The influence of renal function on the enantioselective pharmacokinetics and pharmacodynamics of ketoprofen in patients with rheumatoid arthritis

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- 1 Single oral doses of 100 mg racemic ketoprofen were given to 15 patients (age range: 51–79 years) with rheumatoid arthritis and a range of creatinine clearances (CL_{CR}) from 26 to 159 ml min⁻¹.
- 2 The fractions unbound of (R)- and (S)-ketoprofen in plasma were determined for each subject after *in vitro* addition of rac-ketoprofen (enantiomer range: 1.00–6.00 µg ml⁻¹) to pre-dose plasma.
- 3 An index of the antiplatelet effect of ketoprofen *in vitro* was measured as inhibition of platelet thromboxane B₂ (TXB₂) generation during the controlled clotting of whole blood (pre-dose) spiked with rac-ketoprofen.
- 4 *In vivo* studies revealed significant associations ($P < 0.05$) between the reciprocal of AUC for both unbound and total (bound plus unbound) (S)-ketoprofen and CL_{CR} . Corresponding relationships were also observed for the (R)-enantiomer of ketoprofen. In addition, the half-life of each enantiomer was negatively correlated with CL_{CR} . There was a positive relationship between the 24 h urinary recovery of combined non-conjugated and conjugated (R)-ketoprofen and CL_{CR} while that for the (S)-stereoisomer failed to reach statistical significance ($P > 0.05$).
- 5 There was no difference between AUC for (R)- and (S)-ketoprofen for either unbound or total drug.
- 6 The mean $\pm$ s.d. percentage unbound of (S)-ketoprofen in plasma ($0.801 \pm 0.194\%$) exceeded ($P < 0.05$) the corresponding value for its optical antipode ($0.724 \pm 0.149\%$). The percentage unbound of the (S)-enantiomer was higher at 6.00 µg ml⁻¹ than that at enantiomer concentrations of 3.50 µg ml⁻¹ and below, where it was invariant. The percentage unbound of (R)-ketoprofen was independent of plasma concentration up to 6.00 µg ml⁻¹. There were no correlations between the percentage unbound of each enantiomer and either serum albumin concentration or CL_{CR} .
- 7 The relationship between the serum concentration of unbound (S)-ketoprofen and the percentage inhibition of platelet TXB₂ generation was described by a sigmoidal E_{max} equation for each patient. There was no correlation between the unbound concentration of (S)-ketoprofen in serum required to inhibit platelet TXB₂ generation by 50% (EC_{50}) and CL_{CR} . The mean $\pm$ s.d. EC_{50} was 0.216 ± 0.143 ng ml⁻¹.
- 8 These data indicate that diminished renal function is associated with an increased exposure to unbound (S)-ketoprofen, presumably due to regeneration of parent aglycone arising from the hydrolysis of accumulated acyl-glucuronide conjugates. The apparent sensitivity of platelet cyclo-oxygenase to the inhibitory effect of (S)-ketoprofen was not influenced by renal function.

Keywords ketoprofen enantiomers renal function creatinine clearance acyl-glucuronides rheumatoid arthritis stereoselectivity pharmacokinetics thromboxane B₂ cyclo-oxygenase protein binding

Introduction

Like most congeners of the 2-arylpropanoic acid class of non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen is used clinically as the racemate. It is generally accepted that NSAIDs exert their main pharmacological actions by inhibition of prostaglandin biosynthesis [1], and *in vitro* studies have shown that such activity of chiral NSAIDs resides predominantly or exclusively with the (S)-enantiomers [2–4]. Only drug not bound to plasma protein (unbound drug) is thought capable of interacting with an effector site to elicit an action. Moreover, since ketoprofen (in common with most NSAIDs) has been characterized as a restrictively cleared drug [5], its clearance will depend upon the unbound fraction in plasma [6]. Consequently, it is important to evaluate both the pharmacokinetics and pharmacodynamics of chiral NSAIDs with respect to the unbound (S)-enantiomer concentration, since perturbations of such concentrations may be associated with changes in clinical response.

