Equipotent inhibition by R(–)-, S(+)- and racemic ibuprofen of human polymorphonuclear cell function *in vitro*

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- 1 The effects of racemic (rac) ibuprofen and its S(+)- and R(–)-enantiomers on functions of human polymorphonuclear cells (PMN) and platelets were studied *in vitro*.
- 2 Rac-ibuprofen inhibited PMN functions (O_2^- generation, β -glucuronidase release, LTB_4 formation). Similar IC_{50} values (40–100 μM) were obtained for the S(+)- and R(–)-enantiomers.
- 3 All forms of ibuprofen inhibited cyclooxygenase-related platelet functions (aggregation, thromboxane formation). The S(+)-enantiomer was about twice as active as the racemate while the R(–)-enantiomer was at least 10-fold less active. This demonstrates that the S(+) is the only cyclooxygenase inhibitory component of the racemate.
- 4 The concentrations of rac-ibuprofen in PMN and platelets were similar to those in the incubation medium and represented equal concentrations of the enantiomers. This indicates that neither interconversion nor tissue accumulation of the compounds occurred.
- 5 These data indicate that antineutrophil effects of ibuprofen on human PMN are independent of cyclooxygenase inhibition. Therefore, R(–)-ibuprofen may be superior to the S(+)-isomer for the treatment of PMN-dependent inflammatory diseases. However, effective free drug concentrations may not be obtained *in vivo*.

Keywords ibuprofen chirality human neutrophils leukotrienes
thromboxane platelet function

Introduction

It is generally considered that non-steroidal anti-inflammatory drugs (NSAIDs) improve the vascular symptoms of acute inflammation, i.e. pain, erythema and oedema formation, through inhibition of cyclooxygenase and subsequent prostaglandin formation. Unfortunately, this action is not limited to the site of tissue injury. There is also a general suppression of prostaglandin biosynthesis, resulting in a number of side effects after systemic administration of NSAIDs. These include a bleeding tendency, gastrointestinal discomfort and mucosal microlesions as well as disturbances of renal sodium handling.

There is evidence that certain NSAIDs may exert effects on inflammatory cells that are independent of inhibition of prostaglandin biosynthesis. For example, sodium salicylate inhibits white cell function and chemotactic mediator release *in vitro* by a mechanism unrelated

to inhibition of cyclooxygenase product formation (Abramson *et al.*, 1985). Twomey & Dalke (1992) recently demonstrated that the actions of 12 structurally different NSAIDs on superoxide anion formation by human neutrophils were independent of their inhibitory effect on cyclooxygenase. Recent clinical evidence also suggests that at least the analgesic actions of NSAIDs are poorly correlated with inhibition of prostaglandin generation (McCormack & Brune, 1991). Thus, the question arises whether it is necessary to accept all of the unwanted side effects of NSAIDs resulting from systemic prostaglandin synthesis inhibition for the clinical treatment of pain and inflammation.

Several classes of NSAIDs, including the 2-aryl-propionic acid derivatives, are chiral compounds and, therefore, exist in two stereoisomeric forms. Inhibition of cyclooxygenase by these compounds resides almost

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exclusively in the S(+)-enantiomer (Adams *et al.*, 1976; Kean *et al.*, 1991). Since ibuprofen, like most of the other agents in this group, is used as a racemate, the question arises whether both stereoisomers contribute to the analgetic and antiinflammatory efficacy.

The aim of the present study was to measure the antiinflammatory effects of racemic ibuprofen and its S(+)- and R(-)-stereoisomers *in vitro* in terms of inhibition of human polymorphonuclear cell (PMN) function subsequent to stimulation by a chemotactic mediator. Activated PMN contribute to the inflammatory process and inflammation-related tissue injury (Weiss, 1989). In these cells, O_2^- generation and cytosolic enzyme release (β -glucuronidase) were measured. Leukotriene (LT) B_4 formation was measured as an index of PMN-related eicosanoid generation. The potency of ibuprofen and its enantiomers in inhibiting cyclooxygenase was measured in human platelets.

