Effects of ibuprofen enantiomers and its coenzyme A thioesters on human prostaglandin endoperoxide synthases

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1   Ibuprofen enantiomers and their respective coenzyme A thioesters were tested in human platelets and blood monocytes to determine their selectivity and potency as inhibitors of cyclo-oxygenase activity of prostaglandin endoperoxide synthase-1 (PGHS-1) and PGHS-2.

2   Human blood from volunteers was drawn and allowed to clot at 37°C for 1 h in the presence of increasing concentrations of the test compounds (**R**-ibuprofen, **S**-ibuprofen, **R**-ibuprofenoyl-CoA, **S**-ibuprofenoyl-CoA, NS-398). Immunoreactive (ir) thromboxane B_2 (TXB_2) concentrations in serum were determined by a specific EIA assay as an index of the cyclo-oxygenase activity of platelet PGHS-1.

3   Heparin-treated blood from the same donors was incubated at 37°C for 24 h with the same concentrations of the test compounds in the presence of lipopolysaccharide (LPS, 10 μg ml^{-1}). The contribution of PGHS-1 was suppressed by pretreatment of the volunteers with aspirin (500 mg; 48 h before venepuncture). As a measure of LPS induced PGHS-2 activity immunoreactive prostaglandin E_2 (irPGE$_2$) plasma concentrations were determined by a specific EIA assay.

4   **S**-ibuprofen inhibited the activity of PGHS-1 (IC$_{50}$ 2.1 μM) and PGHS-2 (IC$_{50}$ 1.6 μM) equally. **R**-ibuprofen inhibited PGHS-1 (IC$_{50}$ 34.9) less potently than **S**-ibuprofen and showed no inhibition of PGHS-2 up to 250 μM. By contrast **R**-ibuprofenoyl-CoA thioester inhibited PGE$_2$ production from LPS-stimulated monocytes almost two orders of magnitude more potently than the generation of TXB$_2$ (IC$_{50}$ 5.6 vs 219 μM).

5   Western blotting of PGHS-2 after LPS induction of blood monocytes showed a concentration-dependent inhibition of PGHS-2 protein expression by ibuprofenoyl-CoA thioesters.

6   These data confirm that **S**-ibuprofen represents the active entity in the racemate with respect to cyclo-oxygenase activity. More importantly the data suggest a contribution of the **R**-enantiomer to therapeutic effects not only by chiral inversion to **S**-ibuprofen but also via inhibition of induction of PGHS-2 mediated by **R**-ibuprofenoyl-CoA thioester.

7   The data may explain why racemic ibuprofen is ranked as one of the safest non-steroidal anti-inflammatory drugs (NSAIDs) so far determined in epidemiological studies.

Keywords: Ibuprofen; enantiomers; coenzyme A thioester; prostaglandin endoperoxide synthase; chiral inversion

Introduction

Ibuprofen, a chiral 2-arylpropionic acid derivative is an analgesic, antipyretic and anti-inflammatory drug which is available over-the-counter in many countries. Since the experiments of Vane in the early 1970s it has been accepted that the modes of anti-inflammatory action and of the most important side effects of aspirin and related compounds are mediated by inhibition of prostaglandin endoperoxide synthase (PGHS), the key enzyme in conversion of arachidonic acid to prostaglandin H_2 (PGH$_2$) (Vane, 1971). At least two isoforms of PGHS exist. PGHS-1 is a constitutive enzyme and has physiological functions in the gastrointestinal tissue, the kidney and other organs (DeWitt & Smith, 1988; Simmons et al., 1992; Smith, 1992). About five years ago another PGHS, referred to as PGHS-2, was described which is induced by a number of pro-inflammatory stimuli (Kujubu et al., 1991; Fletcher et al., 1992; O'Banion et al., 1992; Kennedy et al., 1993). Thus over the last few years the hypothesis that inhibition of PGHS-2 and PGHS-1 accounts for the therapeutic activity and for the side effects of NSAIDs, respectively, became an attractive theory (Vane & Botting, 1996). Accordingly, NSAIDs with a more favourable PGHS-2/PGHS-1 ratio should have a good anti-inflammatory activity with fewer side effects e.g. on the gastrointestinal tract. In a recent collaborative meta-analysis with individual NSAIDs ibuprofen proved to have the lowest

risk of serious gastrointestinal complications (Henry et al., 1996). However, several independent studies, in which different in vitro approaches were used to monitor inhibition of PGHS-1 and PGHS-2, did not suggest a preferred inhibition of PGHS-2 by ibuprofen (Mitchell et al., 1993; Laneuville et al., 1994; Gierse et al., 1995; Brideau et al., 1996; Young et al., 1996).

