Human Pharmacology of Naproxen Sodium

Marta L. Capone, Stefania Tacconelli, Maria G. Sciulli, Paola Anzellotti, Luigia Di Francesco, Gabriele Merciaro, Patrizia Di Gregorio, and Paola Patrignani

Department of Medicine and Center of Excellence on Aging, School of Medicine, “G. d’Annunzio” University, Chieti, Italy (M.L.C., S.T., M.G.S., P.A., L.D.F., P.P.); “G. d’Annunzio” University Foundation, Ce.S.I., Chieti, Italy (M.L.C., S.T., M.G.S., P.A., L.D.F., P.P.); and SS Annunziata Hospital, Chieti, Italy (G.M., P.D.G.)

Received March 5, 2007; accepted April 30, 2007

ABSTRACT

We compared the variability in degree and recovery from steady-state inhibition of cyclooxygenase (COX)-1 and COX-2 ex vivo and in vivo and platelet aggregation by naproxen sodium at 220 versus 440 mg b.i.d. and low-dose aspirin in healthy subjects. Six healthy subjects received consecutively naproxen sodium (220 and 440 mg b.i.d.) and aspirin (100 mg daily) for 6 days, separated by washout periods of 2 weeks. COX-1 and COX-2 inhibition was determined using ex vivo and in vivo indices of enzymatic activity: 1) the measurement of serum thromboxane (TX)B2 levels and whole-blood lipopoly-saccharide-stimulated prostaglandin (PG)E2 levels, markers of COX-1 in platelets and COX-2 in monocytes, respectively; 2) the measurement of urinary 11-dehydro-TXB2 and 2,3-dinor-6-keto-PGF1α levels, markers of systemic TXA2 biosynthesis (mostly COX-1-derived) and prostacyclin biosynthesis (mostly COX-2-derived), respectively. Arachidonic acid (AA)-induced platelet aggregation was also studied. The maximal inhibition of platelet COX-1 (95.9 ± 5.1 and 99.2 ± 0.4%) and AA-induced platelet aggregation (92 ± 3.5 and 93.7 ± 1.5%) obtained at 2 h after dosing with naproxen sodium at 220 and 440 mg b.i.d., respectively, was indistinguishable from aspirin, but at 12 and 24 h after dosing, we detected marked variability, which was higher with naproxen sodium at 220 mg than at 440 mg b.i.d. Assessment of the ratio of inhibition of urinary 11-dehydro-TXB2 versus 2,3-dinor-6-keto-PGF1α showed that the treatments caused a more profound inhibition of TXA2 than prostacyclin biosynthesis in vivo throughout dosing interval. In conclusion, neither of the two naproxen doses mimed the persistent and complete inhibition of platelet COX-1 activity obtained by aspirin, but marked heterogeneity was mitigated by the higher dose of the drug.

Recent lines of evidence sustain that aspirin cardioprotection is the result of an almost complete and persistent suppression of platelet thromboxane (TX)A2 biosynthesis throughout the dosing interval, for irreversible inhibition of platelet cyclooxygenase (COX)-1 activity (Patrignani et al., 1982; Patrono et al., 2005). In fact, even tiny concentrations of TXA2 can cause platelet activation (Maree et al., 2005; Pulcinelli et al., 2005; Minuz et al., 2006; Sciulli et al., 2006). All other reversible COX inhibitors, i.e., traditional nonsteroidal anti-inflammatory drugs (tNSAIDs) and NSAIDs selective for COX-2 (coxibs), cause an incomplete and intermittent inhibition of platelet COX-1 insufficient to afford cardioprotection (Patrignani et al., 2001, 2005). In a recent meta-analysis of randomized clinical trials, it has been reported that high dose of the tNSAID naproxen—with a balanced inhibitory effect on COX-1 and COX-2 (Capone et al., 2007)—confers a smaller cardiovascular risk than high-dose regimens of some tNSAIDs, such as diclofenac and ibuprofen ( Kearney et al., 2006). The meta-analysis of Kearney et al. (2006) could not establish whether high-dose naproxen confers a small cardiovascular benefit because of the substantial statistical uncertainty surrounding the point estimate of rate ratio versus placebo. However, it is reasonable to assume that the sustained inhibition of platelet COX-1 ex vivo, in some but not all individuals, by naproxen administration at high doses b.i.d., continuously and regularly (Capone et al., 2004), as it occurs inside the rigor of randomized clinical trials, might be associated with a modest benefit. In contrast to aspirin, the persistence of the inhibitory effect on platelet COX-1 by naproxen can be affected in real life for reduced compliance. This is consistent with the uncertainty of the results of epidemiological studies, showing that naproxen is neutral or somewhat cardioprotective (Hernández-Díaz et al., 2006; McGettigan and Henry, 2006). The possible small