Acyl-glucuronidation is a metabolic pathway common to many 2-arylpropanoic acid NSAIDs including ketoprofen [7]. A low percentage of the dose of such drugs is cleared renally in unchanged form yet, paradoxically, a number of them have diminished clearance in patients with renal dysfunction, or in elderly patients in whom renal function is reduced [8–14]. Moreover, co-administration of probenecid has caused a decrease in the plasma clearance of these NSAIDs [15–19]. It has been suggested by Meffin [20], from mechanistic studies in animals [21, 22], that such observations are consistent with a decrease in the renal elimination of acyl-glucuronides leading to persistence of these labile metabolites in the body followed by their systemic hydrolysis back to the parent aglycone. With such a reversible cycle of acyl-glucuronidation in operation, net drug clearance of the aglycone is due to conjugation and competition between elimination of glucuronide by renal and hydrolytic clearance pathways.

Few studies have examined the influence of renal function on the disposition of 2-arylpropanoic acid NSAIDs, and still fewer have addressed this issue with regard to unbound drug concentrations [11, 23, 24]. No studies have described the disposition of ketoprofen enantiomers in terms of unbound drug. This study was designed to examine the enantioselective pharmacokinetics and pharmacodynamics of the unbound species in a clinical setting. Patients with rheumatoid arthritis were recruited for this single oral dose study and relationships were examined between dispositional parameters and renal function, particularly, in terms of unbound (S)-ketoprofen. While factors such as age, disease severity and concurrent medication may directly influence ketoprofen disposition, in light of recent evidence of diflunisal acyl-glucuronide recycling in patients with renal failure [25], it was postulated that recycling of labile glucuronides

should lead to decreased net clearance of ketoprofen in patients with diminished renal function. In addition, *in vitro* studies were conducted on pre-dose blood obtained from these patients to investigate the relationship between the unbound concentration of (S)-ketoprofen and inhibition of platelet cyclo-oxygenase and the influence thereon of renal function.

Methods

Subjects

Fifteen subjects with definite or classical rheumatoid arthritis [26] aged between 51 and 79 years (mean $\pm$ s.d.: 68.1 ± 8.5 years) gave written informed consent to participate in the study. The study protocol and patient information leaflet were approved by the Research and Ethics Committee of the Repatriation General Hospital, Daw Park, South Australia. Subjects were withdrawn from any non-steroidal anti-inflammatory drug for 7 days prior to the study day (no patients were taking long half-life oxicam NSAIDs or aspirin prior to recruitment). Disease modifying anti-rheumatic agents and other prescribed drugs were continued. All subjects were admitted to the hospital at least 1 day prior to study and were fasted from midnight preceding the study and for 4 h after dosing. On study days, fluid intake was restricted to 300 ml until 4 h post-dose when each volunteer received a standard light lunch. Subject demographics including medications taken as part of continuation of chronic therapy are shown in Table 1.

Ketoprofen administration and blood and urine sampling

Immediately prior to ketoprofen administration, 60 ml of blood was taken from an indwelling cannula sited in a forearm vein. This was used for plasma protein binding experiments and the *in vitro* assessment of the effects of ketoprofen enantiomers on platelet cyclo-oxygenase. A single oral 100 mg dose of racemic ketoprofen (Orudis®, conventional-release capsules, batch number DD2173, May and Baker, Victoria, Australia) was given to each subject with 150 ml of water at approximately 08.00 h. Venous blood samples (10 ml) were collected into heparinized tubes at 15 min intervals for the first 2 h after dosing then at hourly intervals for the following 4 h and finally at 2 hourly intervals up to 10 h.

To avoid *ex vivo* hydrolysis of labile glucuronides of ketoprofen [27], blood samples were immediately placed on ice then centrifuged (2000 g, 10 min, 2° C) within 30 min of collection to allow harvesting of plasma. Aliquots of plasma (1 ml) were placed in glass culture tubes and the pH was stabilized by adding 50 μ l of 2.0 M sulphuric

Table 1 Demographics of the patients studied

Patient	Gender	Age (years)	Weight (kg)	Serum albumin* (g l ⁻¹)	Creatinine clearance (ml min ⁻¹)	Medications during the study
1	F	67	53	31	26	Theophylline, cyclosporin, salbutamol
2	M	71	81	19	45	Verapamil, paracetamol
3	M	74	77	31	50	Isosorbide dinitrate, hydroxychloroquine, chlorothiazide, nifedipine, glyceryl trinitrate
4	F	74	69	36	55	Sulphasalazine, paracetamol, temazepam
5	F	60	62	39	65	Methotrexate
6	M	72	82	34	70	Amiodarone, chlorothiazide, diltiazem, dothiepin, prednisolone, warfarin
7	M	79	83	28	81	Indapamide, glicazide, temazepam, methyl dopa, salbutamol
8	M	67	82	36	87	Methotrexate, paracetamol, nitrazepam
9	F	51	46	32	69	Ranitidine, frusemide, paracetamol, potassium chloride, prednisolone, dextropropoxyphene
10	F	75	57	32	75	Methotrexate
11	M	74	61	34	87	Metformin, glicazide, digoxin, temazepam, paracetamol, aurothioglucose
12	F	77	63	34	83	Atenolol, calcitriol, thyroxine, glyceryl trinitrate
13	M	68	62	26	91	Paracetamol, methotrexate
14	M	57	91	33	120	Aurothiomalate
15	M	56	83	29	159	Paracetamol, methylprednisolone

