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Effect of racemic ibuprofen dose on the magnitude and duration of platelet cyclo-oxygenase inhibition: relationship between inhibition of thromboxane production and the plasma unbound concentration of S(+)-ibuprofen

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1 Four healthy male subjects received racemic ibuprofen (200, 400, 800 and 1200 mg), orally, on four occasions, 2 weeks apart, according to a four-way Latin-square design, in order to investigate the influence of increasing dose of ibuprofen on the magnitude and duration of its antiplatelet effect as well as on the relationship between such effect and drug concentration.

2 The antiplatelet effect of ibuprofen was assessed by measuring the inhibition of platelet thromboxane B_2 (TXB_2) generation during the controlled clotting of whole blood. The plasma unbound concentration of S(+)-ibuprofen, the enantiomer shown in an *in vitro* study to be responsible for the inhibitory effect of platelet TXB_2 generation, was measured using an enantioselective method.

3 The maximum percentage inhibition of TXB_2 generation increased significantly with dose from a mean ± s.d. of 93.4 ± 1.2% after the 200 mg dose to 98.8 ± 0.3% after the 1200 mg dose, and there was an increase with dose in the duration of inhibition of TXB_2 generation. The effect of ibuprofen on platelet TXB_2 generation was transient and mirrored the time-course of unbound S(+)-ibuprofen in plasma; on all but one of the 16 occasions, serum TXB_2 concentrations returned to at least within 10% of the pretreatment concentrations within 24 h of ibuprofen administration.

4 For each subject, the relationship between the percentage inhibition of TXB_2 generation and the unbound concentration of S(+)-ibuprofen in plasma was modelled according to a sigmoidal E_{max} equation. The mean plasma unbound concentration of S(+)-ibuprofen required to inhibit platelet TXB_2 generation by 50% (EC_{50}) was 9.8 ± 1.0 $\mu g\ l^{-1}$.

5 The results indicate that the intensity and duration of the antiplatelet effect of ibuprofen were dose-dependent. The antiplatelet effect was related to the plasma unbound concentration of S(+)-ibuprofen and the small degree of interindividual variability in the EC_{50} values suggests similar sensitivity among the subjects of platelet cyclo-oxygenase to the inhibitory action of S(+)-ibuprofen.

Keywords ibuprofen thromboxane B_2 cyclo-oxygenase

Introduction

Ibuprofen is a chiral non-steroidal anti-inflammatory and analgesic agent which is used clinically in its racemic form in the treatment of rheumatoid arthritis, osteoarthritis and a number of other painful conditions. The desired therapeutic effects of ibuprofen may be the result of its ability to decrease the bio-synthesis of prostaglandins by inhibiting the enzyme cyclo-oxygenase (Day, 1988), an effect which resides almost exclusively

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with the S enantiomer of ibuprofen (Adams *et al.*, 1976).

It is well documented that ibuprofen inhibits the aggregation of platelets in response to a number of stimuli, including adrenaline, arachidonic acid, collagen and thrombin (Brooks *et al.*, 1973; Cox *et al.*, 1987; Cronberg *et al.*, 1984; Ikeda, 1977; Longenecker *et al.*, 1985; McIntyre *et al.*, 1978; O'Brien, 1968; Parks *et al.*, 1981). This anti-platelet activity is thought to be related to the ability of ibuprofen to inhibit platelet cyclo-oxygenase thereby decreasing the synthesis of thromboxane A_2 (TXA_2), a vasoconstrictor and powerful promoter of platelet aggregation. TXA_2 is chemically unstable in biological fluids and is rapidly converted to its stable breakdown product thromboxane B_2 (TXB_2) (Hamberg *et al.*, 1975; Smith *et al.*, 1976).

There are a number of reports on the effect of ibuprofen dosing in humans on platelet aggregation, TXB_2 generation or both (Brooks *et al.*, 1973; Cox *et al.*, 1987; Cronberg *et al.*, 1984; Ikeda, 1977; Longenecker *et al.*, 1985; McIntyre *et al.*, 1978; O'Brien, 1968), and in some of these studies the time-course of the anti-platelet effect of the drug has been examined. Longenecker *et al.* (1985) administered a range of single oral doses of racemic ibuprofen (8, 10, 12 and 14 mg kg^{-1}) to healthy subjects and measured TXB_2 generation, in response to whole blood clotting, prior to, and 2, 4 and 6 h after, drug administration, and again after 7 days. Inhibition of TXB_2 generation did not differ between doses, persisted for at least 6 h, and concentrations of TXB_2 returned to pre-treatment values within 7 days. Cronberg *et al.* (1984) reported that ibuprofen inhibited platelet aggregation at 1.5, 3 and 6 h after a single 800 mg oral dose of racemic ibuprofen, but not after 24 h. Similarly, ibuprofen in single oral doses in the range 300–900 mg inhibited platelet aggregation 2 h after administration and this effect disappeared usually within 24 h (Ikeda, 1977; McIntyre *et al.*, 1978). These studies provide no information on the anti-platelet effects of the drug between 6 and 24 h after administration.