ABBREVIATIONS: TX, thromboxane; COX, cyclooxygenase; tNSAID, traditional nonsteroidal anti-inflammatory drug; NSAID, nonsteroidal anti-inflammatory drug; ADAPT, Alzheimer’s Disease Anti-Inflammatory Prevention Trial; LPS, lipopolysaccharide; PG, prostaglandin; AA, arachidonic acid.
benefit of naproxen might be undermined by the occurrence of intra- and intersubject variability in degree and duration of COX-1 and -2 inhibition, which might lead to a time-dependent divergence in the consequences of suppressing one versus the other COX enzyme (Grosser et al., 2006). Genetic sources of variance, such as polymorphisms detected in COX-1 and cytochrome P450 CYP2C9 (the principal metabolizing enzyme of a wide range of coxibs and NSAIDs, such as naproxen), have been suggested to participate in marked variation in individual response to the COX-2 inhibitors rofecoxib and celecoxib in healthy subjects (Fries et al., 2006).

Different formulations of naproxen, which differ in the pattern of absorption, are available. Naproxen sodium, characterized by a more rapid absorption from the gastrointestinal tract (Brunton et al., 2006), has recently been in the spotlight, because it was administered in the prematurely terminated placebo-controlled trial Alzheimer’s Disease Anti-Inflammatory Prevention Trial [Cardiovascular and Cerebrovascular Events in the Randomized, Controlled Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT), 2006]. The trial involved three treatment arms: low-dose naproxen sodium (220 mg b.i.d.), celecoxib (200 mg b.i.d.), and placebo, acting as a control. The termination of both the celecoxib and naproxen arms of the ADAPT trial “reflected the ADAPT investigators’ reluctance to imply, by continuing the trial, that naproxen was safer than celecoxib when ADAPT data did not support this conclusion” (Cardiovascular and Cerebrovascular Events in the Randomized, Controlled Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT), 2006).

Data on human pharmacology of low-dose naproxen are not available. Thus, in the present study, we explored the variability in degree and recovery from steady-state inhibition of COX-1 and COX-2 both ex vivo and in vivo by two therapeutic doses of naproxen sodium (220 and 440 mg b.i.d.) versus aspirin (100 mg daily) in healthy subjects. These effects were compared with the impact on platelet function by the different therapies.

### Materials and Methods

#### Study Design, Treatments, and Assessment

The study protocol was approved by the Ethic Committee of “G. d’Annunzio” University. Written informed consent was obtained from six healthy subjects. The subjects were between 23 and 30 years of age and within 30% of ideal body weight. They also had an unremarkable medical history, physical examination, and routine hematological and biochemical screen. Smokers and subjects with a bleeding disorder, an allergy to aspirin or any other NSAID, or a history of any gastrointestinal or cerebrovascular disease were excluded. Subjects abstained from the use of aspirin and other NSAIDs for at least 2 weeks before enrollment. The six healthy subjects received consecutively naproxen sodium (Roche, S.p.A., Milan, Italy) 220 mg b.i.d., naproxen sodium 440 mg b.i.d. and aspirin (Bayer AG, Milan, Italy) 100 mg daily for 6 days, separated by washout periods of at least 2 weeks. Blood samples were collected before dosing and on the 6th day after the last dose of the different treatments (at 1, 2, 5, 8, 12, and 24 h after dosing) to assess the inhibition of serum TXB2 (a capacity index of platelet COX-1 activity) (Patrono et al., 1980), lipopolysaccharicide (LPS)-induced prostaglandin (PG)E2 production (a capacity index of monocyte COX-2 activity) (Patrignani et al., 1994), and platelet aggregation induced by 2 mM arachidonic acid (AA) in platelet-rich plasma (Pedersen and FitzGerald, 1985). Urinary samples were collected before treatment (from 8:00 PM to 8:00 AM) and on the 6th day after the last dose of the different treatments from 8:00 AM to 12:00 PM, 12:00 PM to 4:00 PM, 4:00 PM to 8:00 PM, and 8:00 PM to 8:00 AM to evaluate the urinary excretion of 11-dehydro-TXB2 (a major enzymatic metabolite of TXB2 that is an index of systemic TXA2 biosynthesis in vivo mainly platelet COX-1-derived) (Catella and FitzGerald, 1987; Ciabattoni et al., 1989) and of 2,3-dinor-6-keto PGF1α, (a major enzymatic metabolite of prostacyclin that is an index of systemic prostacyclin biosynthesis, mainly vascular COX-2-derived) (FitzGerald et al., 1983; Catella-Lawson et al., 1999; McAdam et al., 1999). Immunoreactive TXB2, PGE2, 11-dehydro-TXB2, and 2,3-dinor-6-keto PGE2 were measured (Patrignani et al., 1980; Ciabattoni et al., 1987; Minuz et al., 1988; Patrignani et al., 1994). Blood concentrations of naproxen were also evaluated (Slattery and Levy, 1979; Santini et al., 1996).