Methods

Blood sampling and preparation of platelet rich plasma and PMN

Blood was taken by venepuncture from healthy donors who had not taken any medication within 2 weeks prior to the study. It was collected into acidic citrate/dextrose (Biostabil[®], 1/10 v/v). Platelet-rich plasma (PRP) was prepared by centrifugation at 300 g at room temperature for 10 min. The pH was adjusted to 7.4 using 0.1 N HCl or 0.1 N NaOH as appropriate. An aliquot of the sample was used to measure the platelet count by phase-contrast microscopy. Subsequently, the platelet count was adjusted to 250,000 platelets μl^{-1} using autologous platelet-poor plasma.

The remaining sediment was redissolved in physiological salt solution (PSS). PMN were prepared as described previously (Hecker *et al.*, 1990). Briefly, erythrocytes were removed by dextran (6% w/v) sedimentation. PMN were separated from other white cells by Ficoll-Paque gradient centrifugation and any remaining red cells were destroyed by hypotonic lysis. PMN were washed twice in phosphate-buffered saline (PBS) and then resuspended in this solution, supplemented with human albumin (0.05 % w/v). The PMN count was determined microscopically and adjusted to a final stock concentration of 5×10^7 cells ml^{-1} . This PMN preparation contained > 95% PMN and their viability, tested by trypan blue staining, was greater than 95%.

Stimulation of PMN and measurement of O_2^- and β -glucuronidase release

PMN (5×10^6 cells) were incubated in PBS, supplemented with Ca^{++} (1 mM) and Mg^{++} (1 mM) (Hank's balanced salt solution, HBS) at 37° C in a total volume of 1.0 ml. After 10 min incubation with the compounds to be studied or the respective vehicles, the cells were stimulated by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) (30 nM). The reaction was stopped after 20 min by centrifugation at 12,000 g at 4° C.

Superoxide anion (O_2^-) generation was measured spectrophotometrically in terms of superoxide dismutase

(SOD, 300 iu ml^{-1}) inhibitable reduction of ferricytochrome C at 550 nm (Babior *et al.*, 1973). The values were corrected for O_2^- generation by unstimulated cells (usually 5–10 nmol $O_2^-/5 \times 10^6$ cells). To determine β -glucuronidase release, PMN were further treated with cytochalasin B (5 $\mu\text{g ml}^{-1}$) for 10 min prior to stimulation. After sedimentation of cells by centrifugation, any released β -glucuronidase activity was determined in the cell-free supernatant by measuring the generation of phenolphthalein from phenolphthalein- β -(D)-glucuronide (Fishman, 1974).

Leukotriene B_4 (LTB $_4$) formation

A suspension of 2.5×10^7 PMN in 10 ml HBS was incubated with the compounds to be studied or the respective vehicles for 10 min at 37° C. The cells were then stimulated by calcimycin (A23187; 10 μM) for a further 10 min. After centrifugation (1,000 g for 10 min at 4° C) the supernatants were extracted with C_{18} -cartridges according to the method of Powell (1982). The Di-HETE fraction was separated by reversed-phase h.p.l.c. and LTB $_4$ and its Ω -oxidized metabolites (LTB $_4$ -M) were measured by u.v.-detection using PGB $_2$ as an internal standard.

Measurement of aggregation and thromboxane generation in platelet-rich plasma

Platelet aggregation was determined photometrically, using a 2-channel aggregometer (Labor, Hamburg, FRG). After an 8 min incubation of 400 μl PRP with 90 μl Tris-buffer (50 mM, pH 7.4), 10 μl of the stimulating agent, i.e. collagen (0.6 and 2.5 $\mu\text{g ml}^{-1}$) or ADP (10 $\mu\text{g ml}^{-1}$) were added to the PRP and the changes in light transmission were recorded with constant stirring of the samples at 1,200 rev min^{-1} and 37° C. In experiments with ibuprofen, the compounds were added at the beginning of the incubation period in a 10 μl volume; the volume of Tris-buffer was decreased accordingly.