Recently, Cox *et al.* (1987) reported on a comprehensive investigation into the relationship between TXB_2 generation, platelet aggregability, and concentrations of ibuprofen. Four healthy subjects were given 200, 400, and 800 mg of ibuprofen, and the serum concentrations of the drug were measured for up to 16 h post-dose. Platelet aggregation studies were performed both prior to, and at selected times after, ibuprofen administration. Platelet aggregability and platelet TXB_2 generation were found to be closely related processes, with plasma TXB_2 concentrations of 40 $\mu g\ l^{-1}$ being necessary for platelet aggregation to occur. The minimum concentration of (unresolved) ibuprofen needed to inhibit platelet aggregation was estimated to be 3 mg l^{-1}. Below this concentration, platelet aggregation proceeded, but the time which elapsed between *in vitro* addition of the aggregatory stimulant (arachidonic acid) and the onset of aggregation was increased. After the 200 mg dose of ibuprofen, platelet aggregation was inhibited for a mean ($\pm$ s.d.) of 6 $\pm$ 2 h, and after the 400 mg and 800 mg doses, the corresponding periods of inhibition were 8 $\pm$ 2 h and 11 $\pm$ 2 h, respectively. An elaborate model was used by Cox *et al.* (1987) to relate platelet aggregation parameters to serum ibuprofen concentration. However, serum ibuprofen concentrations were measured as total (bound plus unbound) unresolved drug, and the model assumed that the unbound concentration of S(+)-ibuprofen was a constant fraction of total unresolved ibuprofen (Cox *et al.*, 1987). Because of the enantioselective disposition of ibuprofen (Evans *et al.*, 1989a, 1990; Lee *et al.*, 1985) and the concentration-dependent plasma protein binding of ibuprofen enantiomers (Evans *et al.*, 1989b), such an assumption is not valid.

The present study was designed to examine the effect of a range of single oral doses of racemic ibuprofen on the time-course of inhibition of platelet TXA_2 synthesis. In addition, it was intended to study the relationship between the degree of inhibition of TXA_2 synthesis and the unbound plasma concentration of S(+)-ibuprofen, the enantiomer shown to possess the inhibitory activity. The pharmacokinetic aspects of the study, including the relationship between the pharmacokinetics of ibuprofen enantiomers and ibuprofen dose, have been reported previously (Evans *et al.*, 1990).

Methods

Subjects and study design

Details of the subjects and study design have been described (Evans *et al.*, 1990). Briefly, four healthy non-smoking men (ranges: 23–28 years; 65–78 kg) each received 200, 400, 800 and 1200 mg of racemic ibuprofen, orally, on four separate occasions (2 weeks apart) according to a four-way Latin-square design. Blood samples (10 ml) were collected from an arm vein immediately prior to (two samples collected), and at various times up to 48 h after (17 samples collected), ibuprofen dosing. One portion of each blood sample was placed into a heparinised tube from which plasma was harvested for the measurement of ibuprofen enantiomers; another aliquot was subjected to controlled clotting for measurement of serum TXB_2 concentration. The subjects were instructed to abstain from all other drugs during the study. The study protocol was approved by the Ethics Committees of the Royal Adelaide Hospital and the University of Adelaide.

Drug analyses in biological fluids

All plasma samples were analysed for total (bound plus unbound) concentrations of R(−)-ibuprofen (R-I) and S(+)-ibuprofen (S-I), as reported previously (Evans *et al.*, 1990), using an enantioselective assay (Evans *et al.*, 1989a). For each subject, the unbound concentrations of R-I and S-I were measured in six plasma samples collected after each dose (Evans *et al.*, 1990) using a previously reported method (Evans *et al.*, 1989b). The six samples were chosen on the basis that they provided accurate representations of the plasma concentration-time profiles of total R-I and total S-I.