*Laboratory reference range: 32–42 g l⁻¹.

acid. The samples were stored at -20° C until assay. In preliminary experiments, involving addition of ketoprofen acyl-glucuronides to plasma, it was verified that the sample processing, storage and subsequent analysis within 1 month of collection did not lead to cleavage of the glucuronides of (R)- or (S)-ketoprofen.

Urine was collected from 0–5 h and 10–24 h after dosing with ketoprofen and aliquots were stored at -20° C until assay. No attempt was made to prevent spontaneous deconjugation of ketoprofen glucuronides in urine during either collection or storage.

Creatinine clearance was calculated by dividing the urinary creatinine excretion over the 24 h urine collection by the plasma creatinine concentration in the 10 h blood sample. An additional blood sample was taken at this time and allowed to clot prior to harvesting of serum for the measurement of albumin. Creatinine and albumin concentrations were measured by standard auto-analyzer colourimetric methods (Synchron CX[®] System, Beckman, Sydney, Australia and BCP System for Hitachi[®] 704, Boehringer Mannheim, Sydney, respectively).

Drug assay

Plasma concentrations of total (bound plus unbound) (R)- and (S)-ketoprofen aglycone were measured by the h.p.l.c. method of Hayball *et al.* [28]. The precision of the analytical method was assessed by replicate assay of quality control plasma samples containing 0.20 and 9.00 µg ml⁻¹ of each isomer present as racemic drug. The inter-day coefficients of variation ($n = 12$) for (R)- and (S)-ketoprofen at the lower quality control concentration were 4.7% and 4.2%, respectively, and for the upper concentration they were 3.9% and 5.0%, respectively. To measure the urinary concentrations of combined aglycones and glucuronides of ketoprofen enantiomers, urine samples (100 µl volumes of the 0–5 h and 5–10 h collections and 1.00 ml from the 10–24 h collection) were

made alkaline by treating aliquots with 100 µl of 1.0 M sodium hydroxide for 15 min at room temperature. The hydrolyzed urine samples were acidified with 0.50 ml of 2.0 M sulphuric acid, extracted with internal standard and then assayed for ketoprofen isomers. These hydrolysis conditions led to complete cleavage without racemization of the aglycone [27]. Since we could not control the potential for conjugate hydrolysis in the bladder [7], no attempt was made to separate aglycone and glucuronide urinary excretion. Unfortunately, we were unable to assay ketoprofen glucuronides reliably in *ex vivo* plasma samples.

Plasma protein binding

The protein binding of ketoprofen enantiomers (concentration range: 1.00–6.00 µg ml⁻¹) was measured by the method of Hayball *et al.* [29]. The reproducibility of the method was assessed by adding 2.0 µg ml⁻¹ of [1-¹⁴C]-rac-ketoprofen to drug-free plasma from a healthy volunteer; the coefficients of variation ($n = 6$) for the determination of f_u (R) and f_u (S) were 9.9% and 10.5%, respectively [29]. The presence of labile ketoprofen conjugates has been reported in *ex vivo* plasma from elderly subjects dosed with the drug [30]. This necessitated the use of pre-dose (metabolite-free) plasma for determining the protein binding of ketoprofen enantiomers, as discussed previously [29]. Additionally, given the specific activity of our radiolabelled ketoprofen, the minimum quantity of 'tracer' required for radiometric detection of unbound isomers was equivalent to 2.0 µg ml⁻¹ of rac-ketoprofen [29] and hence does not represent a tracer quantity if added to *ex vivo* plasma aliquots.