**Platelet TXB2 Production in Whole Blood.** Duplicate whole-blood samples (3 ml) were collected by venipuncture into glass vacutainers containing no anticoagulant and immediately allowed to clot for 1 h at 37°C. Serum was collected after centrifugation at 3000 rpm for 10 min, and it was stored at −80°C until assayed for TXB2 (Patrignani et al., 1980).

**LPS-Stimulated PGE2 Production in Whole Blood.** Duplicate 1-ml aliquots of 10 μM heparinized blood samples were incubated in polypropylene tubes (containing 50 μg of dry aspirin) for 24 h at 37°C in the absence or the presence of LPS (Escherichia coli 026:B6; Sigma-Aldrich, St. Louis, MO) at 10 μg/ml. Plasma was separated by centrifugation at 2000 rpm for 10 min, and it was kept at −80°C until assayed for PGE2 (Patrignani et al., 1994).

**In Vitro Study.** Naproxen sodium (0.02−90 μM), dissolved in saline, was incubated with 1-ml aliquots of human whole blood withdrawn from the same subjects in the absence and in the presence of 10 μM/1ml sodium heparin for 1 h or 24 h with 10 μM LPS, respectively. Serum and plasma samples were assayed for TXB2 and PGE2, respectively (Patrignani et al., 1980; Patrignani et al., 1994).

**Eicosanoid Analyses.** Urinary 11-dehydro-TXB2 and 2,3-dinor-6-keto-PGF1α, plasma PGE2 and serum TXB2 were assessed and validated by previously described radioimmunoassays (Patrignani et al., 1980; Ciabattoni et al., 1987; Minuz et al., 1988; Patrignani et al., 1994).

**Naproxen Blood Levels.** Aliquots of 5 μl of serum samples were added to 195 μl of methanol/water [50:50 (v/v)] and injected directly into a Nova-Pak C18 column (Waters, Milford, MA) of a Beckman System Gold high-performance liquid chromatography (Slattery and Levy, 1979; Santini et al., 1996). The mobile phase consisted of acetonitrile/acetate acid [100:0.1 (v/v)] and water/acetate acid [100:0.1 (v/v)], as follows: 60 and 40%, respectively, at a flow rate of 1 ml/min. Absorbance was assessed at 250 nm. Naproxen eluted with a retention time of 5.5 min.

**Statistical Analysis.** The data are expressed as mean ± S.D. The primary endpoint of the present study was the assessment of serum TXB2 levels ex vivo; the secondary endpoints were the assessment of turbidometric platelet aggregation induced by AA, the urinary excretion of 11-dehydro-TXB2 and 2,3-dinor-6-keto PGF1α, and LPS-induced PGE2 production ex vivo. The primary hypothesis was that the administration of naproxen at 220 mg b.i.d. would cause a lower inhibition of platelet COX-1 activity versus naproxen at 440 mg b.i.d. and aspirin 100 mg daily, as assessed by the measurement of serum TXB2 on day 6 of therapy. It was anticipated on the basis of previous studies that a sample size of six (six per treatment) would afford a power in excess of 90% to detect a difference of 20% or greater in serum TXB2 measurements with two-tailed tests of the hypothesis associated with a type I error rate of less than 0.05 for all the main effects (Catella-Lawson et al., 2001). Values were compared by means of an analysis of variance model for repeated measures, using the PROC MIXED model (SAS Institute, Inc., Cary, NC). Due to marked heterogeneity in response to naproxen, primarily at 12 and 24 h after dosing, data did not pass normality test (by the method Kolmogorov and Smirnov) (Young, 1977) on some occasions; thus, we analyzed the data also by nonparametric tests, i.e., Friedman test.
and Wilcoxon matched pairs test. A probability value of $P < 0.05$ was considered to be statistically significant. Concentration-response curves were fitted, and $IC_{50}$ (drug concentration required for obtaining 50% inhibition) values were analyzed with Prism (GraphPad Software Inc., San Diego, CA).

**Results**

The primary objective of the study was to compare the degree of steady-state inhibition and time-dependent recovery of platelet COX-1 activity ex vivo by two analgesic doses of naproxen sodium, i.e., 220 and 440 mg b.i.d., versus a cardioprotective dose of aspirin, i.e., 100 mg daily. Serum TXB$_2$ levels assessed in the six subjects, on three consecutive free-drug occasions, i.e., before treatment with naproxen sodium at 220 mg b.i.d. and 440 mg b.i.d. or aspirin at 100 mg daily, were not significantly different (277 ± 127, 322 ± 197, and 323 ± 151 ng/ml, respectively). Two hours after the last administration of naproxen sodium at 220 and 440 mg, serum TXB$_2$ levels were comparably reduced by 95.9 ± 5.1 and 99.2 ± 0.4%, respectively ($P < 0.01$ versus predrug values). These values were not significantly different from the average of inhibition detected at 1 and 24 h after the last dose of aspirin, i.e., 99.1 ± 0.9 and 98.7 ± 1%, respectively (Fig. 1A; Table 1). We set the lowest limit of inhibition of platelet COX-1 activity by aspirin at 97% (indicated by the broken line in Fig. 1A), as estimated by mean minus 2 S.D. of values measured at 1 and 24 h after dosing.