Assessment of platelet cyclo-oxygenase inhibition

To monitor the effects of ibuprofen dosing on platelet cyclo-oxygenase, whole blood was allowed to clot under controlled conditions. The amount of TXA_2 generated

during clotting (an index of cyclo-oxygenase activity) was assessed by measuring the concentration of its stable breakdown product, TXB_2, in the harvested serum. The method was based on that described by Patrono *et al.* (1980). Immediately after collection of each blood sample, a 1 ml aliquot was placed into a pre-calibrated Pyrex® borosilicate culture tube (12 mm × 75 mm) which had been pre-warmed to 37° C in a thermostatically-controlled metal heating block. The tube was returned to the heating block and maintained at 37° C for 1 h (the generation of TXB_2 during the clotting of whole blood is a time- and temperature-dependent process; and at 37° C, constant concentrations are achieved within 15 min; Patrono *et al.*, 1980). After the 1 h incubation, the tube was rimmed with a wooden spatula to detach any unretracted portions of the clot. After centrifugation at 1000 *g* for 15 min, a 200 μl aliquot of serum was stored at −18° C until analysed for TXB_2 using the radioimmunoassay method of Fitzpatrick (1982). For each dose, for each subject, ten serum samples were selected for the analysis of TXB_2. These ten samples comprised: two pre-dose samples; the six serum samples corresponding to those plasma samples which had been analysed for the unbound concentrations of R-I and S-I; and the 24 and 48 h serum samples. Each serum sample was assayed for TXB_2 in two replicates and the values averaged, unless they differed by more than 20%, in which case, the serum sample was re-analysed.

Pharmacodynamic analysis of TXB_2 data

For each subject, the pre-dose serum concentration of TXB_2 in each phase was determined by averaging the concentration of TXB_2 determined in the two independent pre-dose serum samples. For samples obtained after ibuprofen administration, inhibition of TXB_2 production during whole blood clotting was calculated as the percentage decrease in the serum concentration of TXB_2, relative to the pre-dose concentration.

The possible existence of a time lag between the observed concentration of unbound S-I in plasma and the magnitude of inhibition of platelet cyclo-oxygenase was investigated by performing hysteresis analysis. This involved plotting the percentage decrease in serum TXB_2 concentration against plasma unbound S-I, according to the time sequence in which the samples were collected (Holford & Sheiner, 1982).

For each subject, data from all four doses were pooled to examine the relationship between the unbound concentration of S-I in plasma and the percentage inhibition of TXB_2 generation. This was achieved by fitting a standard sigmoidal E_{max} equation to the data with an extended least squares modelling computer program (MK Model, Elsevier-Biosoft®, United Kingdom). The sigmoidal E_{max} equation (Hill equation) is

$$E = \frac{E_{max}\, C^n}{EC_{50}{}^n + C^n} + E_0 \qquad (1)$$

where E is the measured effect at drug concentration C; E_0 is the basal effect in the absence of drug (i.e. when $C = 0$); E_{max} is the maximum effect; EC_{50} represents the concentration of drug required to cause 50% of E_{max}; and n is a steepness factor for the log concentration-effect relationship (Colburn & Brazzell, 1986). In the present case, for each subject in each phase of the study, E was expressed as a percentage of the maximal possible effect of the drug, and hence E_0 was set at zero and E_{max} at 100, thereby simplifying equation 1 to

$$\% \text{ inhibition} = \frac{100\,(C_u{}^{S\text{-}I})^n}{EC_{50}{}^n + (C_u{}^{S\text{-}I})^n} \qquad (2)$$

where $C_u{}^{S\text{-}I}$ is the unbound plasma concentration of S-I. Equation 2 was applied for each subject after pooling data from all four doses.

The computer modelling program was used to obtain an estimate of EC_{50} and n for each subject. These data were then used to calculate each subject's EC_{80} (the unbound concentration of S-I required to inhibit TXB_2 generation by 80%).

For each subject at each dose, the maximum observed percentage inhibition of serum TXB_2 was determined directly from the experimental data. Indices of the duration of TXB_2 inhibition were obtained by determining the time at which the plasma unbound concentration of S-I fell below the determined EC_{80} and EC_{50} values. For each subject, these time values were calculated from the log-linear regression of the terminal portion of the plasma unbound S-I *vs* time profiles, using that subject's EC_{50} and EC_{80} values. In all cases, the EC_{80} and EC_{50} values were below the concentration at which the log-linear regression was initiated.

