

Brief Rapid Communications

Clinical Pharmacology of Platelet, Monocyte, and Vascular Cyclooxygenase Inhibition by Naproxen and Low-Dose Aspirin in Healthy Subjects

Marta L. Capone, PharmD; Stefania Tacconelli, PharmD; Maria G. Sciulli, PhD; Marilena Grana, MD; Emanuela Ricciotti, PharmD; Pietro Minuz, MD; Patrizia Di Gregorio, MD; Gabriele Merciaro; Carlo Patrono, MD; Paola Patrignani, PhD

Background—The current controversy on the potential cardioprotective effect of naproxen prompted us to evaluate the extent and duration of platelet, monocyte, and vascular cyclooxygenase (COX) inhibition by naproxen compared with low-dose aspirin.

Methods and Results—We performed a crossover, open-label study of low-dose aspirin (100 mg/d) or naproxen (500 mg BID) administered to 9 healthy subjects for 6 days. The effects on thromboxane (TX) and prostacyclin biosynthesis were assessed up to 24 hours after oral dosing. Serum TXB₂, plasma prostaglandin (PG) E₂, and urinary 11-dehydro-TXB₂ and 2,3-dinor-6-keto-PGF_{1 α} were measured by previously validated radioimmunoassays. The administration of naproxen or aspirin caused a similar suppression of whole-blood TXB₂ production, an index of platelet COX-1 activity *ex vivo*, by 94 \pm 3% and 99 \pm 0.3% (mean \pm SD), respectively, and of the urinary excretion of 11-dehydro-TXB₂, an index of systemic biosynthesis of TXA₂ *in vivo*, by 85 \pm 8% and 78 \pm 7%, respectively, that persisted throughout the dosing interval. Naproxen, in contrast to aspirin, significantly reduced systemic prostacyclin biosynthesis by 77 \pm 19%, consistent with differential inhibition of monocyte COX-2 activity measured *ex vivo*.

Conclusions—The regular administration of naproxen 500 mg BID can mimic the antiplatelet COX-1 effect of low-dose aspirin. Naproxen, unlike aspirin, decreased prostacyclin biosynthesis *in vivo*. (*Circulation*. 2004;109:1468-1471.)

Key Words: aspirin ■ naproxen ■ thromboxanes ■ eprostenol ■ platelets

Aspirin is the only nonsteroidal antiinflammatory drug (NSAID) known to react covalently with the cyclooxygenase (COX) channel of prostaglandin (PG) G/H synthase-1 and -2 (also referred to as COX-1 and COX-2) through a selective acetylation of a single serine residue (Ser⁵²⁹ in human COX-1 and Ser⁵¹⁶ in human COX-2) that results in the permanent loss of the COX activity of the enzyme.^{1,2} The consistency in dose requirement and saturability of the effects of aspirin in acetylating platelet COX-1, inhibiting thromboxane (TX) A₂ formation, and preventing atherothrombotic complications constitutes the best evidence that the antithrombotic effect of aspirin is largely caused by the suppression of platelet TXA₂ production.^{3,4} However, it is uncertain whether other NSAIDs that act as competitive, reversible inhibitors of both COX-1 and COX-2 share an aspirin-like cardioprotective effect. This question has received considerable attention after publication of the Vioxx Gastrointestinal Outcome Research (VIGOR) trial,⁵ a study of approximately 8000 patients with rheumatoid arthritis

randomized to receive rofecoxib 50 mg/d or naproxen 500 mg BID with a mean duration of follow-up of 9 months. The rates of myocardial infarction were 0.5% and 0.1% in the rofecoxib- and naproxen-treated groups, respectively, raising the possibility of a thrombogenic effect of rofecoxib, a cardioprotective effect of naproxen, and/or the play of chance.⁶ Six of 8 recent observational studies and a meta-analysis of these studies suggest that regular use of naproxen might be associated with a somewhat reduced risk of a first myocardial infarction versus nonuse (L.A. Garcia Rodriguez, Centro Espanol de Investigacion Farmacoepidemiologica, Madrid, Spain, personal communication, October 2003). Because of the paucity of data on the clinical pharmacology of platelet and vascular prostanoid inhibition by naproxen,^{7,8} we performed a crossover, open-label study of low-dose aspirin (100 mg/d) or naproxen (500 mg BID) administered to healthy subjects for 6 days. The primary aim of the study was to compare the extent and duration of steady-state inhibition of platelet COX-1 activity *ex vivo* by low-dose aspirin and

Received December 16, 2003; revision received February 3, 2004; accepted February 6, 2004.

