Disposition and effects of flurbiprofen enantiomers in human serum and blister fluid

R. Oelkers, W. Neupert, K. M. Williams,¹ K. Brune & G. Geisslinger

Department of Experimental & Clinical Pharmacology and Toxicology, University of Erlangen-Nuernberg, Universitaetsstr. 22, D-91054 Erlangen, Germany and ¹Department of Clinical Pharmacology and Toxicology, St. Vincent's Hospital, Sydney, NSW, Australia

Aims To investigate the pharmacokinetics of the enantiomers of flurbiprofen and inhibition of prostanoïd production in blister fluid and serum.

Methods Eleven healthy volunteers received 75 mg R-, 75 mg S-flurbiprofen or no medication in a randomized 3-way cross-over study. Flurbiprofen concentrations were determined by h.p.l.c. TXB₂ and PGE₂ were determined by enzyme immunoassay and chemiluminescence immunoassay respectively.

Results S-flurbiprofen produced almost complete (>99% *vs* baseline) inhibition of thromboxane B₂ (TXB₂) in serum in all volunteers and significant inhibition of prostaglandin E₂ (PGE₂) generation in blister fluid, but there was a considerable inter-individual variation in the response ranging from –78 to +190% change from control PGE₂ AUC. After administration of R-flurbiprofen, there was a mean maximum TXB₂ inhibition of $65.2 \pm 15.0\%$ in serum but no significant changes of PGE₂ levels in blister fluid were observed. The pharmacokinetic parameters in serum and blister fluid were not significantly different between enantiomers. R- to S-inversion did not occur to a clinically relevant extent. For R-flurbiprofen, the complex rate constant of transfer into blister fluid was greater at the u.v.-exposed site (0.110 ± 0.050) than at the control site (0.079 ± 0.026 , $P < 0.05$) which corresponded to a higher AUC and $C_{\max}$ of R-flurbiprofen in u.v.-exposed blister as compared with control. For inhibition of TXB₂ generation after administration of S-flurbiprofen, a sigmoidal log-linear concentration–response relationship was established in all subjects (EC_{50} : $0.123 \pm 0.092 \mu\text{g ml}^{-1}$). In contrast, inhibition of PGE₂ production in blister showed no clear concentration–response relationship when correlated with concentrations of S-flurbiprofen in either serum or blister fluid. After administration of R-flurbiprofen, no concentration–effect relationship could be established.

Conclusions It is concluded that the blister model may have value for studying the pharmacokinetics and pharmacodynamics of antiinflammatory drugs in humans. Interestingly, inter-individual variation in the pharmacokinetics of flurbiprofen enantiomers could not account for the variability in response observed in the blister model.

Keywords: non-steroidal antiinflammatory drugs (NSAIDs), variability, flurbiprofen, enantiomers, pharmacokinetics, pharmacodynamics, prostaglandins, suction blister

Introduction

Rheumatologists have long considered that there is a clinically relevant intersubject variability in response to nonsteroidal antiinflammatory drugs (NSAIDs) [1, 2]. This variability in response is not completely understood. Two possible reasons have been proposed to explain the observed variability: i) differences between NSAIDs with respect to pharmacodynamic actions and ii) inter-subject pharmacokinetic variability [3].

Most attempts to correlate drug concentrations with effects have relied on plasma drug concentrations which do not relate well to the time course of action of antiinflammatory drugs [4]. In the treatment of rheumatic diseases, a major site of action of these drugs is probably the synovium. Thus drug concentrations in synovial fluid may better reflect the time course of effect than do plasma samples [5]. However, the collection of synovial fluid is an invasive procedure and not always possible. The skin suction blister provides a readily accessible extravascular compartment where time courses of drug concentrations resemble those in synovial fluid [5, 6]. Additionally, an 'inflammatory' blister site can be induced by previous u.v. exposure of the skin [7]. U.v. irradiation is known to increase the

Correspondence: Dr Rieke Oelkers, Department of Experimental & Clinical Pharmacology and Toxicology, University of Erlangen-Nuernberg, Universitaetsstrasse 22, D-91054 Erlangen, Germany

concentrations of various inflammatory mediators, mainly cyclooxygenase products, in blister fluid [8]. Thus the u.v.-irradiated skin suction blister may allow an integrated study of inflammatory mediators and its modulation by drugs in humans.