In vitro *determination of the influence of R(−)-ibuprofen, S(+)-ibuprofen and RS-ibuprofen on TXB_2 generation during whole blood clotting*

A series of calibrated Pyrex® tubes, identical to those used for the ibuprofen dosing study above, containing a range of quantities of R-I and/or S-I were prepared. Two tubes containing no R-I or S-I served as controls. The tubes were placed into a heating block set at 37° C. Blood (20 ml) was collected by venepuncture from an arm vein of subject 1. With the needle removed, 1 ml of blood was promptly transferred from the syringe into each tube. The final blood concentrations of R-I, S-I and racemic ibuprofen (RS-I) in each tube were as follows: control samples, containing no R-I or S-I; R-I at 1, 2, 5, 10 and 20 mg l^{-1}; S-I at 0.5, 1, 2, 5 and 10 mg l^{-1}; and RS-I at 1, 2, 5, 10 and 20 mg l^{-1}. After gentle mixing, each tube was incubated at 37° C for 1 h, and the serum concentrations of TXB_2 were measured, as described above. The percent inhibition of TXB_2 generation, in tubes containing ibuprofen, was taken to be the percentage decrease in the serum concentration of TXB_2 relative to the mean of the control samples.

Statistical analysis

All data are presented as arithmetic mean ± s.d. In the ibuprofen dose-ranging study, analysis of variance was used to test for changes with dose in the time taken for the plasma concentration of unbound S-I to fall below EC_{80} and EC_{50}. The *a priori* level of significance was 0.05. If a difference between the four doses was detected,

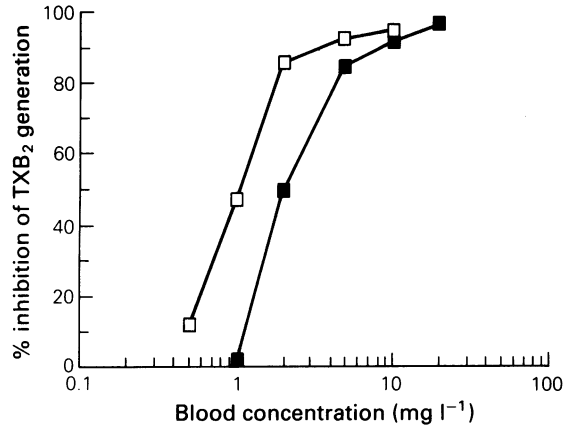

Figure 1 *In vitro* relationship between the percentage inhibition of TXB_2 generation during the controlled clotting of whole blood and log blood concentration of S-ibuprofen (□), and racemic ibuprofen (■).

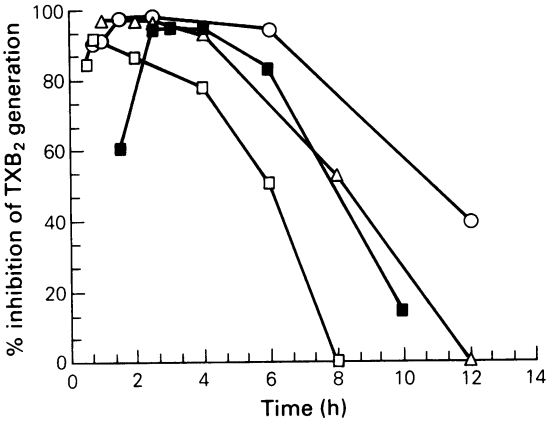

Figure 2 Percentage inhibition of TXB_2 generation *vs* time after oral administration of racemic ibuprofen, in doses of 200 mg (□), 400 mg (■), 800 mg (△) and 1200 mg (○), in subject number 1.

Fisher's least significant difference test was used to test for differences between the individual doses. Because of the discrete limit of 100% for the maximum observed inhibition of TXB_2, a non-parametric statistic (Kruskal-Wallis) was used to test for dose dependency of this parameter.

Results

The effect of S-I and RS-I, added *in vitro*, on TXB_2 generation during the controlled clotting of whole blood, is presented in Figure 1. Serum TXB_2 concentrations were unaffected by R-I over the range of blood concentrations examined (1 to 20 mg l^{-1}); the mean concentration of TXB_2 in the five samples containing R-I was 170 ± 12 μg l^{-1}, compared with 156 μg l^{-1} for the control sample. In contrast, S-I reduced serum TXB_2 in a concentration-dependent manner. When the effect of S-I on TXB_2 generation was modelled, according to a sigmoidal E_{max} equation, the blood concentration of S-I leading to a 50% inhibition of TXB_2 generation was found to be 1.1 mg l^{-1}. When the modelling was performed for racemic ibuprofen, a concentration of 2.2 mg l^{-1} (1.1 mg l^{-1} of each enantiomer) corresponded to a 50% inhibition. For both S-I and RS-I, the slope factor of the log concentration-effect curve (n) was 1.6.