Pharmacodynamic measurements

Inhibition of platelet thromboxane A₂ generation during the controlled clotting of whole blood in the presence of a range of rac-ketoprofen concentrations (5.00, 10.00, 20.0,

50.0, 100, 200, 1000 and 2000 ng ml⁻¹) was measured as described previously [31]. For each subject, the relationship between the percentage inhibition of thromboxane B₂ (TXB₂) generation and the unbound concentration of (S)-ketoprofen in serum was examined by non-linear least-squares regression (with reciprocal weighting) according to a sigmoidal E_{max} model. Previously, we had established that the (S)-enantiomer of ketoprofen was solely active at inhibiting human platelet TXB₂ production and this activity was not modified by the presence of its optical antipode [31]. The method for deriving the corresponding unbound concentration of (S)-ketoprofen in serum after spiking blood with racemic drug was based on preliminary studies which confirmed that the unbound fraction of (S)-ketoprofen in plasma (*fu*(S)) *in vitro* was equivalent to the unbound fraction in serum. This has also been demonstrated for the protein binding of naproxen [11].

The blood to serum concentration ratio of (S)-ketoprofen (*C_b*(S)/*C_s*(S)) was calculated for each subject as the ratio of the concentration of (S)-ketoprofen in serum derived from spiking serum harvested from pre-dose blood with 2.0 µg ml⁻¹ of rac-ketoprofen to the concentration of (S)-ketoprofen in serum subsequent to spiking whole blood with 2.0 µg ml⁻¹ of racemic drug. This parameter was used in conjunction with *fu*(S) to derive the unbound (S)-ketoprofen concentration in serum from the corresponding whole blood spike concentration of drug as described previously [31].

Pharmacokinetic analyses

For each subject, AUC values (bound plus unbound) for each ketoprofen enantiomer were calculated using the linear trapezoidal rule with extrapolation to infinity. The extrapolated area was determined by dividing the final plasma concentration (interpolated from log-linear regression analysis) by the terminal rate constant, and in all cases this area was less than 15% of the total area. The terminal slope, determined by log-linear regression of at least the final five data points, was used to calculate the terminal rate constant and half-life (*t*_{1/2}) for each enantiomer.

The AUC values for unbound (R)- and (S)-ketoprofen (AUC_u(R) and AUC_u(S)) were calculated by multiplying the unbound fraction of drug enantiomer in plasma by the respective AUC for total enantiomer. Values of *fu*(R) and *fu*(S) were calculated for each subject as the arithmetic mean of five individual unbound fraction estimates from pre-dose plasma with 2.00, 3.25, 4.50, 7.00 and 12.0 µg ml⁻¹ of rac-ketoprofen added.

Statistical methods

Correlations between subject characteristics (creatinine clearance and serum albumin) and various pharmacokinetic and pharmacodynamic parameters were investigated using the Spearman rank-order correlation coefficient, or where appropriate, linear regression. Other data sets were compared statistically by analysis of variance or the Student's paired *t*-test, as appropriate, with differences considered significant at *P* < 0.05 for 2-tailed distributions. Unless otherwise indicated, grouped data are expressed as mean ± s.d.

Results

Plasma protein binding of (R)- and (S)-ketoprofen

Figure 1 shows the mean ± s.d. percentages unbound of (R)- and (S)-ketoprofen in plasma from 15 subjects. Values for (S)-ketoprofen exceeded those for the (R)-enantiomer at all but the lowest concentration (*P* < 0.05, Fisher's least significant difference (LSD) test). The percentage unbound of (R)-ketoprofen was constant across the concentration range examined but that of (S)-ketoprofen was significantly greater (*P* < 0.05, Fisher's LSD test) at an enantiomer concentration of 6.00 µg ml⁻¹ than at 3.50 µg ml⁻¹ and below. The arithmetic mean percentage unbound values (Table 2) of (R)- and (S)-ketoprofen were 0.724 ± 0.149% and 0.801 ± 0.194%, respectively (*P* < 0.0002, paired *t*-test). There were no correlations (*P* > 0.05) between the percentage unbound of either enantiomer of ketoprofen and either creatinine clearance (CL_{CR}) or serum albumin concentration.

Disposition of ketoprofen enantiomers

Plasma concentrations of total (bound plus unbound) (R)- and (S)-ketoprofen in two subjects are shown in Figure 2. Pharmacokinetic parameters are listed for individual subjects in Table 2. Blood collections were incomplete for one subject (9) owing to poor patency of the intravenous cannula.