Flurbiprofen, a 2-arylpropionic acid derivative, is a chiral NSAID which is in clinical use as the racemate for the treatment of rheumatic diseases and other painful conditions. While some of the 2-arylpropionates undergo potentially clinically significant *in vivo* chiral inversion of the R-enantiomer to the S-enantiomer, this is not the case for flurbiprofen in healthy volunteers [9]. This feature makes the drug a useful probe of the mechanisms of drug action because it is only the S-enantiomer which has been shown to inhibit cyclooxygenases [10].

The present study was designed to investigate the concentration-effect relationship of flurbiprofen enantiomers with respect to drug concentrations not only in serum but also at an extravascular site. This extravascular site, the blister compartment, was investigated in both u.v.-treated and unexposed skin to allow a comparison of the concentration-effect relationship in an 'inflammatory' and 'non-inflammatory' model, and to assess the value of the blister model for *in vivo* human studies.

Methods

Reference compounds

R- and S-flurbiprofen were kindly supplied by PAZ (Arzneimittelentwicklungsgesellschaft Frankfurt/Main, Germany) with chemical and optical purities of greater than 99%. R-flurbiprofen (lot 20422) and S-flurbiprofen (lot 20959) were administered in gelatine capsules containing 75 mg of the single enantiomer.

Protocol

Twelve healthy male volunteers aged between 22 and 30 years and weighing 80.2 ± 13.3 kg were recruited into the study. One subject dropped out because of insufficient blister formation. The study was conducted according to the Declaration of Helsinki (Hongkong Amendment 1989). Written informed consent from the subjects and approval from the University of Erlangen Medical Ethics Review Committee were obtained. According to a randomized 3-way cross-over design, the subjects took either 75 mg S-flurbiprofen, 75 mg R-flurbiprofen or no medication with 200 ml water following an overnight fast, after which food and fluid were withheld for a further 4 h. The washout interval between each dose was at least 4 weeks. Venous blood samples were collected prior to flurbiprofen dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h. Blood drawn by a plastic syringe was immediately transferred into a glass tube and allowed to clot at 37° C for 30 min. Serum was separated by centrifugation (10 min at 1915 g) and kept at -20° C or -70° C until analysis of flurbiprofen and TXB₂, respectively. Sets of blisters ($n=9$) were induced under temperature control (37° C) on both forearms using gentle suction (-260 mbar) over about 2 h according to the method of Kiistala *et al.* [11]. Blister induction started immediately after drug administration, and samples of blister fluid were drawn at 2, 2.5, 3, 5, 8, 10, 12

and 24 h after drug administration in the cases where blister induction was completed within 2 h. Otherwise samples were collected at 0, 0.5 and 1 h starting with the end of blister induction and the other time-points were then adapted to the time-points of blood sampling. Samples were immediately frozen and stored at -70° C until analysis. The day before the study, inflammation was produced on one forearm by exposure of skin (area 35*50mm) to u.v. light stimulation (280-320 nm, 0.066 J/cm²).

Drug analysis in biological fluids

R- and S-flurbiprofen concentrations in serum and blister fluid were measured by stereoselective h.p.l.c. as described previously [12]. The limit of quantification (lowest concentration that could be determined with either precision or accuracy of less than or equal to 10%) was 10 ng ml⁻¹ for both enantiomers. The coefficient of variation over the calibration range of 10 ng ml⁻¹ to 10 µg ml⁻¹ of each enantiomer was less than 12%.

Thromboxane concentrations in serum

To monitor the effects of flurbiprofen enantiomers on platelet cyclooxygenase, the amount of TXA₂ generated during clotting (an index of cyclooxygenase activity) was assessed by measuring the concentration of its stable breakdown product TXB₂ according to the method of Patrono *et al.* [13]. TXB₂ was assayed using a commercially available TXB₂ EIA kit (Cayman-Chemicals, Ann Arbor, Michigan, USA) with samples diluted appropriately in buffer. The limit of quantification (lowest concentration that could be determined with either precision or accuracy of less than or equal to 20%) was 20 pg ml⁻¹. The coefficient of variation over the calibration range of 20-600 pg ml⁻¹ was 19.2-5.8%.