An unusual feature of ibuprofen, which is marketed in the majority of countries as an equal mixture of **R**- and **S**-ibuprofen (racemate), is the unidirectional metabolic chiral inversion of the in vitro inactive (not prostaglandin synthesis inhibiting) **R**-enantiomer to the prostaglandin synthesis inhibiting **S**-form (Wechter et al., 1974; Caldwell et al., 1988; Williams, 1990). Only **R**-ibuprofen is stereoselectively activated to an ibuprofenoyl-CoA thioester by the long chain fatty acid synthetase (Brugger et al., 1996; Mayer, 1996), an enzyme expressed in several isoforms and showing broad tissue distribution. Subsequently, the **R**-ibuprofenoyl-CoA thioester was shown to be a substrate for a recently discovered epimerase that is responsible for chiral inversion (Reichel et al., 1995; 1997). By contrast **S**-ibuprofen is not activated to a CoA thioester and therefore not inverted to **R**-ibuprofen (Figure 1).

The aim of the present study was to evaluate the potency and selectivity of intermediate products of ibuprofen inversion, namely **R**- and **S**-ibuprofenoyl-CoA thioesters in inhibiting the activity of PGHS-1 and PGHS-2. NS-398 a known rather selective inhibitor of PGHS-2 was used as a reference compound for the PGHS-2 system (Futaki et al., 1993). Inhibition of
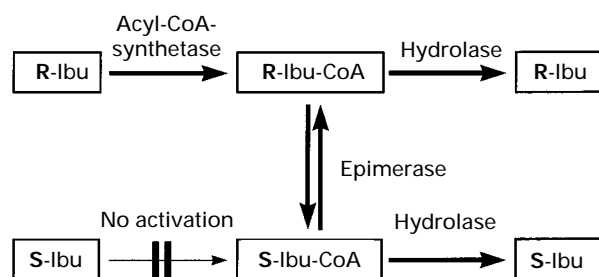
Figure 1 *In vivo* **R**-ibuprofen (**R**-Ibu) is unidirectionally inverted to the **S**-enantiomer. A key step in the inversion process is the activation of **R**-ibuprofen to the **R**-ibuprofenoyl-CoA thioester catalysed by an acyl-CoA synthetase. The **S**-enantiomer is not able to form a CoA thioester *in vivo*. A recently characterized enzyme 'epimerase' catalyses the epimerization of **R**-ibuprofenoyl-CoA thioester to **S**-ibuprofenoyl-CoA thioester. The CoA thioesters hydrolyse in a non-stereoselective manner.

PGHS-2 and PGHS-1 by the test compounds was measured *in vitro* in human whole blood. Patrignani *et al.* (1994) have shown that production of PGE_2 by LPS-stimulated human monocytes corresponds to PGHS-2 induction, while generation of TXB_2 during clotting of whole blood corresponds to the activity of PGHS-1.

A preliminary account of this work was presented at the Annual Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology (Mainz, Germany, March 11-13, 1997) and published in abstract form (Neupert *et al.*, 1997).

Methods

Incubation of **R**-ibuprofen in human whole blood

To exclude a possible inversion of **R**-ibuprofen to **S**-ibuprofen in the test system human whole blood (1 ml aliquots) was spiked with **R**-ibuprofen and **R**-ibuprofenoyl-CoA thioester at a final concentration of about 100 μM. After an incubation period of 2, 4, 6 and 24 h the concentrations of **R**-ibuprofen and **S**-ibuprofen and its thioesters were determined by stereoselective h.p.l.c. analysis as described previously (Menzel-Soglowek *et al.*, 1990; Tracy & Hall, 1991; Brugger *et al.*, 1996).