Significant associations were observed between the reciprocal of AUC_u(S) and CL_{CR} (*r_s* = 0.761, *P* < 0.005) and between the reciprocal of AUC_u(R) and CL_{CR} (*r_s* = 0.647, *P* < 0.02) (Figure 3). In addition, there were significant correlations between the reciprocal of AUC(S) and CL_{CR} (*r_s* = 0.783, *P* < 0.002) and between the reciprocal of AUC(R) and CL_{CR} (*r_s* = 0.594, *P* < 0.05). These relationships were also highly significant (*P* < 0.0005) when assessed by linear regression. Negative correlations of statistical significance were also observed between the half-lives of (R)- and (S)-ketoprofen and CL_{CR} (*r_s* = -0.805, *P* < 0.001 and *r_s* = -0.625, *P* < 0.02; respectively). There were no correlations (*P* > 0.05) between the AUC(R):AUC(S) ratios for either unbound or total drug and CL_{CR}. A significant positive correlation was observed between

Figure 1 Mean (± s.d.) percentage of unbound (R)-ketoprofen (○) and (S)-ketoprofen (●) in plasma from the 15 patients after addition of rac-ketoprofen *in vitro*. To improve clarity the data have been displaced horizontally.

Table 2 Individual pharmacokinetic parameters for (R)- and (S)-ketoprofen

Patient	fu(R) (%)	fu(S) (%)	AUC(R) (mg l ⁻¹ h)	AUC(S) (mg l ⁻¹ h)	AUCu(R) (μg l ⁻¹ h)	AUCu(S) (μg l ⁻¹ h)	t _{1/2} (R) (h)	t _{1/2} (S) (h)	Ae(R) (mg)	Ae(S) (mg)
1	0.890	1.01	19.2	19.9	171	201	2.66	3.82	13.5	23.9
2	0.990	1.17	8.98	10.4	88.9	122	2.55	3.20	5.80	13.3
3	0.830	0.870	11.6	11.4	93.1	99.2	2.23	2.95	9.23	17.5
4	0.752	0.747	19.8	17.3	149	129	2.34	2.74	23.2	31.7
5	0.697	0.755	14.0	15.1	97.6	114	1.87	2.33	27.5	35.1
6	0.526	0.556	15.3	13.3	80.5	73.9	1.88	1.63	23.1	30.6
7	0.528	0.656	10.4	8.74	54.9	57.3	1.83	1.76	17.1	23.2
8	0.693	0.728	10.2	9.64	70.7	70.2	1.41	1.48	29.6	36.3
9	0.738	0.766	*	*	*	*	*	*	14.9	24.8
10	0.625	0.683	12.4	10.6	77.5	72.4	4.14	4.23	23.0	32.5
11	0.760	0.885	9.35	9.99	71.1	88.4	1.77	2.48	24.9	33.7
12	0.618	0.660	15.5	14.0	95.8	92.4	1.42	1.47	25.4	33.4
13	1.01	1.21	11.2	9.31	113	113	1.69	2.58	24.1	30.0
14	0.598	0.630	8.45	7.61	50.5	47.9	1.64	1.41	25.0	34.0
15	0.639	0.686	4.82	4.88	30.8	33.5	1.57	1.89	20.7	29.5

*This patient withdrew from the blood sampling part of the study 3 h after receiving the ketoprofen dose and insufficient data points were obtained to define the terminal portion of the semi-logarithmic plasma drug concentration-time profile. Urine collections were continued for 24 h.

Figure 2 Plasma concentrations of total (bound plus unbound) (R)-ketoprofen (○) and (S)-ketoprofen (●) in two patients (panel a and b refer to patients 3 and 13, respectively) following oral ingestion of single 100 mg doses of rac-ketoprofen.

the 24 h urinary recovery of (R)-ketoprofen ($Ae(R)$) and CL_{CR} ($r_s = 0.554$, $P < 0.05$) but not between $Ae(S)$ and CL_{CR} ($r_s = 0.481$, $0.05 < P < 0.10$). Significant differences (paired t -test) were found between enantiomers for $t_{1/2}$ ($P < 0.01$) and Ae ($P < 0.0001$). However, there were no differences in AUC for either total or unbound drug.

Pharmacodynamics of (S)-ketoprofen

The relationship between the percentage inhibition of TXB_2 generation and serum unbound (S)-ketoprofen

Figure 3 Relationship between the reciprocal of a) $AUCu(S)$ and b) $AUCu(R)$ and CL_{CR} .

concentration is shown for two representative subjects in Figure 4. The effect of pharmacologically active unbound (S)-ketoprofen, added as rac-ketoprofen *in vitro*, on platelet TXB_2 generation during the controlled clotting of whole blood is presented for individual subjects in Table 3. These data are presented as sigmoidal E_{max} model parameter estimates. The data from one subject (13) were not included since they were not amenable to modelling.