At 8 min, a 100 μl aliquot of the PRP was removed, indomethacin (30 μM) and EDTA (7.7 mM) (final concentrations) were added, the samples were centrifuged at 12,000 g for 10 min at room temperature and the platelet-free supernatant was removed and stored frozen until radioimmunoassay for thromboxane (TX) B_2 , the stable degradation product of TXA $_2$, as described previously (Schrör & Seidel, 1988). In samples containing no immunoreactive material, the detection limit (10% displacement of the ligand) of TXB $_2$ (20 pg ml^{-1}) was used for calculation of thromboxane levels.

Studies with washed platelets

The pH of PRP was adjusted to 6.5 with acidic citrate dextrose (Biostabil[®]). After 10 min at room temperature, PGE $_1$ was added (50 nM) and the platelets were sedimented by centrifugation at 400 g for 10 min. The supernatant was discarded and the platelet pellet washed twice in an isotonic buffer (pH 6.5) of the following composition [mM]: NaCl [113], $\text{Na}_2\text{HPO}_4 \times 2 \text{H}_2\text{O}$ [4], $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$ [24], KH_2PO_4 [4], glucose [5], supplemented with 50 nM PGE $_1$. The platelet pellet was finally resuspended in a PGE $_1$ -free buffer solution of the

following composition [mm]: NaCl [120], NaOH [30], $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$ [1], KCl [5], $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ [1], $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ [0.1], glucose [5], supplemented with $50 \mu\text{g ml}^{-1}$ apyrase and $300 \mu\text{g ml}^{-1}$ fibrinogen at pH 7.4. The platelet count was adjusted to $250,000 \text{ cells } \mu\text{l}^{-1}$.

Aggregation and TX generation were measured in these washed platelet suspensions following the procedures described above.

Reagents

Biostabil® (Biotest, Frankfurt/M, FRG); formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) (Calbiochem GmbH, Frankfurt/M, FRG); racemic ibuprofen and its S(+)- and R(-)-enantiomers (Dolorgiet, St Augustin, FRG); collagen (Hormon Chemie, München, FRG); indomethacin (Luitpold-Pharma, München, FRG); [^3H]-TXB₂ (New England Nuclear, Dreieich, FRG); phenolphthalein- β -(D)-glucuronide (Serva, Heidelberg, FRG); calcimycin, apyrase, cytochalasin B, ferricytochrome c, fibrinogen, human serum albumin, nordihydroguaiaretic acid (NDGA), PGB₂, superoxide dismutase (Sigma Chemicals, Deisenhofen, FRG); PGE₁ (Schwarz-Pharma, Monheim, FRG); TXB₂ (Upjohn Diagnostics, Kalamazoo, MI, USA). All other substances and solvents were from E. Merck (Darmstadt, FRG) and were of the highest purity available.

Stock solutions of FMLP and NDGA were prepared in DMSO and diluted in HBS. Indomethacin (10 mg) was dissolved in $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer as described previously (Schrör & Seidel, 1988). Ibuprofen was dissolved in Tris-buffer (50 mM, pH 7.4). Calcimycin was dissolved in ethanol at 10 mM. The final concentrations of the solvents did not affect the assay.

Assays of ibuprofen

R(-)-, S(+)- and racemic ibuprofen were measured by h.p.l.c. and u.v.-detection (u.v.-detector Hitachi L-4250) at 220 nm. [Column: Cyclobond I; eluent: methanol/triethylamine acetate (pH 6) buffer (880:120; v/v).] The detection limit was $1 \text{ ng } 20 \mu\text{l}^{-1}$ injection volume and the mean recovery was 90–107%.

Calculations and statistics

The molar potency of the antagonists was calculated from individual concentration-response curves. Differences between the compounds were assessed in terms of IC₅₀ values. The data are mean $\pm$ s.e. mean of *n* experiments. Statistical analysis was performed with the two-tailed *t*-test. *P* levels of < 0.05 were considered significant.