From the Department of Medicine and Center of Excellence on Aging, G. d'Annunzio University, School of Medicine, and G. d'Annunzio University Foundation, Chieti (M.L.C., S.T., M.G.S., M.G., E.R., C.P., P.P.); the Department of Pharmacology, University of Rome La Sapienza (C.P.); SS. Annunziata Hospital, Chieti (P.D., G.M.); and the Department of Biomedical and Surgical Sciences, University of Verona (P.M.), Italy.

Correspondence to Paola Patrignani, PhD, Dipartimento di Medicina e Scienze dell'Invecchiamento, Università G. d'Annunzio, Via dei Vestini 31, 66013 Chieti, Italy. E-mail ppatrignani@unich.it

© 2004 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000124715.27937.78

naproxen. The secondary aim was to evaluate the effects of these drugs on systemic biosynthesis of TXA_2 and prostacyclin in vivo and on monocyte COX-2 activity ex vivo.

Methods

Study Subjects

The study protocol was approved by the Ethics Committee of the G. d'Annunzio University of Chieti. Informed consent was obtained from each subject. The volunteers were 9 healthy subjects (8 men, 1 woman, 23 to 58 years old) with a negative medical history and physical examination and with routine hematological and biochemical parameters within the normal range. Smokers and subjects with a bleeding disorder, allergy to aspirin or any other NSAID, or a history of any gastrointestinal disorder were excluded. Subjects abstained from the use of aspirin and other NSAIDs for at least 2 weeks before enrollment.

Design of the Study

This was a crossover, open-label study of low-dose aspirin (100 mg/d in an enteric-coated formulation, Bayer SpA) or naproxen (500 mg BID, Recordati SpA) for 6 consecutive days, with a washout period of at least 14 days. The inhibition of platelet COX-1 was assessed by measurements of whole-blood TXB_2 production.⁹ Monocyte COX-2 activity was assessed through the measurement of lipopolysaccharide (LPS)-induced PGE_2 production in whole blood.¹⁰ Measurements were performed before and at 1, 12, and 24 hours after the last dose of aspirin and at 3, 12, and 24 hours after the last dose of naproxen. Urinary samples were collected for 12 hours before dosing and in 3 postdosing aliquots: 0 to 6, 6 to 12, and 12 to 24 hours to evaluate the excretion of 11-dehydro- TXB_2 and 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$, major enzymatic metabolites of TXA_2 and PGI_2 , respectively, that are indexes of their systemic biosynthesis in vivo.^{9-11,12}

Biochemical Analyses

Immunoreactive TXB_2 , PGE_2 , 11-dehydro- TXB_2 , and 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$ were measured by previously validated radioimmunoassay techniques.^{9-11,13}

Statistical Analysis

The data are expressed as mean \pm SD. Statistical comparisons were made by ANOVA followed by Student-Newman-Keuls test. A probability value of $P < 0.05$ was considered to be statistically significant. Assuming an intersubject coefficient of variation (CV) of 25% for serum TXB_2 (primary end point) in healthy subjects, 8 subjects would allow detection of a difference of 40% in its postdosing concentrations versus baseline with a power of 90%, on the basis of 2-tailed tests, with probability values less than the type I error rate of 0.05. Thus, 9 healthy volunteers were enrolled, but 1 male subject refused to take aspirin after completing treatment with naproxen.

