■), synovial fluid (SF; ●) and blister fluid (B; ▲) in two patients after a dose of 1200 mg rac-ibuprofen. In patient 4 (upper plots), the rate constants of transfer of both enantiomers into and out of synovial fluid were greater than the corresponding rate constants in and out of blister fluid. The reverse was the case in patient 5 (lower plots). The curves represent best fits of equations 1, 2 and 3.

uptake of S-ibuprofen (Figure 2a) and loss from synovial fluid (Figure 2b) were quite different from the comparable rate constants in the blister fluid. However, the values of AUC_s and AUC_b were quite similar in several patients, differing by less than 10% in patients 1, 5, 6, and 7. The reason for these similar

values lies in the factors controlling the AUC values. From the compartmental model, it can be shown that the ratio of the AUC values is controlled by the ratio of clearances into and out of synovial fluid [14,15]. Thus:

$$AUC_s/AUC_b = k_{ps} \cdot V_c / k_{sp} \cdot V_s \quad (4)$$

Figure 2 Left panel: Comparison of the kinetics of S-ibuprofen in synovial fluid (open histograms) and blister fluid (hatched histograms): a) rate constants of loss of S-ibuprofen from synovial fluid and from blister fluid (k_{sp} and k_{bp} , respectively); b) complex rate constants of entry of S-ibuprofen into synovial fluid and into blister fluid ($k_{ps} \cdot V_c/V_s$ and $k_{pb} \cdot V_c/V_b$, respectively); c) ratio of clearances of S-ibuprofen into and out of synovial fluid and blister fluid (AUC_s and AUC_b , respectively). Right panel: Comparison of kinetics of R-ibuprofen in synovial fluid (open histograms) and blister fluid (hatched histograms): a) rate constants of loss of R-ibuprofen from synovial fluid and from blister fluid (k_{sp} and k_{bp} , respectively); b) complex rate constants of entry of R-ibuprofen into synovial fluid and into blister fluid ($k_{ps} \cdot V_c/V_s$ and $k_{pb} \cdot V_c/V_b$, respectively); c) ratio of clearances of R-ibuprofen into and out of synovial fluid and blister fluid; d) areas under the time courses of concentrations of R-ibuprofen in synovial fluid and blister fluid (AUC_s and AUC_b , respectively).

A similar relationship exists for the ratio AUC_b/AUC_s . The influence of these factors on the AUC values can be seen, for example, in patient 5. Both the complex rate constant of uptake into and the rate constant of loss from synovial fluid are smaller than the comparable rate constants of blister fluid (Figure 2a,b). However, the ratios of the constants, the clearance ratios, are similar and the values of AUC_s and AUC_b are, therefore, nearly identical (Figure 2c,d). Because of the faster transfer of S-ibuprofen both into and out of blister fluid, the concentration of S-ibuprofen peaks earlier in blister fluid but is less sustained than in synovial fluid (Figure 1, Patient 5). The values of AUC_s and AUC_b of R-ibuprofen were not as close as was the case with the S-enantiomer. In only two patients was AUC_b of R-ibuprofen within 15% of the value of AUC_s .

Discussion

The pharmacokinetics of antiinflammatory drugs have been investigated in synovial fluid because the concentrations at this site might reflect those in the presumed site of action of the drug, the synovium, better than concentrations in plasma. Multiple, sequential sampling of the synovial cavity can be carried out safely, but does pose a potential, although very small, risk of infection and may not be acceptable to all patients. Consequently, there has been an interest in suction blisters as a model to simulate extravascular compartments such as the synovial cavity and to assess the kinetics and dynamics of drugs. Suction blisters allow sequential sampling with virtually no morbidity and greater acceptability, but limit samples to very small volumes, typically 30–50 μ l from each blister. This presents analytical problems but these were overcome in the present study by developing a sensitive, stereospecific assay based on preparation of the diastereomers of ibuprofen after reaction of the enantiomers with a well known, chiral reagent, S- α -methylbenzylamine and the subsequent use of GC/MS for resolution and detection. These drugs are generally administered as their racemates but antiinflammatory activity is believed to reside entirely in the S-enantiomers [16]. Additionally, there is stereospecific chiral inversion of R-ibuprofen to S-ibuprofen in humans [17]. Consequently, stereoselective analysis is important for a proper understanding of the disposition of the 2-arylpropionates. However, surprisingly, while S-ibuprofen is an effective antiinflammatory agent [18], correlations between plasma concentrations and measures of clinical efficacy were not improved when the concentration data were expressed in terms of the S-ibuprofen as compared with R,S-ibuprofen [19].