Results

Inhibition of platelet cyclooxygenase and aggregation by ibuprofen in the presence and absence of plasma

Figure 1 shows the concentration-response curves for inhibition of collagen ($2.5 \mu\text{g ml}^{-1}$)-stimulated platelet thromboxane formation by ibuprofen in plasma and

Figure 1 Concentration-dependent inhibition of TXB₂ formation in platelet rich plasma (open symbols) and washed platelet suspensions (filled symbols) by racemic ibuprofen (circles) and its S(+)- (triangles) and R(-)- (squares) enantiomers. Platelets were stimulated by collagen ($2.5 \mu\text{g ml}^{-1}$).

buffer medium. TXB₂ formation in PRP and washed platelets amounted to $154.8 \pm 16.4 \text{ ng ml}^{-1}$ (*n* = 4) and $61.9 \pm 13.6 \text{ nm}$ (*n* = 6), respectively. As expected, there was a significant and concentration-dependent inhibition of TXB₂ and platelet function by all compounds studied. The IC₅₀ of the racemate for inhibition of TX generation was $50 \pm 27 \mu\text{M}$ in PRP decreasing to $0.3 \pm 0.1 \mu\text{M}$ in washed platelets. This difference was probably due to the extensive protein binding of ibuprofen.

Stereoselectivity of inhibition of platelet function and thromboxane formation

As also shown in Figure 1, there was a clear difference in inhibition of thromboxane formation by the stereoisomers of ibuprofen. Tables 1 and 2 summarize the data on inhibition of platelet aggregation and TXB₂ release by racemic ibuprofen and its stereoisomers in PRP and washed platelets, respectively. The S(+)-isomer was the most potent compound, followed by the racemate and

Table 1 Inhibition by racemic ibuprofen and its enantiomers of collagen ($2.5 \mu\text{g ml}^{-1}$)-stimulated aggregation and thromboxane (TXB₂) formation in PRP—IC₅₀ values (μM)

	Aggregation		TXB ₂	
Rac-ibuprofen	134 ± 63	(1.0)	50 ± 27	(1.0)
S(+)-ibuprofen	88 ± 44	(1.5)	26 ± 15	(1.9)
R(-)-ibuprofen	$1260 \pm 670^*$	(0.10)	$380 \pm 60^*$	(0.13)

Data are mean $\pm$ s.e. mean of 3–6 experiments in each group. The numbers in brackets indicate the relative potency (rac-ibuprofen = 1).

**P* < 0.05 (S(+)-ibuprofen or rac-ibuprofen vs R(-)-ibuprofen).

Table 2 Inhibition by racemic ibuprofen and its enantiomers of collagen ($2.5 \mu\text{g ml}^{-1}$)-stimulated aggregation and thromboxane (TXB₂) formation in washed platelets (WP)—IC₅₀ values (μM)

	Aggregation		TXB ₂	
Rac-ibuprofen	1.3 ± 0.3	(1.0)	0.3 ± 0.1	(1.0)
S(+)-ibuprofen	0.6 ± 0.4	(2.2)	0.2 ± 0.1	(1.5)
R(-)-ibuprofen	$33.0 \pm 0.8^*$	(0.04)	$15.6 \pm 1.2^*$	(0.02)

Data are mean $\pm$ s.e. mean of 3–6 experiments in each group. The numbers in brackets indicate the relative potency (rac-ibuprofen = 1).

**P* < 0.05 (S(+)-ibuprofen or rac-ibuprofen vs R(-)-ibuprofen).

the R(–)-form. Relative to the racemate, the R(–)-isomer was 10–50-fold less active while the S(+)-form was 1.5–2.2-fold more active. Similar results were obtained with low-dose ($0.6 \mu\text{g ml}^{-1}$) collagen and ADP ($10 \mu\text{g ml}^{-1}$) as stimulating agents (not shown).