Results

We compared the time course of recovery from steady-state inhibition of platelet COX-1 activity by low-dose aspirin (100 mg/d) and naproxen (500 mg BID) administered for 6 days to 9 healthy subjects. As shown in Figure 1A, at 1, 12, and 24 hours after the last dose of aspirin, platelet COX-1 activity was suppressed by $99 \pm 0.3\%$, $99 \pm 0.3\%$, and $99 \pm 1\%$ (mean \pm SD, $n=8$, $P < 0.01$ versus predrug values reported in the Table). The persistent suppression of platelet TXB_2 production by low-dose aspirin up to 24 hours reflects the irreversible inactivation of platelet COX-1 activity. At 3 and 12 hours after the last dose, naproxen caused comparably profound suppression of platelet COX-1 activity ($94 \pm 8\%$ and $94 \pm 3\%$, respectively; mean \pm SD, $n=9$) (Figure 1A). Thereafter, a slow recovery of platelet COX-1 activity was detect-

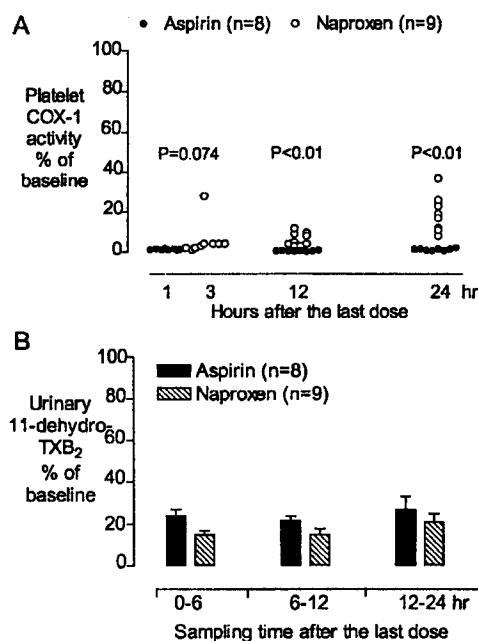


Figure 1. Recovery from steady-state inhibition of platelet COX-1 activity, as assessed by measurement of serum TXB_2 (A), and TXA_2 biosynthesis in vivo, as assessed by measurement of urinary 11-dehydro- TXB_2 excretion (B), after administration of low-dose aspirin (100 mg/d) or naproxen (500 mg BID) for 6 days to 9 healthy subjects. Probability values are for comparison between aspirin and naproxen at each time point.

able (at 24 hours after dosing, serum TXB_2 was reduced by $80 \pm 9\%$) (Figure 1A) that is consistent with the reversible interaction of naproxen with COX-1.

As shown in Figure 1B, both aspirin and naproxen caused a comparable and persistent inhibition of the biosynthesis of TXA_2 in vivo, as reflected by the urinary excretion of 11-dehydro- TXB_2 . In 3 consecutive urine collections performed after the last dose of aspirin or naproxen (ie, 0 to 6, 6 to 12, and 12 to 24 hours after dosing), urinary 11-dehydro- TXB_2 was reduced by $76 \pm 9\%$, $79 \pm 7\%$, and $74 \pm 16\%$, and $85 \pm 5\%$, $85 \pm 8\%$, and $79 \pm 11\%$, respectively.

As shown in Figure 2A, the administration of low-dose aspirin did not affect LPS-induced PGE_2 production in whole blood, an index of monocyte COX-2 activity, to any statistically significant extent. In contrast, naproxen significantly reduced COX-2 activity at 3, 12, and 24 hours after the last dose; COX-2 activity was reduced by $68 \pm 18\%$, $62 \pm 9\%$, and $56 \pm 21\%$, respectively ($P < 0.01$ versus predrug values). As shown in Figure 2B, aspirin and naproxen had markedly different effects on systemic prostacyclin biosynthesis, as reflected by urinary 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$ excretion. In the 3 consecutive urine collections, 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$ levels were not significantly affected by aspirin, whereas naproxen reduced 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$ by $78 \pm 9\%$

1470 *Circulation* March 30, 2004

Baseline Measurements of Whole Blood TXB₂ and PGE₂ Production Ex Vivo and TXA₂ and PGI₂ Biosynthesis In Vivo in Healthy Volunteers

Serum TXB ₂ , ng/mL	LPS-induced PGE ₂ , ng/mL	Urinary 11-dehydro-TXB ₂ , pg/mg Creatinine	Urinary 2,3-dinor-6-keto-PGF _{1α} , pg/mg Creatinine
514±183	42±35	472±237	123±70

All values are mean±SD (n=9).