In contrast to aspirin, the suppression of platelet COX-1 activity by naproxen sodium recovered in a time- and dose-dependent manner (Fig. 1A; Table 1). In fact, at 12 and 24 h after dosing with naproxen sodium at 220 mg b.i.d., the degree of inhibition of serum TXB$_2$ was significantly ($P < 0.01$) lower than that detected at the corresponding times after naproxen at 440 mg b.i.d. and after aspirin as well (Fig. 1A; Table 1). The inhibition of serum TXB$_2$ by the administration of naproxen sodium at 440 mg b.i.d. was significantly ($P < 0.05$) different from aspirin only at 12 h after dosing, but the use of a nonparametric test showed a statistically significant divergence at 24 h as well (Fig. 1A; Table 1).

The values of serum TXB$_2$ detected at the different times after dosing with the three treatments are reported in Fig. 1B. In contrast to aspirin, marked heterogeneity in serum TXB$_2$ generation was detected after dosing with naproxen at 220 and 440 mg b.i.d. The frequency of samples with serum TXB$_2$ levels higher than 10 ng/ml (corresponding to the upper extreme value of TXA$_2$ generated in whole blood of healthy subjects with complete inhibition of platelet COX-1 activity by aspirin; Sciulli et al., 2006) increased in a time-dependent manner. After naproxen at 220 and 440 mg b.i.d., serum TXB$_2$ values started to move away from aspirin response, in a statistically significant manner, at 5 and 12 h after dosing, respectively (Fig. 1B).

**Fig. 1.** Comparison of degree and duration of steady-state inhibition of COX-1 activity and platelet function ex vivo by naproxen sodium at 220 and 440 mg b.i.d. or low-dose aspirin for 6 days. A, inhibition of platelet COX-1 activity ex vivo, as assessed by the measurement of serum TXB$_2$ in six healthy subjects. The open symbols represent the values detected in each individual, whereas the closed symbols represent the mean ± S.D. $\dagger$, $P < 0.05$ versus aspirin at 440 mg b.i.d. at corresponding times; $\ddagger$, $P < 0.05$ and $\dagger\ddagger$, $P < 0.01$ versus aspirin at 24 h; broken line indicates inhibition of platelet COX-1 activity by 97%. Using a nonparametric test, $\dagger$, $P < 0.05$ versus aspirin 24 h. B, serum TXB$_2$ levels detected in each individual up to 24 h after dosing with the different treatments (open symbols). The colored closed symbols represent the mean ± S.D. The broken line indicates the serum TXB$_2$ value of 10 ng/ml. $\dagger$, $P < 0.05$ and $\ddagger$, $P < 0.01$ versus aspirin at 24 h; C, inhibition of AA-induced platelet aggregation detected in six healthy subjects up to 24 h after dosing with the different treatments (open symbols). The colored closed symbols represent the mean ± S.D. The broken line indicates the serum TXB$_2$ value of 10 ng/ml. $\dagger$, $P < 0.05$ and $\ddagger$, $P < 0.01$ versus aspirin at 24 h.
To verify whether time-dependent recovery of platelet COX-1 activity from steady-state inhibition by naproxen translated into a functional effect, we studied platelet aggregation induced by AA. As shown in Fig. 1C, aspirin caused a statistically significant ($P < 0.01$ versus predrug values) reduction of AA-induced platelet aggregation at 1 h, which persisted up to 24 h after dosing. None of subjects responded to AA after dosing with 100 mg of aspirin for 6 days. In contrast, at 12 and 24 h after naproxen sodium at 220 mg and at 24 h after naproxen sodium at 440 mg, platelet function was not significantly reduced versus predrug values. The inhibition of AA-induced platelet aggregation recorded at 24 h after naproxen sodium at 220 mg b.i.d., but not after naproxen sodium at 440 mg b.i.d., was significantly ($P < 0.05$) different from aspirin (Fig. 1C). At 24 h after naproxen at 220 mg, naproxen at 440 mg, and aspirin, the number of subjects who responded to AA with a complete aggregation in platelet-rich plasma was four of six, one of six, and zero of six subjects who responded to AA after dosing with 100 mg of aspirin for 6 days. In contrast, at 12 and 24 h after naproxen sodium at 220 mg and at 24 h after naproxen sodium at 440 mg, platelet function was not significantly reduced versus predrug values. The inhibition of AA-induced platelet aggregation recorded at 24 h after naproxen sodium at 220 mg b.i.d., but not after naproxen sodium at 440 mg b.i.d., was significantly ($P < 0.05$) different from aspirin (Fig. 1C). At 24 h after naproxen at 220 mg, naproxen at 440 mg, and aspirin, the number of subjects who responded to AA with a complete aggregation in platelet-rich plasma was four of six, one of six, and zero of six subjects, respectively. Interestingly, full platelet aggregation was detected in platelet-rich plasma samples obtained from whole blood that generated TXB$_2$ concentrations $\leq 50$ ng/ml when allowed to clot for 1 h at 37°C.