Inhibition of superoxide anion generation and β -glucuronidase release in PMN

Figure 2 summarizes the data on O_2^- generation in human PMN. O_2^- release from unstimulated PMN was $9.2 \pm 0.9 \text{ nM O}_2^-/5 \times 10^6 \text{ cells}$ ($n = 22$). All ibuprofen forms stimulated basal O_2^- formation at the highest concentration used (1 mM) by about 50% ($P < 0.05$). Stimulation with FMLP enhanced O_2^- formation about 3-fold above control, amounting to $27.1 \pm 1.2 \text{ nM O}_2^-/5 \times 10^6 \text{ cells}$ ($n = 20$). All ibuprofen forms caused a concentration-dependent inhibition of FMLP-induced O_2^- generation which became significant at 30 and $100 \mu\text{M}$, respectively ($P < 0.05$). No differences were observed between racemic ibuprofen and its stereoisomers (Table 3).

Similar data were obtained with β -glucuronidase release and are shown in Figure 3. Resting PMN released only a small amount of β -glucuronidase, i.e. $5.8 \pm 0.4 \mu\text{g phenolphthalein}/5 \times 10^6 \text{ cells}$ ($n = 21$). All ibuprofen forms reduced the basal enzyme secretion by about 50% at a 1 mM concentration ($P < 0.05$). Lower concentrations were ineffective.

Stimulation with FMLP increased β -glucuronidase release more than 6-fold above control, amounting to $30.9 \pm 1.7 \mu\text{g phenolphthalein}/5 \times 10^6 \text{ cells}$ ($n = 21$). All ibuprofen forms inhibited FMLP-induced β -glucuronidase release in a concentration-dependent fashion and were equipotent (Table 3). The inhibition was significant ($P < 0.05$) at concentrations of $300 \mu\text{M}$. No differences between the enantiomers were observed.

LTB₄ formation

PMN, stimulated by calcimycin, released $1,012 \pm 79 \text{ ng LTB}_4/2.5 \times 10^7 \text{ cells}$ ($n = 8$). As shown in Table 3 and Figure 4, all ibuprofen forms inhibited LTB_4 formation in a concentration-dependent manner. There was a

Table 3 Inhibition by racemic ibuprofen and its enantiomers of FMLP (30 nM) stimulated O_2^- formation and β -glucuronidase release and calcimycin ($10 \mu\text{M}$)-induced LTB_4 generation of human PMN— IC_{50} values (μM)

	O_2^-		β -glucuronidase		LTB_4	
Rac-ibuprofen	0.60 ± 0.13	(1.0)	0.44 ± 0.08	(1.0)	0.36 ± 0.10	(1.0)
(S+)-ibuprofen	0.50 ± 0.11	(1.2)	0.53 ± 0.12	(0.8)	0.14 ± 0.07	(2.6)
(R-)-ibuprofen	0.43 ± 0.08	(1.4)	0.58 ± 0.09	(0.8)	0.22 ± 0.02	(1.6)

Data are mean $\pm$ s.e. mean of 4–9 experiments in each group. The numbers in brackets indicate the relative potency (rac-ibuprofen = 1).

No significant differences between the groups were found ($P > 0.05$).

Figure 2 Inhibition of FMLP (30 nM) induced O_2^- formation in human PMN by racemic ibuprofen (■) and its S(+)- (▤) and R(–)- (▨) enantiomers. The bars are mean $\pm$ s.e. mean of the number of experiments indicated at the bars. * $P < 0.05$ (treatment vs control).

Figure 3 Inhibition of FMLP (30 nM) induced β -glucuronidase release from human PMN by racemic ibuprofen (■) and its S(+)- (▤) and R(-)- (▦) enantiomers. The bars are mean $\pm$ s.e. mean of the number of experiments indicated at the bars. * $P < 0.05$ (treatment vs control).

Figure 4 Inhibition of calcimycin (10 μ M) induced LTB_4 formation in human PMN by racemic ibuprofen (■) and its S(+)- (▤) and R(-)- (▦) enantiomers. The bars are mean $\pm$ s.e. mean of the number of experiments indicated at the bars. * $P < 0.05$ (treatment vs control).