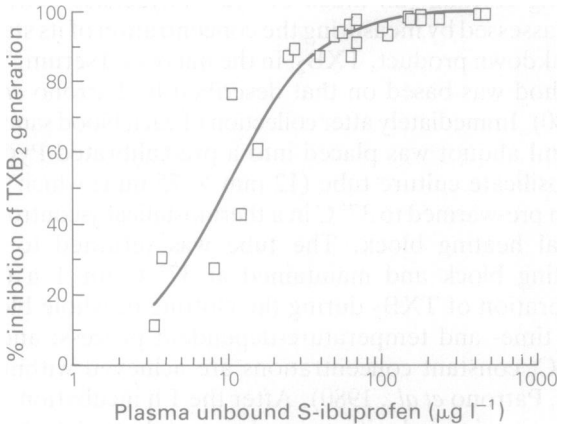

Figure 3 Relationship between the percentage inhibition of TXB_2 generation and log plasma concentration of unbound S-ibuprofen, for subject number 2. The symbols are actual data points, and the line represents the predicted relationship, according to a sigmoidal E_{max} model, from the computer-generated analysis.

Table 1 Computer-generated parameters (EC_{50}, EC_{80} and n) describing the relationship between the unbound plasma concentration of S-I and the percentage inhibition of TXB_2 generation

Subject	EC_{50} (μg l^{-1})	EC_{80} (μg l^{-1})	n
1	10.3	27.0	1.44
2	10.1	25.7	1.48
3	10.3	30.7	1.27
4	8.3	30.8	1.06
Mean	9.8	28.6	1.31
s.d.	1.0	2.6	0.19

In the dose-ranging study, ibuprofen was found to decrease the serum concentration of TXB_2 in all subjects at all dose levels. The time-course of the percent decrease in serum TXB_2 concentration after ibuprofen administration (up to 12 h), for each dose in a representative subject, is presented in Figure 2. The effect of ibuprofen on platelet TXA_2 synthesis was transient, and in all but one case serum TXB_2 concentrations returned to at least within 10% of the pre-treatment concentration within 24 h of ibuprofen administration. In the one exception (1200 mg dose in subject 3), the serum TXB_2 concentration was 32.5% below the pre-treatment value at 24 h but had returned to the pre-treatment level by 48 h.

The relationship between the percentage inhibition of TXB_2 generation and the log concentration of plasma unbound S-I (includes data from all four dose levels), is shown for a representative subject in Figure 3. The EC_{80}, EC_{50} and the slope factor values, determined from concentration-effect modelling of the data from each subject, are presented in Table 1. The EC_{50} was estimated with good precision in each subject with the coefficients of variation for the computer-generated individual estimates ranging from 1.20 to 2.43%.

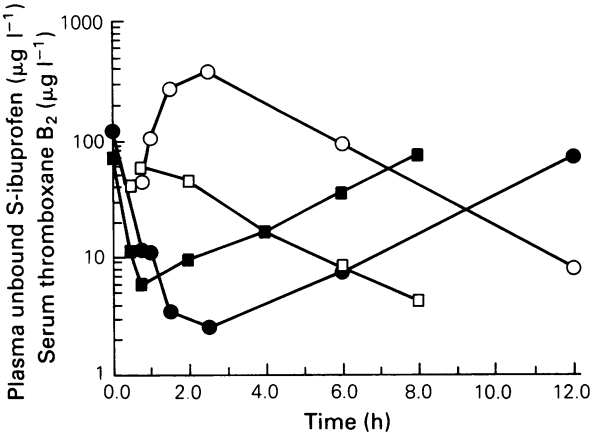

Figure 4 Log concentration-time profiles for both serum TXB_2 (closed symbols) and plasma unbound S-ibuprofen (open symbols) after oral dosing with 200 mg (□, ■) and 1200 mg (○, ●) of racemic ibuprofen, for subject number 1.

Table 2 Maximum observed percentage inhibition of TXB_2 generation *vs* ibuprofen dose

	Maximum % inhibition of TXB_2 generation			
Subject	*200 mg*	*400 mg*	*800 mg*	*1200 mg*
1	91.6	94.7	96.8	99.1
2	93.8	97.5	97.1	98.6
3	94.0	96.6	98.1	98.6
4	94.0	86.8	99.0	99.0
Mean	93.4	93.9	97.8	98.8
s.d.	1.2	4.9	1.0	0.3
Significance*	c,d	c,d	a,b	a,b

*Significantly different ($P < 0.05$) from the corresponding value at the (a) 200 mg (b) 400 mg (c) 800 mg and (d) 1200 mg dose level.