Stability of ibuprofenoyl-CoA thioesters in whole blood

Aliquots of human whole blood (1 ml) were incubated with **R**-ibuprofenoyl-CoA (52 μM) and **S**-ibuprofenoyl-CoA (80 μM) over 24 h (37°C). The concentrations of ibuprofen enantiomers formed by hydrolysis of the respective ibuprofenoyl-CoA thioesters were measured by stereoselective h.p.l.c. analysis.

PGHS-1/-2 activity in human whole blood

Peripheral venous blood was drawn from healthy volunteers before and 2 days after oral administration of 500 mg aspirin. Approval was obtained from the University of Erlangen Medical Ethics Review Committee.

For assessment of PGHS-2 induction, aliquots of whole blood (1 ml) containing 10 iu of sodium heparin were incubated in the presence of LPS (10 μg ml^{-1}) for 24 h at 37°C, as described by Patrignani *et al.* (1994). The contribution of platelet PGHS-1 was suppressed by the aspirin pretreatment. The reaction was stopped by centrifugation (10 min, 2000 r.p.m.) and separation of plasma which was frozen at -70°C until measurement of immunoreactive (ir) PGE_2 (within 2 weeks) as an index of the PGHS-2 activity.

To test the activity of PGHS-1, peripheral blood was drawn from volunteers who had not taken any NSAID for 3

weeks before the study. Aliquots of this blood (1 ml) were immediately transferred to separate tubes and were allowed to clot (37°C for 60 min) as described by Patrignani *et al.* (1994). Serum was collected after centrifugation (10 min, 3000 r.p.m.) and kept at -70°C until measurement of irTXB_2 (within 2 weeks) as a reflection of the PGHS-1 activity.

The effects of ibuprofenoyl-CoA thioesters, ibuprofen enantiomers and NS-398 (used as control) on the activity of PGHS-1 and PGHS-2 in whole blood were studied at a minimum of 5 concentrations.

R-ibuprofenoyl-CoA and **S**-ibuprofenoyl-CoA were dissolved in phosphate buffered saline (PBS) and added to blood samples (1 ml aliquots) to given final concentrations of $0.00052-250$ and $0.0018-250$ μM, respectively. **R**-ibuprofen, **S**-ibuprofen and racemic ibuprofen in PBS (10 μl) were studied similarly at final concentrations of $0.0001-250$ μM. Palmitoyl-CoA and CoA were used as controls in the same way. NS-398, a known selective PGHS-2 inhibitor was tested at a final concentration of $0.0001-100$ μM.

The results are expressed as the percentage inhibition of PGE_2 or TXB_2 biosynthesis relative to control incubations. The inhibitory potencies of the substances are expressed as IC_{50} values (estimated concentration producing half-maximum inhibition of the PGHS-1 or -2 activity).

Preparation of mononuclear cells

Mononuclear cells were separated from human whole blood containing ammonium heparin (15 iu ml^{-1} blood) by density gradient centrifugation with Histopaque-1077 (Sigma, Deisenhofen, Germany) according to Brideau *et al.* (1996). The mononuclear cells were washed twice with PBS (pH 7.4) to remove contaminating platelets. Subsequently, the cells were resuspended in RPMI-1640 complete medium (Sigma, Deisenhofen, Germany) and diluted to 2.0×10^6 cells ml^{-1}. Viability of cells was examined by Trypan Blue exclusion as described by Baader *et al.* (1988). Mononuclear cells were incubated with different concentrations of the test compounds in the absence and presence of LPS (10 μg ml^{-1}) for 24 h at 37°C in 5% CO_2-humified atmosphere. Incubation was stopped by centrifugation. Thereafter the cells were examined by Western blot analysis and irPGE_2 concentrations were measured in the supernatant.