No correlation was found between the concentration of unbound (S)-ketoprofen required to cause a 50% inhibition of TXB_2 generation (EC_{50}) and CL_{CR} ($r_s = -0.129$, $P > 0.05$). The mean $\pm$ s.d. EC_{50} for the 14 subjects examined was 0.216 ± 0.143 ng ml⁻¹ and the corresponding slope (n) for the concentration-effect relationship was 2.26 ± 1.97 .

Figure 4 Relationships between the percentage inhibition of TXB₂ generation and the concentration of unbound (S)-ketoprofen in serum from two representative patients (panel a and b refer to patients 3 and 14, respectively). The symbols (•) represent experimental data and the line is the sigmoidal E_{max} model-predicted relationship.

Table 3 Pharmacodynamic parameters ($\pm$ s.e. of the estimates) derived from sigmoidal E_{max} modelling of individual subject data together with the experimentally derived blood to serum concentration ratio of (S)-ketoprofen used (together with *fu*(S)) for calculating corresponding unbound serum drug concentrations after adding racemic drug to whole blood

Patient	$C_b(S)/C_s(S)$	EC_{50} (ng ml ⁻¹)	n
1	0.554	0.254 $\pm$ 0.030	2.99 $\pm$ 0.41
2	0.742	0.044 $\pm$ 0.007	0.91 $\pm$ 0.17
3	0.622	0.484 $\pm$ 0.072	2.61 $\pm$ 0.43
4	0.695	0.243 $\pm$ 0.009	8.25 $\pm$ 1.99
5	0.766	0.113 $\pm$ 0.022	0.67 $\pm$ 0.11
6	0.670	0.142 $\pm$ 0.030	1.64 $\pm$ 0.32
7	0.626	0.218 $\pm$ 0.066	0.79 $\pm$ 0.20
8	0.588	0.117 $\pm$ 0.024	3.72 $\pm$ 1.12
9	0.783	0.355 $\pm$ 0.090	1.96 $\pm$ 0.53
10	0.769	0.138 $\pm$ 0.012	1.02 $\pm$ 0.08
11	0.644	0.508 $\pm$ 0.087	2.50 $\pm$ 0.45
12	0.654	0.084 $\pm$ 0.007	0.87 $\pm$ 0.08
13	0.773	*	*
14	0.618	0.189 $\pm$ 0.053	2.52 $\pm$ 0.72
15	0.769	0.135 $\pm$ 0.021	1.21 $\pm$ 0.19
Mean	0.685	0.216	2.26
s.d.	0.077	0.143	1.97

*Data from this subject were not amenable to modelling.

Discussion

Given the concentration-dependent plasma protein binding of (S)-ketoprofen in our patients at some total drug concentrations achieved within the first hour of dosing, it would have been desirable to estimate *fu*(S) and AUC_u(S) from serial *ex vivo* samples. However, this was not possible given the presence of ketoprofen

glucuronides in post-dose plasma [30]. While these metabolites do not directly influence the protein binding of the parent enantiomers [29], they are susceptible to deconjugation under the handling conditions (physiological pH and temperature) necessary to measure the unbound species [27, 29]. Furthermore, we have observed stereoselective hydrolysis of ketoprofen conjugates in plasma under physiological conditions [27], thereby spuriously altering the (R):(S) aglycone ratio (in addition to increasing the parent drug concentration) in unquenched *ex vivo* samples. Thus, the calculated AUC_u(S) values may differ slightly from true values. However, the magnitude of this discrepancy would be expected to be small based on a mean 11% increase in *fu*(S) at 6.00 μ g ml⁻¹ of (S)-ketoprofen vs the corresponding value at 3.50 μ g ml⁻¹ (Figure 1) and the fact that *fu*(S) was invariant at most *ex vivo* plasma enantiomer concentrations.