Table 3 The effect of ibuprofen dose on the time taken for the unbound plasma concentration of S-I to fall below EC_{80}

	Time for $C_u^{S\text{-}I}$ to fall below EC_{80} (h)			
Subject	*200 mg*	*400 mg*	*800 mg*	*1200 mg*
1	2.44	6.38	6.63	9.00
2	4.32	5.07	9.58	8.65
3	3.50	4.42	7.22	8.15
4	2.92	5.46	6.53	8.28
Mean	3.30	5.33	7.49	8.52
s.d.	0.81	0.82	1.43	0.38
Significance*	b,c,d	a,c,d	a,b	a,b

*Significantly different ($P < 0.05$) from the corresponding value at the (a) 200 mg (b) 400 mg (c) 800 mg and (d) 1200 mg dose level.

Table 4 The effect of ibuprofen dose on the time taken for the unbound plasma concentration of S-I to fall below EC_{50}

	Time for $C_u^{S\text{-}I}$ to fall below EC_{50} (h)			
Subject	*200 mg*	*400 mg*	*800 mg*	*1200 mg*
1	5.32	8.73	8.61	11.4
2	7.13	7.38	12.9	11.4
3	7.06	8.80	10.0	11.4
4	5.56	16.9	9.73	11.7
Mean	6.27	10.5	10.3	11.4
s.d.	0.96	4.4	1.8	0.2
Significance* (all subjects)	NS	NS	NS	NS
(excluding subject 4)	c,d	d	a	a,b

*Significantly different ($P < 0.05$) from the corresponding value at the (a) 200 mg (b) 400 mg (c) 800 mg and (d) 1200 mg dose level. NS = not significant from data at any other dose.

Inhibition of platelet TXB_2 generation consistently exceeded 50% within 90 min of ibuprofen administration (e.g. Figure 2). There was no evidence of a time lag between the appearance of unbound S-I in plasma and the decrease in serum TXB_2 concentration (Figure 4). Hysteresis analysis was not possible in most of the 16 data sets because TXB_2 generation was near maximally inhibited soon after ibuprofen administration and, as a result, there was a paucity of data during the onset of the inhibitory effect (for example, see Figure 2). Where such analysis was possible, however, negligible hysteresis was observed.

The maximum percentage inhibition of TXB_2 generation increased significantly ($P < 0.05$) with dose (Table 2) from a mean of 93.4% after the 200 mg dose, to 98.8% after the 1200 mg dose. In addition, the time at which the plasma unbound concentration of S-I fell below EC_{80} increased significantly ($P < 0.001$) with dose (Table 3). Although the time taken for unbound plasma S-I to fall below EC_{50} tended to increase with dose, the change was not statistically significant (Table 4). This was because, after the 400 mg dose for subject 4, the time taken for unbound plasma S-I to fall below EC_{50} was 16.9 h; as discussed previously (Evans *et al.*, 1990), the absorption of ibuprofen appeared to be delayed in this isolated case. When the data for subject 4 were excluded from the statistical analysis, on the basis that the data for this individual were not representative, the change in the time for unbound S-I to fall below EC_{50} was significant ($P < 0.05$; Table 4).

The time-points chosen for the measurement of plasma unbound S-I and therefore serum TXB_2, differed between subjects, and between doses. Hence, to illustrate the overall effect of dose on the average magnitude and duration of TXB_2 inhibition, it was necessary to categorise the data according to the time intervals over which they were collected (samples collected up to 1 h after dosing were included in the 0–1 h interval; those collected after 1 h, and up to 2 h, were included in the 1–2 h interval, and so on). Figure 5 depicts the mean percent inhibition of TXB_2 generation, over each of the selected time-intervals, as a function of the magnitude of ibuprofen dose.