Prostanoid concentrations in blister fluid

Blister fluid samples were assayed for immunoreactive PGE₂ using a chemiluminescence immunoassay (CLIA) as described previously [14]. The limit of quantification (lowest concentration that could be determined with either precision or accuracy of less than or equal to 20%) was 100 pg ml⁻¹. The coefficient of variation over the calibration range of 100-50 000 pg ml⁻¹ was less than 18%.

Pharmacokinetic modelling

Pharmacokinetic models were chosen using statistical methods to assess the validity of the models for describing the experimental data (*F*-ratio test, Akaike information, Schwarz and Imbimbo criteria) [15]. Serum concentrations of flurbiprofen enantiomers were fitted using a 2-compartment disposition model with a 2-segment input to a polyexponential equation of the following general form:

$$C_S = A * e^{-k_{a1} * (t - t_{lag1})} + B * e^{-k_A * (t - t_{lag1})} + C * e^{-\alpha * (t - t_{lag1})} + D * e^{-\beta * (t - t_{lag1})} + E * e^{-k_{a2} * (t - t_{lag2})} + F * e^{-k_A * (t - t_{lag2})} + G * e^{-\alpha * (t - t_{lag2})} + H * e^{-\beta * (t - t_{lag2})} \quad (1)$$

where A–H are coefficients (amplitudes) of the exponential terms, k_{a1} and k_{a2} are dissolution rate constants for the two fractions of the split dose, k_A defines the absorption rate of both fractions, α and β are disposition constants and t_{lag1} and t_{lag2} are the lag times between administration of drug and its detection at the sampling site for input segment 1 and 2, respectively. The constants were determined by an iterative least squares program with a weighting factor of $1/y$ (TOPFIT® Program package, version 2.0, [15]).

It is assumed that flurbiprofen enantiomers are transferred between the central compartment of distribution and blister fluid by first order processes and that the amounts so transferred do not alter the concentrations in the central compartment significantly [16, 17]. Accordingly, concentrations of flurbiprofen enantiomers in blister fluid were fitted to the following equation:

$$C_B = k_{SB} * V_C / V_B * \left[A * \frac{e^{-k_{a1} * (t - t_{lag1})} - e^{-k_{BS} * (t - t_{lag1})}}{k_{BS} - k_{a1}} + B * \frac{e^{-k_A * (t - t_{lag1})} - e^{-k_{BS} * (t - t_{lag1})}}{k_{BS} - k_A} + C * \frac{e^{-\alpha * (t - t_{lag1})} - e^{-k_{BS} * (t - t_{lag1})}}{k_{BS} - \alpha} + D * \frac{e^{-\beta * (t - t_{lag1})} - e^{-k_{BS} * (t - t_{lag1})}}{k_{BS} - \beta} + E * \frac{e^{-k_{a2} * (t - t_{lag2})} - e^{-k_{BS} * (t - t_{lag2})}}{k_{BS} - k_{a2}} + F * \frac{e^{-k_A * (t - t_{lag2})} - e^{-k_{BS} * (t - t_{lag2})}}{k_{BS} - k_A} + G * \frac{e^{-\alpha * (t - t_{lag2})} - e^{-k_{BS} * (t - t_{lag2})}}{k_{BS} - \alpha} + H * \frac{e^{-\beta * (t - t_{lag2})} - e^{-k_{BS} * (t - t_{lag2})}}{k_{BS} - \beta} \right] \quad (2)$$

where k_{SB} is the first order rate constant of transfer from the central compartment to blister fluid, while V_C and V_B are the volumes of the central compartment and blister fluid, respectively. The rate constant of loss from blister fluid, k_{BS} , and the complex constant $k_{SB} * V_C / V_B$ were determined by iterative least square analysis with a weighting factor of $1/y$ using the SigmaPlot® program package (version 2.01, Jandel corporation).