Western blot analysis

After incubation isolated human mononuclear cells were washed three times with PBS containing 1% Triton X-100 according to Klein *et al.* (1994). Cell lysates with equal amounts of protein were separated by a 7.5% SDS-PAGE and blotted for 2 h to a nitrocellulose membrane. Transfer efficiency was checked with a solution of 5% Ponceau S. Subsequently the membranes were destained and blocked with 5% milk powder in PBS/0.1% Tween 20 for 2 h. A polyclonal anti-PGHS-2 antibody was diluted 1:500 and added to the blot for 2 h. The blot was washed three times with PBS/0.1% Tween 20 and incubated with anti-goat antibody (diluted 1:10,000) for 2 h. The blot was developed with a chemiluminescence technique with enhanced luminol (ECL) according to the instructions of the supplier (Boehringer Mannheim GmbH, Mannheim, Germany).

Measurement of irPGE₂ and irTXB₂

IrTXB_2 and irPGE_2 concentrations in serum and plasma, respectively, and in cell culture media were measured by enzyme immunoassay. The coefficients of variance were <18% in the range $30-1,000$ pg ml^{-1} for PGE_2 and <12% in the range $20-1,000$ pg ml^{-1} for TXB_2 (inter- and intra-day validation). Unextracted samples of serum, plasma and cell culture media were diluted with assay buffer (PBS, 0.5% bovine serum albumin) for quantification.

Materials

R- and **S**-ibuprofen were kindly provided by Pharma Trans Sanaq AG (Basel, Switzerland). The optical purity of the enantiomers exceeded 98.5%. **R**- and **S**-ibuprofenoyl-CoA thioesters were synthesized as previously described (Brugger *et al*., 1996). NS-398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide; used as a selective PGHS-2 inhibitor), PGHS-2 (human, ovine) and immunoassays (EIA) for PGE$_2$ and thromboxane B$_2$ (TXB$_2$) were from Cayman Chemicals (Ann Arbor, MI, U.S.A.). Polyclonal anti-PGHS-2 antibody (from goat) was obtained from Santa Cruz Biotechnology Inc. (Heidelberg, Germany). Coenzyme A (CoA), palmitoyl-CoA, lipopolysaccharide (LPS) from *E. coli*, peroxidase conjugated anti-goat IgG, Histopaque 1077, Triton X-100 and all other compounds were from Sigma Chemie (Deisenhofen, Germany).

Synthesis of **R**- and **S**-*ibuprofenoyl-CoA*

R- and **S**-ibuprofenoyl-CoA thioesters used for inhibition experiments and as chromatographic standards were prepared as described by Brugger *et al*. (1996). Briefly, the respective enantiomers of ibuprofen (10 μmol) were dissolved in thionyl chloride (16.8 mmol) and 180 μl dimethylformamide. After heating the reaction mixture to 55°C for 1 h the excess thionyl chloride was removed under reduced pressure to yield the acyl chloride. NaHCO$_3$ buffer (10 ml, 0.1 mM, pH 7.5) was flushed with nitrogen for 30 min and CoA (100 mg) was dissolved in the buffer. The oily solution was added dropwise to the NaHCO$_3$ buffer under controlled and readjusted pH. The reaction was allowed to proceed for 2 h. The formation of thioester was monitored by high-performance liquid chromatographic (h.p.l.c.) analysis. The reaction was stopped by adjusting to pH 3 (1 M HCl). The oily product was filtered and the thioester purified by semi-preparative h.p.l.c. as described previously (Brugger *et al*., 1996). The chemically synthesized ibuprofenoyl-CoA thioesters were characterized by impact mass spectrometry (Brugger *et al*., 1996). The products were quantified after hydrolysis with NaOH (2 M) and the amounts of **R**- and **S**-ibuprofen were determined by GC/MS according to Rudy *et al*. (1991).

Data analysis

Each concentration was tested in at least 6 replicate experiments. The IC$_{50}$ values for inhibition of PGHS-1/-2 were analysed and calculated with the Marquardt-Levenberg algorithm (Marquardt, 1963) by use of the sigmoidal E$_{max}$-model running under Jandel Sigmal Plot (Jandel GmbH, Erkrath, Germany).

Results

Approximately 35% of the ibuprofenoyl-CoA thioesters were hydrolyzed to the respective ibuprofen enantiomer over the 24 h incubation period in whole blood. However, no inversion of **R**-ibuprofen to **S**-ibuprofen was detected. The very small proportion (1.8%) of **S**-ibuprofen detected after incubation of **R**-ibuprofen can be explained by impurity with the optical antipode. Incubating human whole blood with **R**-ibuprofenoyl-CoA and **S**-ibuprofenoyl-CoA over 24 h resulted in less than 2% epimerization.