A greater percentage unbound of (S)-ketoprofen in plasma relative to the (R)-isomer in our elderly patients contrasts with a lack of enantioselective plasma protein binding noted previously in healthy young volunteers [29]. Moreover, in those young subjects (none of whom was taking any medication), concentration-dependent binding of (S)-ketoprofen was evident only at a supra-therapeutic enantiomer concentration of 19.0 μ g ml⁻¹. The magnitude of the percentage unbound values are similar in both groups of subjects. Recent studies with flurbiprofen [24, 32] have shown a decrease in plasma protein binding of both enantiomers in uraemic patients, compared with normal subjects, which was attributed to a significantly lower plasma albumin concentration in the former. Some of our patients (none of whom was clinically uraemic) had serum albumin concentration values below the normal range. However, we did not observe any correlation between the percentage unbound of each enantiomer and either serum albumin or CL_{CR}. An unavoidable complicating factor in this and most other studies examining protein binding in elderly patients with rheumatoid arthritis is the potential effects of concurrent medication. Perhaps this, together with reported alterations to binding of acidic drugs in patients with reduced renal function and accumulated endogenous anions [33] and the low serum albumin concentrations in this elderly patient group, might explain the appearance of non-linear protein binding of (S)-ketoprofen at lower total concentrations than observed in healthy young subjects.

For ketoprofen, which is cleared almost exclusively by metabolism [7], the AUC (bound plus free) after a single oral dose (*D*) is determined by the fraction of the administered dose absorbed from the gastrointestinal tract (*f_a*), the fraction of drug unbound in plasma, and the intrinsic clearance of unbound drug from plasma (CL_{u_{int}}). For each enantiomer, given the minimal chiral inversion of this 2-arylpropanoate in man,

$$AUC = \frac{f_a \cdot D}{fu \cdot CL_{u_{int}}} \quad (1)$$

The corresponding relationship for the unbound species of each enantiomer simplifies to:

$$AUC_u = \frac{f_a \cdot D}{CL_{u_{int}}} \quad (2)$$

Equation (2) predicts that disease-induced changes to AUC_u would result from alterations to either oral absorption or intrinsic drug clearance or both and are independent of changes to the fraction unbound in plasma.

The data shown in Figure 3 demonstrate that although a negligible amount of ketoprofen is cleared renally as unchanged drug [7] the AUCs of both unbound (R)- and unbound (S)-ketoprofen were inversely related to renal function. While the potential confounding influence of co-medication is recognised, these findings support the mechanism of accumulation of renally eliminated, labile acyl-glucuronides and their subsequent systemic hydrolysis back to the parent aglycone, demonstrated by Meffin *et al.* [21, 22] for clofibrate. This was described as a 'futile cycle' and was proposed originally to account for the lower clearance of diflunisal in patients with impaired renal function [34]. In the present case, declining renal function induces an apparent reduction in net CL_{u,int} (i.e. the resultant of conjugative and other clearances of ketoprofen enantiomers and their regeneration by hydrolysis of the unstable glucuronconjugates) and hence an increase in AUC_u (equation(2)). Sallustio *et al.* [35] were unable to establish a relationship between AUC, in terms of total ketoprofen enantiomers at steady state, and CL_{CR} in an elderly cohort of patients with rheumatoid arthritis upon multiple dosing with a sustained-release formulation of the drug. In the absence of protein binding data for those patients it is possible that changes in *fu* offset alterations in net CL_{u,int}, thereby masking a relationship between renal function and AUC. In the present study, both total and unbound AUC (for each enantiomer) were inversely correlated with CL_{CR}. Furthermore, in the presence of the 'futile cycle', renal dysfunction should cause greater elevations in AUC_u for those drugs (such as ketoprofen) cleared predominantly by acyl-glucuronidation relative to drugs subject to significant phase I (non-conjugative) metabolism. This might explain why Knadler and associates [24] were unable to demonstrate 'futile cycling' based upon measurement of unbound flurbiprofen enantiomers in uraemic patients, since elimination of this drug proceeds by substantial oxidative metabolic pathways in addition to glucuronide conjugation.

An alternative explanation of the relationships shown in Figure 3 might be an increase in oral absorption as a function of degree of renal impairment. However, this seems unlikely since the oral bioavailability of ketoprofen in healthy subjects has been reported to be close to unity [36]. Indeed, the urinary recovery of (R)-ketoprofen, as combined aglycone and conjugate, decreased with diminishing renal function and if this is indicative of reduced (rather than increased) oral absorption, then the relationships between AUC_u and CL_{CR} would have been dampened by changes to *f_a*. Hence these data (Figure 3) could be more compelling in support of the conjugate recycling postulate. Previously, it has been suggested that decreased systemic clearance of ketoprofen in elderly subjects is due to a diminished capacity to conjugate the aglycone with glucuronic acid [9]. While declining renal function might be associated with a synchronous impairment of liver function, studies of a number of drugs (forming stable ether glucuronides) administered to patients with renal failure have shown

normal glucuronidation capacity [37].