We then verified whether the intersubject variability in the response to naproxen was driven by fluctuations of circulating drug levels. As shown in Fig. 2A, at each time studied after naproxen at 440 mg b.i.d., circulating drug levels were significantly higher than those detected after naproxen at 220 mg b.i.d. Individual circulating concentrations and the corresponding degree of COX-1 inhibition measured ex vivo at each time point were reported on the same graph depicting the sigmoidal concentration-response curve obtained in vitro (Fig. 2B). Naproxen inhibited platelet COX-1 activity in vitro in a concentration-dependent manner, with an IC$_{50}$ value of 5.8 $\mu$g/ml and IC$_{97}$ value (97% is the lowest limit of platelet COX-1 inhibition by aspirin) of 70 $\mu$g/ml (Fig. 2B). As shown in the same figure, the pharmacokinetic-pharmacodynamic relationship after dosing with naproxen fitted the concentration-response curve for inhibition of platelet COX-1 obtained in vitro. However, marked heterogeneity in drug response was detected at lower circulating drug levels.

We assessed the impact of chronic dosing with naproxen sodium at 220 and 440 mg b.i.d. and aspirin at 100 mg daily on urinary 11-dehydro-TXB$_2$, a biomarker of systemic biosynthesis of TXA$_2$ in vivo—prominently of platelet origin (Cattella and FitzGerald, 1987). At predrug on three different occasions, i.e., before naproxen sodium at 220 mg, 440 mg, and aspirin, overnight urinary excretion of 11-dehydro-TXB$_2$ did not differ in a statistically significant manner (400 $\pm$ 138, 431 $\pm$ 108, and 428 $\pm$ 114 pg/mg creatinine, respectively). On day 6, after the last administration of aspirin and naproxen sodium at 220 and 440 mg, systemic TXA$_2$ biosynthesis was

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**TABLE 1**

Effects of naproxen at 220 and 440 mg b.i.d. or aspirin at 100 mg daily on COX-1 and COX-2 activities ex vivo

Values are reported as mean ± S.D.

<table>
<thead>
<tr>
<th>Time</th>
<th>Naproxen, 220 mg b.i.d.</th>
<th>Naproxen, 440 mg b.i.d.</th>
<th>Aspirin, 100 mg Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>95.9 ± 5.1</td>
<td>99.2 ± 0.4</td>
<td>99.1 ± 0.9</td>
</tr>
<tr>
<td>2 h</td>
<td>90.8 ± 8.6</td>
<td>98.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>5 h</td>
<td>88.9 ± 10</td>
<td>96.7 ± 1</td>
<td></td>
</tr>
<tr>
<td>8 h</td>
<td>86.6 ± 7.1$^*$</td>
<td>92.9 ± 3.1$^*$</td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td>69.1 ± 19.9$^*$</td>
<td>85.3 ± 5.1$^*$</td>
<td>98.7 ± 1</td>
</tr>
<tr>
<td>24 h</td>
<td>75.8 ± 7.4$^*$</td>
<td>87.5 ± 5.8$^*$</td>
<td></td>
</tr>
</tbody>
</table>

# Inhibition of LPS-Induced PGE$_2$ (COX-2 Activity ex Vivo)

<table>
<thead>
<tr>
<th>Time</th>
<th>1 h</th>
<th>2 h</th>
<th>5 h</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>86.7 ± 10$^{**}$</td>
<td>86.7 ± 11**</td>
<td>86.2 ± 15**</td>
<td>75.8 ± 7.4$^{**}$</td>
<td>67.8 ± 16**</td>
<td>1.3 ± 3</td>
</tr>
</tbody>
</table>

$^*$ $P < 0.05$, $^{**}P < 0.01$ versus aspirin at 24 h.
$^*$ $P < 0.01$ and $^{**}P < 0.05$ versus naproxen at 440 mg b.i.d. at the corresponding times (nonparametric test).
$P < 0.05$ (parametric test).
$P < 0.05$ versus aspirin at 24 h (nonparametric test).