Racemic ibuprofen inhibited PGHS-1 more potently than PGHS-2 (Figure 2). IC$_{50}$ values for PGHS-1 and PGHS-2 were 6.5 $\pm$ 2.5 μM and 46.7 $\pm$ 19.7 μM, respectively (Table 1). **S**-ibuprofen had an equipotent effect on PGHS-1 and -2 (IC$_{50}$ values: 2.1 $\pm$ 1.3 μM vs 1.6 $\pm$ 0.8 μM; Table 1). **R**-ibuprofen exhibited no inhibitory effect on PGHS-2 over the tested concentration range and inhibited PGHS-1 with an IC$_{50}$ value of 34.9 $\pm$ 13.2 μM. **S**-ibuprofenoyl-CoA inhibited PGHS-1 with

Figure 2 (a–d) Show the relationship between percentage inhibition of TXB$_2$ generation (as reflection of PGHS-1 activity) or PGE$_2$ generation (as reflection of PGHS-2 activity) and log concentrations of **R**-ibuprofen (a), **R**-ibuprofenoyl-CoA thioester (b), **S**-ibuprofen (c) and **S**-ibuprofenoyl-CoA (d), respectively.

Table 1 Computer fitted IC_{50} values describing the relationship between concentrations of the various test compounds and the percentage inhibition of TXB_2 generation (as reflection of PGHS-1 activity) and PGE_2 generation (as reflection of PGHS-2 activity) in the human whole blood assay

Test compound	IC_{50} (PGHS-1)	IC_{50} (PGHS-2)	$IC_{50(PGHS-2)}/IC_{50(PGHS-1)}$
R-ibuprofenoyl-CoA	219.3 ± 95	5.6 ± 2.5	0.026
S-ibuprofenoyl-CoA	22.5 ± 3.9	11.8 ± 4.8	0.52
R-ibuprofen	34.9 ± 13.2	>250	–
S-ibuprofen	2.1 ± 1.3	1.6 ± 0.8	0.76
Ibuprofen, racemic	6.5 ± 2.5	46.7 ± 19.7	7.2
Palmitoyl-CoA	>250	89.2 ± 38	–
CoA	>250	>250	–
NS-398	9.2 ± 1.4	0.08 ± 0.04	0.0087

Values are expressed in μM and are means $\pm$ s.d. ($n=6$).

an IC_{50} value of 22.5 ± 3.9 μM while **R**-ibuprofenoyl-CoA showed no inhibitory effect on PGHS-1 over the concentration range tested (Figure 2). Interestingly, both ibuprofenoyl-CoA thioesters inhibited PGHS-2 (IC_{50} values of 5.6 ± 2.5 and 11.8 ± 4.8 μM for the **R**- and **S**-enantiomer, respectively). In this system the selective PGHS-2 inhibitor NS-398 inhibited PGHS-2 and PGHS-1 with a ratio of 0.0087. The results are summarized in Table 1.

Immunoblot analysis of LPS stimulated human mononuclear cells by use of a polyclonal antibody reacting exclusively with human PGHS-2 showed a distinct band of approximately 72 kD (Figure 3). Coincubation of LPS stimulated mononuclear cells with **R**-ibuprofen, **S**-ibuprofen or with racemic ibuprofen over 24 h (concentration range $0.001 - 1.0$ mM) showed no effect on the induced expression of PGHS-2 protein (Figure 3). By contrast both thioesters of ibuprofenoyl-CoA suppressed the expression of PGHS-2 in a dose-dependent manner. At concentrations higher than 50 μM hardly any PGHS-2 protein was detected. Quantification of irPGE$_2$ levels in supernatants were in line with these findings. Following LPS stimulation (24 h) the mean level of irPGE$_2$ was 1256 ± 293 pg/10^6 cells (means $\pm$ s.d., $n=6$). **R**-ibuprofenoyl-CoA inhibited the generation of irPGE$_2$ in LPS-stimulated monocytes with an IC_{50} of 13.2 ± 6.8 μM ($n=6$).