($P<0.001$), $77\pm 19\%$ ($P<0.001$), and $66\pm 17\%$ ($P<0.001$), respectively (Figure 2B).

Discussion

The aim of the present study was to explore the pharmacodynamic plausibility of an aspirin-like cardioprotective effect of naproxen, a nonselective NSAID with a long half-life. We found that the chronic administration of a therapeutic antiinflammatory dose of naproxen (500 mg BID) to healthy subjects caused persistent and almost complete suppression of platelet TXB₂ production throughout the 12-hour dosing interval that was indistinguishable from that of low-dose aspirin (100 mg/d). However, whereas aspirin pharmacokinetics is dissociated from pharmacodynamics and 100 mg represents approximately a 3-fold excess versus the lowest effective dose to saturate platelet COX-1 activity,³ naproxen

pharmacodynamics is strictly related to its systemic bioavailability, as shown by the significant recovery of platelet COX-1 activity at 24 hours after dosing. Moreover, 500 mg BID is probably close to but not quite at the top of the dose-response curve for COX-1 inhibition. This suggests that compliance and daily dose are likely to represent the main determinants of the clinical efficacy of naproxen for cardiovascular protection. Thus, the apparently conflicting results of a randomized clinical trial, like VIGOR,⁵ and observational studies¹⁴⁻¹⁸ may reflect markedly different rates of compliance and regular use of a high dose of the drug in the 2 settings.

The results of 2,3-dinor-6-keto-PGF_{1α} measurements confirm earlier studies^{19,20} suggesting that an important component of the basal rate of PGI₂ biosynthesis is COX-2-dependent, as reflected in the present study by the consistent differential effects of naproxen versus low-dose aspirin on monocyte COX-2 activity and urinary 2,3-dinor-6-keto-PGF_{1α} excretion.

The inhibitory effects of naproxen on PGI₂ biosynthesis are unlikely to counteract its potential cardioprotective effect, in light of the demonstrated efficacy of aspirin at high doses having a similar effect on PGI₂.^{3,4} However, the clinical relevance of simultaneous suppression of TXA₂ and PGI₂ by naproxen in patients with cardiovascular disease remains to be determined. Moreover, the impact of naproxen on COX-2-dependent sources of thromboxane biosynthesis might contribute to its clinical effects in preventing myocardial infarction.

In conclusion, the present study demonstrates the pharmacodynamic plausibility of a COX-1-dependent cardioprotective effect of naproxen and contributes to the interpretation of the VIGOR cardiovascular findings. Although our results are mechanistically informative of what may happen under the best-case scenario of a randomized clinical trial of regular, prolonged use of a high-dose reversible COX inhibitor, practicing physicians should not assume that the same holds true in the less-than-ideal circumstances of real-life use of these drugs, which is neither regular nor continuous nor necessarily at high doses.

Acknowledgments

This study was supported by a grant from the Italian Ministry of University and Research (MURST) to the Center of Excellence on Aging, G. d'Annunzio University of Chieti. We thank the medical students of G. d'Annunzio University and the personnel of the Blood Transfusion Center of SS. Annunziata Hospital for their generous cooperation.

References

1. Roth GJ, Majerus PW. The mechanism of the effect of aspirin on human platelets. I: acetylation of a particulate fraction protein. *J Clin Invest*. 1975;56:624-632.