The maximum measured drug concentration C_{max} and the corresponding timepoint t_{max} in serum and blister fluid were determined directly from the data. The terminal disposition rate constant (λ_z) was estimated by unweighted linear least-squares regression analysis of the last linear segment of the log serum drug-concentration time data which was identified by visual inspection. The terminal elimination half-life was calculated from $\ln 2 / \lambda_z$. Apparent systemic clearance (CL/F) was calculated by dividing the dose by its AUC. The half-life of return in blister fluid ($t_{1/2B}$ of return) was for each subject derived from $\ln 2 / k_{BS}$ (rate constant of loss from blister fluid). The area under the

drug concentration-time curve in either serum (AUC_S) or blister fluid (AUC_B) and the PGE_2 concentration-time course in blister fluid (PGE_2 AUC) were calculated using the linear trapezoidal rule. AUC_S was extrapolated to infinity from $C_{(last)}$, whereas PGE_2 AUC and AUC_B were calculated between 0 and 24 h because the extrapolated area to infinity from $C_{(last)}$ exceeded 10% of the total AUC in most subjects. AUC_B was extrapolated to zero (time of flurbiprofen administration) from the first time-point of blister fluid sampling.

Pharmacokinetic/pharmacodynamic modelling

Inhibition of TXB_2 generation during whole blood clotting was calculated in samples obtained after flurbiprofen administration as the percentage decrease in serum concentration of TXB_2 , relative to the pre-dose concentration. For inhibition of PGE_2 synthesis in blister fluid, the PGE_2 time course without medication was used as reference to calculate percentage change from control after flurbiprofen administration. For each subject, the percentage inhibition of TXB_2 generation in serum and PGE_2 production in blister fluid was related to the drug concentration in serum and blister fluid, respectively, using the sigmoidal E_{max} model

$$E = \frac{E_{max} * c^S}{c^S + EC_{50}^S} \quad (3)$$

and a non-linear least-squares modelling program (BOOMER, [18]) to estimate the EC_{50} (concentration of drug which causes a 50% reduction in the maximum effect) and the steepness factor (S).

Statistical analysis

Group data are presented as mean $\pm$ standard deviation (s.d.), if not marked otherwise. Results were compared by analysis of variance (ANOVA) and Student's paired two-tailed t -test. In addition, 95% confidence intervals for differences were calculated. $P < 0.05$ was considered to be statistically significant.

Results

TXB_2 generation in serum

S-flurbiprofen caused nearly complete inhibition of serum TXB_2 (range of maximum observed percentage inhibition 99.6 to 99.9%) in all subjects. The time that the serum concentrations of S-flurbiprofen exceeded the EC_{98} for inhibition of TXB_2 generation was 12.5 ± 4.3 h. After administration of R-flurbiprofen, highly variable serum TXB_2 inhibition occurred with maximum observed inhibition ranging from 34.6 to 83.6% (mean $\pm$ s.d. $65.2 \pm 15.0\%$).

PGE_2 concentrations in blister fluid

Time courses of mean PGE_2 concentrations are given in Figure 1. Without medication, PGE_2 concentrations in the

Figure 1 Concentration-time course of PGE_2 concentration in blister fluid (mean $\pm$ s.e.mean) in the u.v.-exposed arm (a) and at the control site (b) after peroral administration of 75 mg S-flurbiprofen ($\blacksquare$), 75 mg R-flurbiprofen ($\bullet$) or no medication ($\triangle$).

u.v.-exposed arm exceeded those of the control site over the first hour after blister induction (PGE_2 AUC(0, 1 h) 17 ± 6 vs 9 ± 4 $\text{ng ml}^{-1} \text{h}$, $P < 0.01$, 95% CI for differences -13 to -3). However, over the entire measuring period the area under the PGE_2 concentration-time curve without medication at the inflamed site was not significantly different from the unaffected site (PGE_2 AUC(0, 24 h) 161 ± 70 vs 171 ± 100 $\text{ng ml}^{-1} \text{h}$, $P = 0.68$, 95% CI for differences -63 to $+43$).

S-flurbiprofen reduced PGE_2 concentration significantly ($P < 0.05$) in both the u.v.-exposed and the control arm, but there was a considerable inter-individual variation in response (mean percentage change from control of PGE_2 AUC(0, 24 h) $-22 \pm 41\%$, range -78 to $+190\%$). The corresponding areas under the S-flurbiprofen concentration-time curve in blister fluid were much less variable (range, 14.9 to 68.2 $\mu\text{g ml}^{-1} \text{h}$). R-flurbiprofen caused no significant changes in PGE_2 concentrations, although there was a tendency for R-flurbiprofen to increase PGE_2 levels in the u.v.-exposed arm vs a slight reduction in the control site (mean percentage change from control of PGE_2 AUC (0, 24 h) $+28 \pm 68\%$ and $-9 \pm 48\%$, respectively).