Discussion

Over the last twenty years much attention has been given to explaining inter-subject variability in the efficacy and safety profile of NSAIDs (Day & Brooks, 1987; Furst, 1994). Since differences in the pharmacokinetics of NSAIDs do not appear to be a major factor contributing to different safety profiles of NSAIDs research has focused on pharmacodynamic explanations. The recent discovery of two distinct isozymes of PGHS was a breakthrough in inflammation and pain research (Fu et al., 1990; Masferrer et al., 1990). The theory that prostaglandins, formed by the constitutive PGHS-1 regulate gut mucosal and kidney function and prostaglandins derived from the inducible PGHS-2 are responsible for inflammatory symptoms has provided a convincing relationale for differences in the safety profile of NSAIDs (Vane & Botting, 1996). Accordingly, NSAIDs selectively inhibiting PGHS-2 should show a better safety profile as compared to NSAIDs which inhibit both PGHS isozymes equipotently. Thus, over the last few years several studies have focused on differential effects of clinically used NSAIDs on PGHS-1 and PGHS-2. For the investigation of differential PGHS-1/PGHS-2 inhibition a variety of experimental models have been used (Meade et al., 1993; Mitchell et al., 1993; Laneuville et al., 1994; Gierse et al., 1995; Brideau et al., 1996; Young et al., 1996). However, the results from the different test systems vary with changes in experimental procedures. In the case of ibuprofen, for example relative potencies for inhibition of PGHS-2 and PGHS-1, expressed as ratios of $IC_{50,PGHS-2}/IC_{50,PGHS-1}$ ranged between

Figure 3 Immunoblot analysis of LPS stimulated human mononuclear cells. Isolated mononuclear cells were coincubated with LPS (10 μg ml^{-1}) and test compounds for 24 h. Mononuclear cells were lyzed and proteins analysed by SDS-polyacrylamine gel electrophoresis and Western blot techniques. All lanes contained equal amounts of protein. PGHS-2 was detected by a specific goat antibody against PGHS-2 and peroxidase labelled anti-goat IgG. (a) **R**- and **S**-ibuprofen ($0.001 - 1.0$ mM) was incubated with stimulated mononuclear cells. Also mononuclear cells were incubated with LPS alone. Purified PGHS-2 protein (Cayman Chemicals) was used as control. (b) In the same manner the effects of racemic ibuprofen ($0.01 - 1.0$ mM) and **R**-ibuprofenoyl-CoA thioester ($0.01 - 0.5$ mM) on the expression of PGHS-2 protein were compared. (c) The inhibitory effects of increasing concentrations of **R**-ibuprofenoyl-CoA thioester ($0.01 - 0.5$ mM) and **S**-ibuprofenoyl-CoA thioester ($0.01 - 0.5$ mM) on PGHS-2 protein expression in stimulated mononuclear cells are shown.

0.67 and 15 (Meade et al., 1993; Mitchell et al., 1993; Laneuville et al., 1994; Gierse et al., 1995; Brideau et al., 1996; Young et al., 1996). Recently, Patrignani et al. (1994) and Panara et al. (1995) presented a simple system based on expression of PGHS-1 and PGHS-2 in platelets and LPS-stimulated human whole blood, respectively. Potential advantages and disadvantages of the different models which have been used for the assessment of PGHS-1/PGHS-2 inhibition have been discussed in detail in the literature (Patrignani et al., 1994; Panara et al., 1995; Jouzeau et al., 1997).