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profoundly and persistently depressed versus overnight predrug levels ($P < 0.01$). As shown in Fig. 3A, in urine samples collected from 12 to 24 h after dosing with naproxen at 220 mg b.i.d., the degree of inhibition of 11-dehydro-TXB$_2$ was significantly ($P < 0.01$) lower than that detected at 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h after the last administration of naproxen at 440 mg b.i.d. The degree of inhibition of the urinary excretion of 11-dehydro-TXB$_2$ by naproxen sodium at 440 mg—recorded at each period of collection—was slightly higher versus the other treatments, but it was significantly ($P < 0.01$) different only versus the inhibition detected in the urine samples collected from 12 to 24 h after dosing with naproxen sodium at 220 mg, and from 4 to 12 h after dosing with aspirin (Fig. 3A). Thus, similarly to the results obtained ex vivo, naproxen dose elevation homogenized the inhibitory effect on TXA$_2$ generation in vivo. In contrast to the results obtained ex vivo, it seemed to be a dose dependence for inhibition of urinary 11-dehydro-TXB$_2$ by naproxen.

In the same urine collections, we assessed the levels of 2,3-dinor-6-keto-PGF$_{1\alpha}$, an index of systemic prostacyclin biosynthesis (Catella-Lawson et al., 1999; McAdams et al., 1999). At predrug on three different occasions, i.e., before naproxen sodium at 220 mg, 440 mg, and aspirin, overnight urinary 2,3-dinor-6-keto-PGF$_{1\alpha}$ did not differ in a statistically significant manner (83 ± 25, 100 ± 44, and 79 ± 20 pg/mg creatinine, respectively). Aspirin did not significantly affect systemic prostacyclin biosynthesis (Fig. 3B). The urinary excretion of the prostacyclin metabolite was significantly reduced by the treatment with naproxen sodium at 220 and 440 mg ($P < 0.01$ versus overnight predrug values). Although the average degree of inhibition of the urinary excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$ after dosing with naproxen sodium at 440 mg was higher versus 220 mg b.i.d., the differences between the two treatments were statistically significant only in urine samples collected from 8 to 12 h. However, the proportion of urine samples with degree of inhibition of prostacyclin biosynthesis >50% detected in the 24-h period was significantly ($P < 0.01$) higher in subjects treated with naproxen at 440 mg (18 of 24) than with 220 mg (8 of 24) (Fig. 3B).

The assessment of LPS-induced whole-blood PGE$_2$ generation, a marker to predict drug effects (analgesia) in humans, was performed to verify whether naproxen sodium at 220 and 440 mg b.i.d. were comparable doses for efficacy. In fact, it has been reported that IC$_{50}$ evaluated in vitro correlates directly with the analgesic plasma concentrations of different COX inhibitors (Huntjens et al., 2005). Naproxen inhibited LPS-induced PGE$_2$ generation in vitro in a concentration-dependent manner, with IC$_{50}$ and IC$_{80}$ values of 15 and 30 μg/ml, respectively (Fig. 2B). As shown in Fig. 2A, at both naproxen doses, blood levels were almost always ≥IC$_{50}$ for COX-2 inhibition in vitro throughout the 12-h dosing interval. This was confirmed by the results obtained ex vivo, showing that the proportion of samples with PGE$_2$ levels reduced at clinically relevant ranges (i.e., ≥50%) were 18 of 24 and 24 of 24 after naproxen sodium at 220 and 440 mg, respectively (Fig. 4).

We verified the occurrence of time-dependent divergence of COX selectivity achieved ex vivo and in vivo by the two naproxen doses. The degree of selectivity for COX-2 by naproxen in vitro—a chemical property of the drug—expressed as ratio of IC$_{50}$ values showed that the drug is 2.6-fold more potent for COX-1 than COX-2 (Fig. 2B). However, pharmacokinetic and pharmacodynamic variations between individuals would be expected to affect the degree of COX selectivity actually attained in humans, which can be described by the ratio of COX-1 inhibition versus COX-2 inhibition at any given plasma concentration. When the degree of selectivity attained in subjects was estimated using this measure, naproxen sodium at 220 and 440 mg b.i.d. inhibited COX-1 and COX-2 ex vivo similarly throughout the 24 h. In fact, the ratio of COX-1 inhib-
because urinary 11-dehydro-TXB₂ was persistently reduced by higher in urine collected from 4 to 24 h versus the first 4 h, doses. We found that the ratio was significantly (dosing with aspirin, which is consistent with its preferential, vivo (Fig. 5B). However, the highest ratios were detected after aspirin, whereas prostacyclin metabolite excretion was reversibly reduced in some subjects. In urine samples collected after dosing with naproxen at 220 and 440 mg b.i.d., inhibitory ratio dehydro-TXB₂ inhibition (mostly COX-1-derived) (Catella and low-dose aspirin at 100 mg daily for six consecutive days. The open symbols represent the degree of inhibition of plasma PGE₂ detected in each individual, whereas the colored closed symbols represent the mean ± S.D. In red, green, and blue are the reported mean values of LPS-induced PGE₂ inhibition by naproxen at 220 mg b.i.d., 440 mg b.i.d., and low-dose aspirin, respectively. **, P < 0.01 versus aspirin at 1 and 24 h. Using a nonparametric test, §, P < 0.01 and #, P < 0.05 versus naproxen at 440 mg b.i.d. at the corresponding times.