Table 4 Tissue (PMN) and supernatant concentrations of ibuprofen

Compound	Concentration ($\mu\text{g g}^{-1}$ or $\mu\text{g ml}^{-1}$)	
	PMN	Supernatant
S(+)-ibuprofen	241 $\pm$ 12	216 $\pm$ 4
R(-)-ibuprofen	219 $\pm$ 10	196 $\pm$ 2
Rac-ibuprofen	(S+)	136 $\pm$ 2
	(R-)	131 $\pm$ 4
	Σ	267
		208

The data are mean $\pm$ s.e. mean of $n = 6$ determinations. No significant differences were obtained between PMN and the incubation medium ($P > 0.05$).

tendency for inhibition at 30 μM and a significant inhibition at 100 μM ($P < 0.05$). No differences between the enantiomers were observed. Although the R(-)-enantiomer appeared to be slightly more potent at 30 μM , this was probably not relevant considering all of the other concentrations of the isoforms used.

Measurement of S(-)- and R(-)-ibuprofen in PMN

Table 4 compares the ibuprofen content in PMN with the concentrations in the supernatant. It is evident that the drug concentrations in PMN were similar to those in the incubation medium. There was no interconversion between the enantiomers as indicated by the equal distribution of the two isomers between the tissue (PMN) and bath medium after addition of the racemate. Similar data were obtained with PRP (not shown).

Discussion

This study shows that inhibition of platelet function and thromboxane formation by ibuprofen requires the appropriate steric configuration of the molecule, whereas inhibition of PMN function and LTB₄ formation do not. This suggests that antineutrophil actions of ibuprofen are to be expected from both the racemate and the two stereoisomers to the same extent and cannot be explained by inhibition of cyclooxygenase product formation.

Racemic ibuprofen was half as potent as the S(+)-isomer in inhibiting platelet aggregation and thromboxane formation while the R(-)-enantiomer was about two orders of magnitude less active. Evans *et al.* (1991) have measured thromboxane generation in clotting blood *ex vivo* and also found the S(+)-isomer to be twice as potent as the racemate. These data confirm the enantioselectivity of cyclooxygenase inhibition by ibuprofen (Adams *et al.*, 1976) and show that the inhibition of platelet function and thromboxane formation reside almost exclusively in the S(+)-enantiomer.

There is currently much debate about the clinical use of chiral compounds. About 40% of synthetic drugs are chiral and about 90% of them are currently marketed as racemates (Lennard, 1991). This issue is particularly relevant for antirheumatic drugs because most of them, including ibuprofen, are chiral compounds that are marketed as racemates (Williams, 1990). The obvious

question is, whether the cyclooxygenase inhibitory activity of the S(+)-isomer is not 'diluted' by adding an inactive compound, eventually resulting in 'isomeric ballast and medicinal pollution of the patient' (Ariens, 1990).

Before discussing this issue from a pharmacological point of view, it has to be clarified whether cyclooxygenase inhibition is indeed a prerequisite for the antiinflammatory actions of NSAIDs *in vivo*. This is by no means clear. Cyclooxygenase inhibition by NSAIDs might even result in enhanced PMN-activation because several prostaglandins are potent inhibitors of superoxide anion formation, lysosomal enzyme release and LTB₄ production of human PMN (Fantone & Kinnes, 1983; Hecker *et al.*, 1990; Ney & Schrör, 1989; O'Flaherty *et al.*, 1979). It has also been shown that E-type prostaglandins and NSAIDs may act synergistically to inhibit neutrophil function (Kitsis *et al.*, 1991). In addition, human leukocytes, including PMN (Naccache *et al.*, 1989) and lymphocytes (Goldyne, 1989) do not generate any significant amount of cyclooxygenase products. Our study has shown that ibuprofen also blocks calcium ionophore-induced LTB₄ formation with an equal potency of the enantiomers and the racemate.