Induction of suction blisters appears to be primarily a non-inflammatory event with few inflammatory cells present in the blister fluid at least up to 24 h after blister induction [20]. Inflammation can be induced in the blister to give a comparative inflammatory exudate model. The present study pro-

vided data with respect to a non-inflammatory model and results may differ when inflammation is present. However, it was felt that study of the non-inflammatory model was more appropriate in the first instance. Blister fluid also differs from synovial fluid in that, although hyaluronic acid concentrations are approximately 100 times higher in blister fluid than in plasma [21], they are still orders of magnitude lower than in synovial fluid. However, this difference may not be important because ibuprofen does not bind to hyaluronate.

The concentrations of the ibuprofen enantiomers in blister fluid and synovial fluid followed a qualitatively similar pattern consistent with data reported in a recent preliminary study [5]. Both the synovial fluid and blister fluid behaved pharmacokinetically as peripheral compartments of distribution of the ibuprofen enantiomers, and both required lag times to accommodate the data. The delay in the appearance of ibuprofen in blister fluid is consistent with data reported for other drugs such as antibiotics [4,22].

The mean rate constants for transfer of the ibuprofen enantiomers into and out of synovial fluid (Table 1) were within 25% of the values found previously during the chronic administration of ibuprofen [7] although the range of values was much smaller. Furthermore, as again observed previously [7] the complex rate constant describing transfer of S-ibuprofen into synovial fluid was greater than that for R-ibuprofen. This difference is consistent with a lower binding of the S-enantiomer to plasma proteins as reported previously (percent unbound, 0.419 *c.f.* 0.613, R- and S-ibuprofen, respectively, [23], and indicates that the enantiomers diffuse into synovial fluid mainly in the protein free forms. A similar contrast was seen with blister fluid (Table 1), but the transfer of S-ibuprofen was, in addition, faster out of blister fluid. Unfortunately, the limitations of small blister fluid volumes and analytical sensitivity precluded an assessment of unbound enantiomer concentrations in this study which may have given a better understanding of the relative distribution of drug between the compartments.

Despite the general lack of significance in the difference in the kinetics of transfer of ibuprofen between the compartments, the kinetics of the enantiomers in the two fluids cannot be said to be identical, there being a poor correlation between the rate constants for the compartments. The interpatient differences in the kinetic constants of transfer into or out of blister fluid were not paralleled by the corresponding rate constants for synovial fluid and, in individual patients, the kinetics in blister fluid were not predictive of the kinetics in synovial fluid (Figure 1, Table 1). The values of AUC_s and AUC_b were more closely related than the individual rate constants for the individual enantiomers, but there is no guarantee that clinical response is related to AUC values. However, even here the correlation was still not precise. Overall, detailed analysis of the kinetics of transfer confirms that there are interpatient differences in the patterns of concentrations of the ibuprofen enantiomers. Contrary to earlier findings [5],

the skin blister model did not appear to simulate quantitatively the pharmacokinetics of ibuprofen enantiomers in synovial fluid, at least in individuals, although the blister does more closely simulate the time course of concentrations of drug in synovial fluid than do plasma concentrations. An inflammatory blister model might simulate synovial fluid kinetics even better.

The clinical relevance of blister concentrations of NSAIDs is unclear. One would predict that the concentrations of NSAIDs in a peripheral location such as blister fluid might relate better to the clinical efficacy of the drug than plasma concentrations.

Unbound concentrations may provide an even better correlation with effect and the best measure of these e.g. AUC, peak concentrations etc needs to be studied. Clinical trials to examine the correlation between efficacy of NSAIDs and unbound concentrations in blister fluid would be of value.