Consistent with the 'futile cycle' hypothesis, we observed a reduction in the urinary recovery of combined (conjugated and non-conjugated) (R)-ketoprofen as renal function decreased (the correlation between the urinary recovery of (S)-ketoprofen and CL_{CR} just failed to reach statistical significance). Urine collections were not extended beyond 24 h after dosing since most of the aglycone and metabolites recovered were found in the initial 0–5 h aliquot with only 14% in the remainder (mean result from the five patients with the lowest CL_{CR}). In addition, a previous study examining the disposition of tritiated ketoprofen in elderly subjects found negligible urinary excretion of radioactivity after 24 h [38], and a study of ketoprofen in patients with chronic renal failure also demonstrated negligible excretion of drug aglycone or conjugate beyond 24 h [8]. This and other studies [30, 35], together with our data show considerably less urinary recovery of unconjugated and conjugated ketoprofen in the elderly compared with young healthy subjects [7, 39]. Biliary excretion of ketoprofen and its acyl-glucuronide has been ruled out as a significant route of elimination in man [16]. It is possible that hydroxylation, as reported in early studies with ketoprofen [40], may be of greater importance in patients with diminished renal function.

While metabolic chiral inversion of the (R)- to the (S)-enantiomer has been demonstrated for ketoprofen in man [41], the extent of this inversion was reported to be small (9–12% in healthy subjects). Obviously, the (R):(S) AUC ratio is not a measure of the absolute extent of chiral inversion. However, based on the 'futile cycle' model proposed for a chiral structural homologue of ketoprofen, 2-phenylpropanoic acid [23], a renally-induced net decrease in clearance of the (R)-enantiomer would disproportionately increase the plasma concentrations of its optical antipode due to increased fractional inversion. The extent of this will depend on the degree of chiral inversion of the 2-arylpropanoate. In the absence of a direct effect of renal function on the intrinsic inversion process and given the minor role of inversion for ketoprofen in man, it is not surprising that we are unable to detect any relationship between the AUC(R):AUC(S) ratio for either total or unbound ketoprofen and CL_{CR}.

The unbound and total AUC estimates for (R)- and (S)-ketoprofen (Table 2) show a lack of enantioselectivity in terms of the plasma concentration-time course of this drug. Our dose-normalised AUC values for ketoprofen enantiomers were similar to those observed in elderly patients with arthritis as were the half-life estimates for (R)- and (S)-ketoprofen [30]. The higher *Ae*(S) relative to *Ae*(R) values (Table 2) has also been noted by others [30, 35, 39]. We have observed rapid, stereoselective hydrolysis of ketoprofen glucuronides in human plasma *in vitro* [27]. Thus, the faster deconjugation of (R)-ketoprofen glucuronide, prior to its urinary excretion, may be reflected in the higher urinary recovery of (S)-ketoprofen observed in this study. Spahn *et al.* [42] have noted that stereoselective degradation of acyl-glucuronide conjugates could be misinterpreted as a difference in glucuronide formation rates.

With respect to the *in vitro* inhibition of platelet cyclooxygenase by unbound (S)-ketoprofen, it appeared that renal function was not a determinant of this effect, based

on the estimated concentration of pharmacologically active moiety required to inhibit the production of platelet TXA₂ by 50%. Moreover, the mean value of EC₅₀ was similar (although discrete estimates were subject to greater inter-individual variability) to values obtained in young healthy subjects [31]. It is also apparent, based on estimates of (S)-ketoprofen concentrations achieved during chronic dosing of racemic drug [39] and applying our present and previous [31] *in vitro* data, that the thromboxane-related antiplatelet effects of ketoprofen should persist throughout the dosing interval.

In conclusion, exposure to active unbound (S)-ketoprofen is increased and the corresponding elimination

half-life is prolonged in patients with decreased renal function. Dosage adjustment may be warranted in these patients.

This study was supported by a research grant from the Central Health and Medical Research Council of the Department of Veteran Affairs of Australia. The authors are indebted to the patients and the ward 20 staff at the Repatriation General Hospital, Daw Park. We would also like to thank Ms Elizabeth Duncan (Department of Haematology, Institute of Medical and Veterinary Science, Adelaide, South Australia) for performing the serum TXB₂ assays and Miss Donna Lapins for her excellent secretarial assistance.

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(Received 22 December 1992,
accepted 11 May 1993)