Pharmacokinetics of flurbiprofen enantiomers in serum and blister fluid

The concentrations of the enantiomers in blister fluid were initially lower than those in serum but generally equalled and exceeded the serum concentrations from about 10 h after dosing. The pharmacokinetic parameters in either serum or blister fluid, including the rate constants of transfer ($k_{\text{SB}} \cdot V_{\text{C}} / V_{\text{B}}$ and k_{BS}), were not significantly different between the enantiomers (Table 1). After administration of R-flurbiprofen, concentrations of S-flurbiprofen in serum were always lower than 0.070 $\mu\text{g ml}^{-1}$ indicating little or no inversion of R-flurbiprofen to S-flurbiprofen.

The data were well described by the model equations (Figure 2) and blister fluid behaved pharmacokinetically as a peripheral compartment of distribution. Two peaks in the rising part of the serum concentration versus time curve were identifiable in most subjects indicating a splitting into two dose fractions at the site of absorption. According to the statistical methods for model selection, a two-segment input with lag-times for absorption was introduced to fit the data. For R-flurbiprofen, the complex rate constant of transfer into blister fluid was significantly ($P < 0.05$) greater

at the u.v.-exposed site than at the control site (95% CI for differences 0.006 to 0.056). This corresponded to a significantly ($P < 0.05$) higher AUC (95% CI for differences 1.12 to 11.28 $\mu\text{g ml}^{-1} \text{ h}$) and C_{max} (95% CI for differences 0.11 to 0.72 $\mu\text{g ml}^{-1}$) of R-flurbiprofen in u.v.-exposed blisters as compared with control.

Concentration–response relationship

For inhibition of platelet-derived TXB_2 after administration of S-flurbiprofen, a sigmoidal log-linear concentration–response relationship could be established in all volunteers with coefficients of variation for the individual fits ranging from 0.97 to 17% and correlation coefficients greater than 0.97 in all subjects (Figure 3). The estimated mean EC_{50} was $0.123 \pm 0.092 \mu\text{g ml}^{-1}$ and the mean steepness factor S was 1.71 ± 0.89 . In contrast, correlation of blister PGE_2 inhibition with concentrations of S-flurbiprofen in serum was poor. EC_{50} and S could be estimated within reasonable limits for less than half of the subjects. Data were not improved by correlating PGE_2 inhibition with drug concentrations in blister fluid itself; a concentration–response relationship could be established only in the u.v.-exposed arm of two subjects.

Neither inhibition of TXB_2 in serum (Figure 3b) nor changes of PGE_2 generation in blister fluid could be correlated with drug concentrations after administration of R-flurbiprofen.

Discussion

Several studies have investigated the pharmacokinetics of NSAIDs in responding and non-responding groups of patients suffering from rheumatoid arthritis. However,

variability in plasma concentrations appeared to account for only a small proportion of the inter-individual variation in response in these groups, and a clear plasma concentration–effect relationship could not be established [4, 19, 20]. It was considered likely that concentrations of drug at the site of action (e.g. synovial fluid) would better reflect the time course of drug action than do plasma concentrations [21]. As the suction blister offers an accessible extravascular site [5, 6], this model might prove to be useful to investigate concentration–response relationships of antiinflammatory drugs at their site of action in humans. Induction of suction blister has been reported to be a non-inflammatory event [22] on the basis of the lack of cellular infiltration in the early time-frame (up to 24 h) following blister induction. Inflammation can be induced in the blisters by prior u.v.-exposure which produces PGE_2 concentrations in suction blister fluid of between 30 and 60 ng ml^{-1} [23–25]. In the first hour after blister induction (approximately 20 h post irradiation) we found similar concentrations of PGE_2 in the u.v.-exposed arm. These concentrations generally exceeded those of the control i.e. non-exposed site. However, despite this early difference, there was no significant difference between the areas under the PGE_2 concentration–time data between the inflamed and non-inflamed arms with respect to the entire measuring period. This failure to detect a difference was due not only to the large intersubject variability, but also to a delayed peak in the control (non u.v.-exposed) blisters between 3 and 5 h after blister induction (Figure 1). This delayed PGE_2 concentration peak was observed in 10/11 subjects and probably reflects a secondary inflammatory response. Thus, blister induction even without u.v. irradiation may be regarded as a ‘minor’ inflammatory event. The PGE_2 rebound could be related to the induction of cyclooxygenase-2 (COX-2) which has