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References

- Nowak A, Kadykow M, Klimowicz A. Penetration of trimethoprim and sulfamethoxazole into blister fluid. *Eur J clin Pharmac* 1983; **25**: 825–827.
- Mouton JW, Horrevorts AA, Mulder PGH, Prens EP, Michel MF. Pharmacokinetics of cephazidime in serum and suction blisters fluid during continuous and intermittent infusions in healthy volunteers. *Antimicrob Agents Chemother* 1990; **34**: 2307–2311.
- Schreiner A, Hellum KB, Digraanes A, Bergman I. Transfer of penicillin G and ampicillin into human skin blisters induced by suction. *Scand J infect Dis* 1978; Suppl. **14**: 233–237.
- Shyu WC, Quintiliani R, Nightingale CH. An improved method to determine interstitial fluid pharmacokinetics. *J infect Dis* 1985; **152**: 1328–1331.
- Walker JS, Knihinicki RD, Seideman P, Day RO. Pharmacokinetics of ibuprofen enantiomers in plasma and suction blister fluid in healthy volunteers. *J pharm Sci* 1993; **82**: 1–4.
- Kaiser DG, Vangiesen Reischer Wechter WJ. Isomeric conversion of ibuprofen (R)-enantiomer in humans. *J pharm Sci* 1976; **65**: 269–273.
- Lee EJD, Williams KM, Graham GG, Day RO, Champion GD. Stereoselective disposition of ibuprofen enantiomers in man. *Br J clin Pharmac* 1985; **19**: 669–674.
- Adams SS, Bresloff P, Mason CG. Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (–)-isomer. *J pharm Sci* 1976; **28**: 256–257.
- Kiistala U. Suction blister device for the separation of viable epidermis from dermis. *J invest Dermatol* 1968; **50**: 129–137.
- Day RO, Williams KM, Graham GG, Lee EJ, Champion GD. Stereoselective disposition of ibuprofen enantiomers in synovial fluid. *Clin Pharmac Ther* 1988; **43**: 480–487.
- Baillie TA, Adams WJ, Kaiser DG, Olanoff LS, Halstead GW, Harpootlian H, van Giessen GJ. Mechanistic studies of the metabolic chiral inversion of (R)-ibuprofen in humans. *J Pharmac exp Ther* 1989; **249**: 517–523.
- Coburn WA. Simultaneous pharmacokinetic and pharmacodynamic modeling. *J Pharmacokin Biopharm* 1981; **9**: 367–388.
- Noe DA, Kumor KM. Drug kinetics in low flux (small) anatomic compartments. *J pharm Sci* 1983; **72**: 718–719.
- Meyer-Carrive I, Graham GG, Ghosh P. Pharmacokinetics of tiaprofenic acid in normal rabbits and rabbits subjected to joint immobilisation. *Agents Actions* 1993; **39**: 59–68.
- Graham GG. Kinetics of non-steroidal anti-inflammatory drugs in synovial fluid. *Agents Actions Suppl* 1988; **24**: 66–75.
- Williams KM. Enantiomers in arthritic disorders. *Pharmac Ther* 1990; **46**: 273–295.
- Lee EJD, Williams KM, Graham GG, Day RO, Champion GD. Stereoselective disposition of ibuprofen enantiomers in man. *Br J clin Pharmac* 1985; **19**: 669–674.
- Stock KP, Geisslinger G, Loew D, Beck WS, Bach GL, Brune K. S-ibuprofen versus ibuprofen-racemate. *Rheumatol Int* 1991; **11**: 199–202.
- Bradley JD, Rudy AC, Katz BP, Ryan SI, Kalasinski LA, Brater DC, Hall SD, Brandt KD. Correlation of serum concentrations of ibuprofen stereoisomers with clinical response in the treatment of hip and knee osteoarthritis. *J Rheumatol* 1992; **19**: 130–134.
- Kiistala U, Mustakallio KK. Dermo-epidermal separation with suction. *J invest Dermatol* 1967; **48**: 466–467.
- Juhlin L, Tengblad A, Ortonne JP, Lacour J Ph. Hyaluronate in suction blisters from patients with scleroderma and various skin disorders. *Acta Derm Venereol (Stockh)* 1986; **66**: 409–413.
- Fleishaker JC, McNamara PJ. Performance of a diffusional clearance model for β -lactam antimicrobial agents as influenced by extravascular protein binding and interstitial fluid kinetics. *Antimicrob Agents Chemother* 1985; **28**: 369–374.
- Evans AM, Nation RL, Sansom LN. Stereoselective plasma protein binding of ibuprofen enantiomers. *Eur J clin Pharmac* 1989; **36**: 283–290.

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