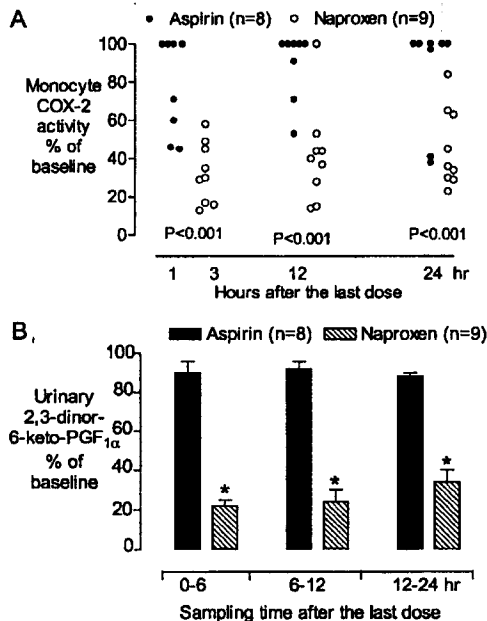


Figure 2. Recovery from steady-state inhibition of monocyte COX-2 activity, as assessed by measurement of whole-blood LPS-induced PGE₂ (A), and systemic prostacyclin biosynthesis, as assessed by measurement of urinary 2,3-dinor-6-keto-PGF_{1α} excretion (B), after administration of low-dose aspirin (100 mg/d) or naproxen (500 mg BID) for 6 days to 9 healthy subjects. Probability values are for comparison between aspirin and naproxen at each time point.

Capone et al COX Inhibition by Naproxen and Low-Dose Aspirin 1471

2. Lecomte M, Lanenville O, Ji C, et al. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *J Biol Chem.* 1994;269:13207-13215.
3. Patrono C, Collier B, Dalen JE, et al. Platelet active drugs: the relationships among dose, effectiveness, and side effects. *Chest.* 2001;119:39S-63S.
4. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ.* 2002;324:71-86.
5. Bombardier C, Laine L, Reicin A, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *N Engl J Med.* 2000;343:1520-1528.
6. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med.* 2001;345:433-442.
7. Van Hecken A, Schwartz JI, Depré M, et al. Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. *J Clin Pharmacol.* 2000;40:1109-1120.
8. Shah AA, Thjodleifsson B, Murray FE, et al. Selective inhibition of COX-2 in humans is associated with less gastrointestinal injury: a comparison of nimesulide and naproxen. *Gut.* 2001;48:339-346.
9. Patrono C, Ciabattini G, Pinca E, et al. Low dose aspirin and inhibition of thromboxane B₂ production in healthy subjects. *Thromb Res.* 1980;17:317-327.
10. Patrignani P, Panara MR, Greco A, et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Ther.* 1994;271:1705-1712.
11. Ciabattini G, Pugliese F, Davi G, et al. Fractional conversion of thromboxane B₂ to urinary 11-dehydrothromboxane B₂ in man. *Biochim Biophys Acta.* 1989;992:66-70.
12. FitzGerald GA, Brash AR, Falardeau P, et al. Estimated rate of prostacyclin secretion into the circulation of normal man. *J Clin Invest.* 1981;68:1272-1275.
13. Minuz P, Covi G, Paluani F, et al. Altered excretion of prostaglandin and thromboxane metabolites in pregnancy-induced hypertension. *Hypertension.* 1988;11:550-556.
14. Mamdani M, Rochon P, Juurlink DN, et al. Effect of selective cyclooxygenase 2 inhibitors and naproxen on short-term risk of acute myocardial infarction in the elderly. *Arch Intern Med.* 2003;163:481-486.
15. Solomon DH, Glynn RJ, Levin R, et al. Nonsteroidal anti-inflammatory drug use and acute myocardial infarction. *Arch Intern Med.* 2002;162:1099-1104.
16. Watson DJ, Rhodes T, Cai B, et al. Low risk of thromboembolic cardiovascular events with naproxen among patients with rheumatoid arthritis. *Arch Intern Med.* 2002;162:1105-1110.
17. Rahme E, Pilote L, LeLorier J. Association between naproxen use and protection against acute myocardial infarction. *Arch Intern Med.* 2002;162:1111-1115.
18. Ray WA, Stein M, Hall K, et al. Non-steroidal anti-inflammatory drugs and risk of coronary heart disease: an observational cohort study. *Lancet.* 2002;359:118-123.
19. McAdam BF, Catella-Lawson F, Mardini IA, et al. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci U.S.A.* 1999;96:272-277.
20. Catella-Lawson F, McAdam B, Morrison BW, et al. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J Pharmacol Exp Ther.* 1999;289:735-741.