Table 1 Pharmacokinetic parameters of flurbiprofen enantiomers in serum and blister fluid (group data are presented as mean $\pm$ s.d. except observed peak concentration (C_{max}) and time to observed peak concentration (t_{max}) which are presented as median and interquartile range in parentheses).

Serum	S-flu	R-flu
C_{maxS} ($\mu\text{g ml}^{-1}$)	9.55 (3.01)	8.52 (3.17)
t_{maxS} (h)	1.5 (1.5)	1.5 (1.5)
$t_{1/2S}$ (h)	5.78 ± 1.15	6.09 ± 1.72
$\text{CL}_{\text{totS}}/F$ (ml min^{-1})	18.98 ± 4.29	19.35 ± 4.26
AUC_S ($\mu\text{g ml}^{-1} \text{ h}$)	69.04 ± 14.78	68.61 ± 21.12

Blister fluid	S-flu		R-flu	
	Control	u.v.	Control	u.v.
C_{Pmax}^B ($\mu\text{g ml}^{-1}$)	1.97 (0.76)	1.91 (1.11)	1.98 (0.72)	2.26 (1.11)*
t_{Pmax}^B (h)	7.25 (6.5)	7.5 (6.0)	7.0 (5.75)	7.5 (6.75)
$t_{1/2}$ of return (h)	6.66 ± 5.04	8.38 ± 6.68	6.20 ± 3.11	5.12 ± 2.55
$k_{\text{SB}} \star V_C / V_B$	0.140 ± 0.193	0.083 ± 0.057	0.079 ± 0.026	0.110 ± 0.050 *
k_{BS}	0.249 ± 0.385	0.118 ± 0.062	0.135 ± 0.062	0.170 ± 0.084
AUC_B (0,24 h) ($\mu\text{g ml}^{-1} \text{ h}$)	33.74 ± 13.40	35.72 ± 14.06	32.66 ± 8.89	38.91 ± 13.20 *

* $P < 0.05$ vs control (i.e. not u.v.-exposed arm).

Figure 2 Representative concentration-time courses of flurbiprofen enantiomers in serum (■), blister fluid of the u.v.-exposed site (●) and blister fluid of the control site (△) in two subjects (a, b) after peroral administration of 75 mg S-flurbiprofen (left panel) and 75 mg R-flurbiprofen (right panel). The curves represent best fits of equation 1 and 2.

been reported to occur with a latency of approximately 4 h to inflammatory stimuli [26], whereas it is believed that the constitutively expressed COX-1 isoenzyme maintains the basal 'housekeeping' concentrations of PGE₂.

The combination of the u.v.-exposure with the blister model allows a simultaneous assessment of concentrations of inflammatory mediators and drug in an 'inflammatory' model and seems therefore useful to screen for antiinflammatory actions of drugs.

Appropriate pharmacokinetic models are available to describe the concentrations of drug in the extravascular compartment and to quantify the drug transfer into and out of blister fluid. The variability of the flurbiprofen enantiomers in serum observed in this study fell within the range of previously reported data [9]. Pharmacokinetic variability in blister fluid was higher than in serum but consistent with data reported for ibuprofen [6]. The delayed times at which peaks in blister fluid were produced are due to the relatively slow entry of the enantiomers into blister fluid. The mean rate constants of transfer into and out of blister fluid were not different between the enantiomers. R-flurbiprofen accumulated in u.v.-exposed blisters in comparison with control as indicated by a significantly greater complex rate

constant of transfer into blister fluid, higher AUC and C_{max} . This accumulation might be due to fenestration of the microvasculature and leakage into the interstitial spaces. These are characteristic features of an acute inflammation. For S-flurbiprofen, no significant differences between the u.v.-exposed blisters and control could be detected. The difference between the enantiomers with regard to distribution into the blister fluid after u.v.-exposure may relate to the differences in the pharmacological actions of the enantiomers. The S-enantiomer has the ability to modulate its own distribution by inhibiting inflammation and thus decreasing permeability and access to the blister compartment. By contrast, the R-enantiomer which lacks intrinsic antiinflammatory activity (not being an inhibitor of cyclooxygenase(s)) has no effect on the permeability of the 'inflamed' membranes at the site of action i.e. the blister, and so is able to diffuse more readily, either as unbound drug or as protein bound drug.