Using the whole blood assay, we evaluated the potency and selectivity of ibuprofen enantiomers to inhibit PGHS-1 and PGHS-2. As an unusual phenomenon of ibuprofen metabolism is represented by the unidirectional chiral inversion from **R**- to **S**-ibuprofen (Wechter et al., 1974, 1994; Caldwell et al., 1988; Williams, 1990), we also tested the intermediate products

of chiral inversion namely the **R**- and **S**-ibuprofenoyl-CoA thioesters. The mechanism of chiral inversion involves three metabolic steps: after thioesterification of **R**-ibuprofen with CoA (Nakamura *et al.*, 1981) via an adenylate intermediate (Menzel *et al.*, 1994) the **R**-ibuprofenoyl-CoA thioester epimerizes via a recently characterized enzyme (Reichel *et al.*, 1997). Hydrolysis of the CoA thioesters occurs in a nonstereoselective manner (Figure 1). We show here, for the first time, that the intermediate product of the inversion process of ibuprofen, namely the **R**-ibuprofenoyl-CoA thioester, inhibited PGE$_2$ production from LPS-induced monocytes (as a reflection of PGHS-2 activity) approximately two orders of magnitude more potently than generation of TXB$_2$, as a reflection of PGHS-1 activity (IC$_{50}$ 5.6 vs 219 μM). The idea that CoA-activated substances may inhibit prostaglandin biosynthesis originates from the study by Fujimoto *et al.* (1992). They described the inhibition of prostaglandin synthesis by palmitoyl-CoA thioester in rabbit kidney microsomes. The respective free acid did not inhibit prostaglandin biosynthesis in this model. These results are in line with our data where palmitoyl-CoA thioester was used as a reference compound (Table 1). CoA, used as a negative control had no effect on the activities of PGHS-1 or PGHS-2 up to 200 μM. Thus the observed effects can be assigned to the CoA activated fatty acid.

The inhibition of PGHS activity by some NSAIDs is time-dependent (Laneuville *et al.*, 1994) and, consequently, the present studies were conducted over 24 h (Patrignani *et al.*, 1994; Panara *et al.*, 1995). However, it could then be argued that the effect of the ibuprofenoyl-CoA thioesters was mediated by the free acids following hydrolysis (half-life of hydrolysis about 30 h). This explanation can be excluded for the **R**-ibuprofenoyl-CoA thioester, since the free acid, **R**-ibuprofen did not affect the activity of PGHS-2 (IC$_{50,PGHS-2}$ >250 μM vs 5.6 μM for **R**-ibuprofenoyl-CoA thioester). By contrast the 10 fold greater potency of **S**-ibuprofenoyl-CoA compared to **R**-ibuprofenoyl-CoA can be explained by partial hydrolysis of the thioester. Furthermore, in keeping with these data, both ibuprofenoyl-CoA thioesters inhibited the expression of PGHS-2 in a concentration-dependent manner, as determined

by Western Blot analysis of LPS-stimulated human monocytes (Figure 3). The nonactivated enantiomers of ibuprofen did not show inhibition of PGHS-2 expression. These are the first data to our knowledge to demonstrate inhibition of PGHS-2 expression by a metabolite of a commercially available NSAID.

Since the concentrations of the test compounds were in the μM range, our results may also be of clinical relevance. It has been assumed that **R**-ibuprofen is an isomeric ballast only contributing to the therapeutic effects of ibuprofen racemate by chiral inversion to **S**-ibuprofen (Williams, 1990; Malmberg & Yaksh, 1995). Our results confirm previous data that indeed **R**-ibuprofen neither inhibits PGHS-2 nor PGHS-1 at therapeutically relevant concentrations (Boneberg *et al*, 1996). However, the present data further suggest that **R**-ibuprofen may contribute to the therapeutic effects of the racemate following metabolism to the CoA thioester. A prerequisite of this hypothesis is that concentrations of the ibuprofenoyl-CoA esters, formed during chiral inversion are in the μM range. However, so far, no data are available focusing on the determination of ibuprofenoyl-CoA esters following administration of clinically used doses of ibuprofen racemate. Moreover, parameters of ibuprofenoyl-CoA esters such as protein binding are also lacking. Thus, further studies should aim at the assessment of the pharmacokinetic profile of the metabolites of ibuprofen inversion.

One may speculate that inhibition of PGHS-2 induction by **R**- and **S**-ibuprofenoyl-CoA thioesters may be one reason why ibuprofen racemate is ranked as one of the safest NSAIDs, as determined by epidemiological studies (Henry *et al.*, 1996). Further work is needed to study the mechanism of inhibition of PGHS-2 induction and whether or not the observed results are unique for ibuprofen or also hold true for other 2-aryl-propionic acids,

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