Discussion

The effect of ibuprofen on platelet activity was monitored by measuring TXB_2 production during controlled clotting of whole blood, in which endogenous thrombin was the stimulus for TXB_2 generation. This technique has been used by many other workers to monitor the anti-platelet activity of non-steroidal anti-inflammatory agents

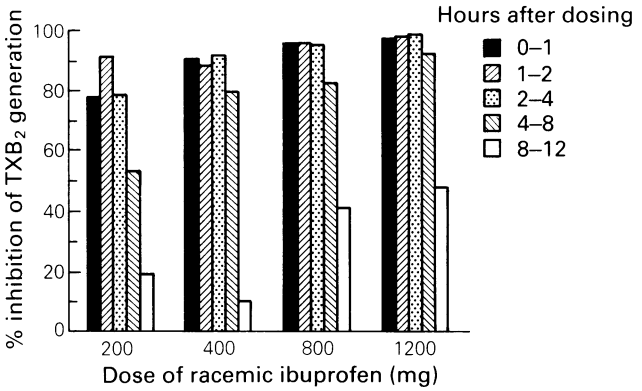

Figure 5 Mean percentage inhibition of TXB_2 generation at various times after oral dosing with a range of doses of racemic ibuprofen, using the pooled data from all four subjects.

(Cerletti *et al.*, 1987; Longenecker *et al.*, 1985; Nuotto *et al.*, 1983; Patrignani *et al.*, 1982; Patrono *et al.*, 1980).

Using a bovine seminal vesicle microsomal preparation of prostaglandin synthetase, Adams *et al.* (1976) demonstrated that S-I was about 160 times more potent than R-I as an inhibitor of this enzyme system. It was expected, therefore, that S-I would be the active enantiomer with respect to inhibition of platelet TXB_2 generation. This was confirmed by the *in vitro* experiment, in which it was shown that S-I at a blood concentration of 1.1 mg l^{-1} inhibited platelet TXB_2 generation, in response to whole blood clotting, by 50%. For racemic ibuprofen, the corresponding blood concentration was 2.2 mg l^{-1} (1.1 mg l^{-1} of each enantiomer). There was no detectable effect of R-I on TXB_2 generation. Hence the results of the *in vitro* experiment indicate firstly, that S-I is solely active at inhibiting platelet TXB_2 generation, and secondly, that the activity of S-I is not altered by the presence of its optical antipode, suggesting that the binding of S-I to the active site(s) on cyclo-oxygenase is not modified by the presence of R-I. The *in vitro* anti-platelet and/or anti-TXB_2 activity of the chiral 2-phenylpropionic acid (2-PPA) derivatives fenoprofen (Rubin *et al.*, 1985), flurbiprofen (Kulmacz & Lands, 1985) and naproxen (Kean *et al.*, 1989) were also found to reside almost exclusively with the S(+)-enantiomer. Because of the enantioselective activity of the 2-PPA derivatives, it is essential, when relating anti-platelet effects to the concentration in plasma or plasma water, that the concentrations of the S(+)-enantiomer should be monitored.

The results of the present study indicate that in healthy subjects, an average (± s.d.) of 9.8 ± 1.0 μg l^{-1} of unbound S-I was necessary to inhibit TXA_2 synthesis by 50%. The mean concentration required to inhibit TXA_2 synthesis by 80% was 28.6 ± 2.6 μg l^{-1}. While only four subjects were investigated, the small degree of inter-individual variability in the EC_{50} and EC_{80} values was notable and suggests similar sensitivity among the subjects of platelet cyclo-oxygenase to the inhibitory action of S-I. For all subjects, the unbound concentrations of S-I corresponding to EC_{50} and EC_{80} were exceeded after oral administration of 200, 400, 800 and 1200 mg of racemic ibuprofen. This suggests that the peak intensity of the inhibition of platelet cyclo-oxygenase should vary little with dose. In this study the maximum observed inhibition of TXB_2 generation did increase as ibuprofen dose increased, although the magnitude of the change with dose was small. As ibuprofen dose increased, the plasma concentrations of unbound S-I remained elevated above EC_{80} and EC_{50} for a longer time. In fact, the increase with dose in the mean time taken for plasma unbound S-I to fall below the EC_{80} was highly significant, with the mean duration increasing by about 160% between the 200 mg and 1200 mg dose levels. When the data for subject 4 were omitted, there was also a significant increase with dose in the time taken for the plasma concentration of unbound S-I to fall below EC_{50}; in this case there was a mean increase of about 75% between the 200 mg and 1200 mg doses. It would appear, therefore, that the duration of TXB_2 inhibition is influenced by the magnitude of ibuprofen dose to a greater extent than is the peak percentage inhibition of TXB_2 generation, a feature which is clearly illustrated by examining the pooled data in Figure 5.

The close temporal relationship between inhibition of TXB_2 generation and plasma unbound S-I concentration (Figure 4) is in keeping with the proximity of the biological receptor (platelet cyclo-oxygenase) to the sampling compartment (plasma water). The lack of significant hysteresis when the 'concentration-effect' data were plotted according to time sequence also suggests that platelet cyclo-oxygenase was unaffected by ibuprofen metabolites (Holford & Sheiner, 1982).