Although the concentrations of ibuprofen necessary to block PMN functions were somewhat higher than those required for inhibition of platelet cyclooxygenase, they were in the range of total plasma concentrations achieved with therapeutic doses of the compound, ranging between 100–300 μM (Ali *et al.*, 1984; Lenhard *et al.*, 1990). By comparing the IC₅₀ levels of inhibition of thromboxane formation in PRP and washed platelets, roughly 1% of total ibuprofen appears to be unbound. Thus effective free concentrations of the compound may not be obtained *in vivo* after administration of the racemate at conventional doses. Nevertheless, from a pharmacological point of view, our IC₅₀ values for inhibition of O₂⁻ and lysosomal enzyme release are similar to those reported by others (Maderazo *et al.*, 1984; Simchowicz *et al.*, 1979) and suggest that 50% inhibition of LTB₄ formation is obtained by R(-)-ibuprofen at a concentration of 0.22 mM. This concentration blocked platelet cyclooxygenase in this study by less than 40%. This degree of inhibition of platelet-derived thromboxane formation is probably without any consequences for haemostasis while the essentially complete inhibition by the same concentration of the S(+)-isomer should result in significant inhibition of thromboxane-dependent platelet functions (Reilly & FitzGerald, 1987).

There is evidence for bioconversion of the R(-)-enantiomer into the S(+)-form *in vivo* (Adams *et al.*, 1976; Lee *et al.*, 1985) and *in vitro* by the 2-arylpropionyl-CoA-epimerase (Chen *et al.*, 1991; Sannins *et al.*, 1990). This may result in a supraproportional bioavailability of S(+)-ibuprofen from the racemate *in vivo* (Romero *et al.*, 1991) as well as a reduced risk of formation of potentially toxic metabolites from the R(-)-isomer (Kean *et al.*, 1991), although it is not entirely clear whether this is really a stereoselective reaction (Freneaux *et al.*, 1990). However, in our study there was no evidence for any interconversion of R(-)-ibuprofen into the S(+)-form *in vitro*, confirming the data of others (Knihinicki *et al.*, 1989).

There are other cyclooxygenase-independent mechanisms of action of ibuprofen which may also cause

antiinflammatory reactions. For example, ibuprofen may interfere with the haem-arachidonic acid interaction, resulting in reduced generation of toxic oxygen metabolites, such as hydroxyl ions, and lipid peroxides. This mechanism has been shown in phosgene toxicity and was explained by the iron chelating properties of the compound (Kennedy *et al.*, 1990). More recently, interference of NSAIDs with G-protein-dependent pathways has been demonstrated in purified PMN membrane preparations, suggesting an uncoupling of post-receptor signalling events by these compounds (Abramson *et al.*, 1991).

In conclusion, we have shown a significant PMN-antagonistic action of racemic ibuprofen. This activity appears to be independent of its inhibitory effect on cyclooxygenase because the R(–)-enantiomer was equipotent to the racemate. Since many of the clinical side effects of NSAIDs result from their inhibition of prostaglandin

formation, the racemate or the R(–)-isomer may be superior to the S(+)-enantiomer because of less inhibition of prostaglandin-formation yet with preserved antineutrophil activity (Wallace *et al.*, 1990). This concept is in line with a recent clinical trial by Bradley *et al.* (1991), demonstrating that the therapeutic efficacy of ibuprofen in patients suffering from osteoarthritis was the same, whether the drug was administered at (low) analgesic or (higher) antiinflammatory doses. This would suggest that it is probably an oversimplification to consider aryl acid isomers without cyclooxygenase inhibitory potential as inactive metabolic ballast.

The authors are most grateful to Dolorgiet Pharmaceuticals (St Augustin, FRG) for kindly providing racemic ibuprofen and its isomers, and to Drs G. Berner and K. Posselt from Dolorgiet Pharmaceuticals for kindly performing the assays of ibuprofen.

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(Received 10 June 1992,
accepted 3 September 1992)