Discussion

Results of human pharmacology have shed some light on the phenotypes induced by aspirin and selective inhibitors of COX-2 translating into opposite risk of thrombotic events: reduced risk by the former and increased risk by the latter (Rodríguez and Patrignani, 2006). Aspirin affords cardioprotection because of two concurrent effects on platelet COX-1, the only COX isoform expressed in mature platelets (Patrignani et al., 1999): 1) complete inhibition and 2) persistence of this effect throughout dosing interval (Patrino et al., 2005). Partial or no reduction of platelet COX-1 by selective COX-2 inhibitors is unable to counter the thrombotic risk associated with profound suppression (≥60%) of COX-2-dependent prostacyclin, which acts as a constraint on endogenous mediators of platelet activation, hypertension, atherogenesis, and cardiac dysfunction (Grosser et al., 2006).

In the present study, we showed that 97% is the lowest limit of inhibition of platelet COX-1 activity by aspirin. This value corresponds to the mean minus 2 S.D. of serum TXB₂ inhibition detected in 40 samples obtained from healthy volunteers treated with 100 mg of aspirin daily, participating in clinical studies that we previously performed (Capone et al., 2004, 2005). Importantly, the occurrence of ≥97% suppression of platelet COX-1 activity was always associated with levels of serum TXB₂ ≥10 ng/ml and a complete suppression of AA-induced platelet aggregation (Scuilli et al., 2006; this study).

Varied scenarios are associated with different tNSAIDs, because they are a cluster of compounds with a wide spectrum of COX selectivity, as assessed in vitro using whole blood assays (Patrino et al., 1980; Patrignani et al., 1994), ranging from drugs with balanced inhibitory effect on COX-1 and COX-2 (e.g., profen and naproxen) to selective inhibitors of COX-2, such as diclofenac (Capone et al., 2007). For the tNSAIDs causing balanced, profound inhibition of the activity of both COX isoforms, the thrombotic risk associated with the suppression of prostacyclin can be neutralized when platelet COX-1 is inhibited to functional range, i.e., ≥97% (Rodriguez and Patrignani, 2006). This effect can be realized at peak plasma concentrations because they are often administered at higher doses than the minimal efficacious dose required for analgesic/anti-inflammatory effects (Capone et al., 2007). Although it has been shown that plasma levels corresponding to the IC₅₀ of exposure-response relationships in vitro for inhibition of LPS-stimulated PGE₂ in whole blood are associated with efficacy (Huntjens et al., 2005), still the selection of doses are driven by clinical endpoints that do not have the power to detect precisely differences among doses (Patrino et al., 2001).