The present study confirms the findings of other workers, that the inhibition of TXA_2 synthesis after oral administration of racemic ibuprofen is relatively short-lived (Cox *et al.*, 1987; Cronberg *et al.*, 1984; Ikeda, 1977; Longenecker *et al.*, 1985; McIntyre *et al.*, 1978). The time-course of inhibition, which reflects that of unbound S-I in plasma, is consistent with the fact that the binding of ibuprofen to platelet cyclo-oxygenase is a reversible process (Ikeda, 1977). In contrast, the function of platelets which have been exposed to aspirin cannot be restored, since this drug irreversibly acetylates the active site on cyclo-oxygenase (Flower *et al.*, 1985). Since platelets are anuclear, they are unable to replace inactivated enzyme and the cyclo-oxygenase inactivation of an aspirin-exposed platelet persists for the life-span of that platelet (8 to 11 days; Flower *et al.*, 1985). This explains why, after a single dose of aspirin, anti-platelet effects can persist for up to 7 days (Nuotto *et al.*, 1983; Patrono *et al.*, 1980). Interestingly, ibuprofen, and other reversible inhibitors of cyclo-oxygenase, have been found to protect platelets against the irreversible inactivation by aspirin, possibly by blocking the access of aspirin to the active site on cyclo-oxygenase (Rao *et al.*, 1983).

In view of the sensitivity of platelet cyclo-oxygenase to S-I as demonstrated in the present study, it would be expected, in patients taking ibuprofen on a chronic basis, that inhibition of platelet thromboxane synthesis would be virtually continual. Because of the potential for accumulation of S-I in adipose tissue (Williams *et al.*, 1986), the time taken for the anti-platelet effects to subside after cessation of chronic dosing is more difficult to predict. The results of the present investigation would suggest that platelet function would, in most cases, return to normal within 12 h. However, the leaching of

S-I from adipose stores may result in more prolonged anti-platelet effects.

Little is known regarding the degree of inhibition of TXA_2 synthesis necessary to produce physiological changes which may be important clinically. In examining the pharmacokinetics and pharmacodynamics of a specific thromboxane antagonist (SQ 28, 668) in humans, Friedhoff *et al.* (1986) found that template bleeding times were prolonged if TXA_2 production was inhibited by greater than 94%. In the present study, ibuprofen, over the therapeutic dose range, was found to elicit this magnitude of inhibition, if only for a short time (Figure 2). However, it is difficult to extrapolate the present observations to platelet aggregation *in vivo*, the latter being a complex process which is controlled by numerous factors, including the anti-aggregatory and vasodilatory compound, prostaglandin I_2 (PGI_2). This substance is produced in large quantities by vascular endothelial cells and is believed to function in concert with platelet TXA_2, to help regulate platelet aggregation (Moncada *et al.*, 1985; Mustard *et al.*, 1980). Parks *et al.* (1981) reported that the synthesis of PGI_2 by cultured human umbilical vein endothelial cells, and of TXA_2 by washed human platelets, were equally sensitive to the inhibitory effects of ibuprofen. Interestingly, Lognenecker *et al.* (1985) studied the effect of orally administered ibuprofen on both platelet TXB_2 production and on the basal blood concentrations of PGI_2 in human subjects. While ibuprofen inhibited TXB_2 generation, the basal blood concentrations of PGI_2 remained unchanged, leading the authors to suggest that ibuprofen induced anti-platelet effects while sparing endogenous anti-platelet mechanisms. The reason for the conflict between the *in vitro* data of Parks *et al.* (1981) and the *ex vivo* data of Longenecker *et al.* (1985) is unknown, and warrants further investigation.

In conclusion, the present study has demonstrated a close relationship between the plasma unbound concentration of S-I and the extent of inhibition of platelet thromboxane synthesis. There was an increase with dose in the maximum observed inhibition of platelet TXB_2 generation, and in the time taken for the unbound plasma concentrations of S-I to fall below those required to inhibit TXB_2 production by 80% and 50%. For the four subjects tested, there was very little variability in the sensitivity of platelets to the actions of S-I, as gauged by the individual values of EC_{50} and EC_{80}. Because of the low concentrations of unbound S-I needed to inhibit platelet cyclo-oxygenase, there is a need for a close examination of the anti-platelet effects of the drug in patients who have been receiving ibuprofen in the long term.

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