Pharmacokinetic-pharmacodynamic modelling in this study was performed without quantifying concentrations of the unbound drug, as the number of assays performed in blister fluid was limited by the sampling volume (one blister generally yielded *circa* 50 µl of exudate). For inhibition of

Figure 3 Relationship between the percentage inhibition of TXB₂ generation and log serum concentrations of flurbiprofen following peroral administration of 75 mg S-flurbiprofen (a) and 75 mg R-flurbiprofen (b). Different symbols represent different subjects. The curve in (a) represents best fit of equation 3.

PGE₂ synthesis, a concentration-response relationship could be established in less than half of the subjects with a better correlation with levels of S-flurbiprofen in serum than in blister fluid. PGE₂ generation responsible for the PGE₂ concentrations in blister fluid may occur primarily in cells supplied from the blood site rather than from cells in direct contact with the blister fluid. Potential sources of eicosanoids in the normal and inflamed skin are both the constituent cells (keratinocytes, fibroblasts, mast cells, vascular endothelium) and the blood cells infiltrating the skin (neutrophils, eosinophils, monocytes, lymphocytes, platelets) [27].

Both isoforms of cyclooxygenase are probably involved in PGE₂ generation in this model. A different contribution of COX-1 and COX-2 to PGE₂ generation with different time courses in expression and/or activity could explain the lack of concentration-response relationships in some subjects which is consistent with data reported previously [21]. In subjects in which concentration-response relationships in both arms could be established the EC₅₀ values after u.v.-exposure exceeded those without irradiation. This might be due to a higher contribution of COX-2 in the u.v.-exposed site consistent with a ratio EC₅₀ COX-1/EC₅₀ COX-2 of 0.03 for S-flurbiprofen *in vitro* [28].

In contrast, only COX-1 is considered to be the key enzyme for generating prostaglandins involved in platelet aggregation [29]. The contribution of only one isoform to thromboxane generation might facilitate pharmacokinetic-pharmacodynamic modelling in contrast to blister fluid, where both isoforms seem to be involved. For inhibition of TXB₂ generation, concentration-response relationships could be established in all subjects consistent with the findings reported previously [21, 30]. The data for the effect of serum concentrations of S-flurbiprofen on platelet-derived TXB₂ indicate considerable inter-individual variability in the EC₅₀, there being a nearly eight-fold range in this parameter. This result is consistent with data from Day *et al.* for naproxen [21].

After administration of R-flurbiprofen, no concentration-response relationship could be established. Inhibition of TXB₂ generation could have been a direct effect of the R-flurbiprofen itself. However, the R-enantiomer is 500-fold less potent than the S-enantiomer [28]. Therefore it is more likely that any effect observed is a consequence of the small (<1%) impurity of S-antipode in the R-enantiomer. However, it cannot be excluded that a pharmacodynamic action not related to direct inhibition of

cyclooxygenase or an interaction between the enantiomers was involved.

In summary, these data indicate firstly the potential value of the suction blister after u.v.-irradiation as a relatively non-invasive model of inflammation for the study of pharmacokinetics and pharmacodynamics of antiinflammatory drugs in humans. Secondly, the data demonstrate considerable inter-subject variability in response expressed as inhibition of blister fluid PGE₂ generation and inter-subject pharmacokinetic variability of the flurbiprofen enantiomers in both serum and blister fluid. However, while concentrations of the S-enantiomer correlated well with pharmacodynamic response in blood, a poor correlation was observed between drug enantiomer concentrations and effect in blister fluid. It is concluded that inter-individual variation in the pharmacokinetics of R- and S-flurbiprofen is not a major factor contributing to variability in response in extravascular compartments.

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