The reversible nature of the interaction of tNSAIDs with COX-1, leads to a time-dependent recovery of platelet TXA₂ generation from steady-state inhibition. This translates into an intermittent suppression of platelet COX-1, throughout the dosing interval, that is inconsistent with cardioprotection. Time-dependent recovery of platelet COX-1 activity can be restrained by the administration of these drugs at intervals shorter than the pharmacokinetic half-life. This is realized by naproxen, which has a pharmacokinetic half-life >12 h (Brunton et al., 2006), administered at high doses b.i.d. However, we showed that naproxen at 500 mg b.i.d. suppresses platelet COX-1 at degree and duration comparable with aspirin only in some, but not all, subjects (Capone et al., 2004), which is compatible with no increased risk or a small reduced risk shown in observational studies (Hernández-Díaz et al., 2006; McGettigan and Henry, 2006). However, the prematurely terminated ADAPT trial [Cardiovascular and Cerebrovascular Events in the Randomized, Controlled Alzheimer’s Disease Anti-Inflammatory Prevention Trial (ADAPT), 2006] led some to speculate that the modest car-
dioprotection detected with naproxen at 500 mg b.i.d. (Hernández-Díaz et al., 2006; Kearney et al., 2006; McGettigan and Henry, 2006) might be dissipated at lower doses. To address this issue, we performed a clinical study comparing the degree and duration of platelet COX-1 inhibition by naproxen sodium at 220 versus 440 mg b.i.d. and aspirin at 100 mg daily. Our results showed that neither of the two naproxen doses mimed the inhibition of platelet COX-1 activity achieved by aspirin, with the major differences on the persistence of the inhibitory effect. Importantly, the maximal inhibition of platelet COX-1 and AA-induced platelet aggregation obtained at 2 h after dosing with naproxen sodium at 220 and 440 mg b.i.d. was indistinguishable in a statistically significant manner, but at 12 and 24 h after dosing, we detected marked variability that was higher with naproxen sodium at 220 mg b.i.d. than at 440 mg b.i.d. At these time points after both naproxen doses, serum TXB₂ levels were frequently higher than 10 ng/ml, which corresponds to the highest value detectable in healthy subjects when complete inhibition of platelet COX-1 occurs (Sciulli et al., 2006). Importantly, time-dependent recovery of TXA₂ biosynthesis from steady-state inhibition by naproxen at 220 mg b.i.d. was detected both ex vivo and in vivo, and it was associated with complete restoration of AA-induced platelet aggregation in some individuals. Large size studies will be required to determine the different sources of variance participating in heterogeneity of platelet COX-1 inhibition by naproxen sodium, such as the occurrence of polymorphisms in COX-1 and in CYP2C9, a major pathway of naproxen metabolism (Brunton et al., 2006). However, we showed that naproxen blood levels (total: bound and unbound to plasma proteins) increased in a dose-dependent manner and that they had comparable coefficients of variation at each time after dosing with naproxen at 220 and 440 mg. This suggests that variability in total drug levels is not the cause of marked heterogeneity in drug response. Because higher variability in COX-1 inhibition was detected at lower circulating levels, we suppose the occurrence of intersubject variability in the unbound fraction of naproxen. Naproxen is extensively (approximately 99.7%) bound to plasma protein (Brunton et al., 2006); thus, small intersubject variability in unbound drug concentrations may translate into a detectable effect when they are lower than the minimal effective concentration required for a full pharmacodynamic effect. In fact, variability in platelet COX-1 inhibition increased when circulating blood levels lowered from 70 μg/ml (Fig. 2A), corresponding to the minimal concentration required for complete inhibition by naproxen.

The extensive binding of naproxen to plasma proteins (Brunton et al., 2006) might restrict the compound largely to the plasma compartment. This mirrors the finding that naproxen sodium at 220 mg caused an inhibition of the systemic biosynthesis of TXA₂ comparable with aspirin at 100 mg, a preferential inhibitor of platelet COX-1 (Cipollone et al., 1997) acting mainly in the presystemic circulation (Pedersen and FitzGerald, 1984). In contrast, naproxen sodium at 440 mg b.i.d. caused a slightly higher suppression of 11-

Fig. 5. Time-dependent COX selectivity achieved ex vivo and in vivo by naproxen sodium at 220 and 440 mg b.i.d. or low-dose aspirin. A, COX selectivity achieved ex vivo was determined by estimating the ratio of serum TXB₂ inhibition versus LPS-induced PGE₂ inhibition. The red, green, and blue open symbols represent the COX selectivity achieved ex vivo in each individual, after treatment with naproxen at 220 mg b.i.d., 440 mg b.i.d., or low-dose aspirin, respectively. The broken line represents the ratio of 1, indicating a similar inhibition on COX-1 and -2 activities ex vivo. **, P < 0.01 versus aspirin at 1 and 24 h. B, COX selectivity achieved in vivo was determined by estimating the ratio of urinary 11-dehydro-TXB₂ (mainly COX-1-derived) inhibition versus 2,3-dinor-6-keto-PGF₁α (mainly COX-2-derived) inhibition. The red, green, and blue open symbols represent the COX selectivity achieved in vivo in each individual, after treatment with naproxen at 220 mg b.i.d., 440 mg b.i.d., or low-dose aspirin, respectively. The broken line represents the ratio of 1, indicating a similar inhibition on TXA₂ and PGI₂ synthesis in vivo. #, P < 0.05 versus aspirin at 0 to 4 h; +, P < 0.05 and **, P < 0.01 versus aspirin at the corresponding times of collection.
prostacyclin by naproxen sodium at 220 mg may mitigate the
and/or vascular cells. This is coherent with the results of

References

We thank the students of the School of Medicine, “G. d’Annunzio” University, and the staff of Centro Trasfusionale of the SS Annunziata Hospital, Chieti, Italy.

Acknowledgments

We thank the students of the School of Medicine, “G. d’Annunzio” University, and the staff of Centro Trasfusionale of the SS Annunziata Hospital, Chieti, Italy. 

References


Address correspondence to: Dr. Paola Patrignani, Sezione di Farmacologia, Dipartimento di Medicina e Scienze dell’Invecchiamento, Università di Chieti “G. d’Annunzio”, Via dei Vestini, 31, 66013 Chieti, Italy. E-mail: ppatrignani@unic.it.