

Platelet function is inhibited by non-selective non-steroidal anti-inflammatory drugs but not by cyclo-oxygenase-2-selective inhibitors in patients with rheumatoid arthritis

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Abstract

Background. Interaction with platelet function by non-steroidal anti-inflammatory drugs (NSAIDs) is related to the inhibition of cyclo-oxygenase-1 (COX-1). In patients with rheumatoid arthritis (RA), only one of the COX-2-selective NSAIDs (nabumetone) has been demonstrated to spare platelet function partially.

Objective. To compare the effects of the COX-2-selective inhibitor, meloxicam, with those of the non-selective NSAID, naproxen, on platelet function and thromboxane levels in RA patients.

Methods. In this randomized, controlled, cross-over trial, 10 RA patients used meloxicam 7.5 mg bid and naproxen 500 mg bid, each during a 2-week period. Washout periods were applied. Before and after each 2-week period of NSAID intake, laboratory studies were performed.

Results. Platelet aggregation was significantly less influenced, thromboxane levels were less inhibited (246 vs 117 pg/ml) and bleeding times were less prolonged with meloxicam than with naproxen (3.2 vs 2.3 min). Moreover, the results of all tests during meloxicam exposure were comparable with baseline values.

Conclusion. In RA patients, meloxicam, a representative of the selective COX-2 inhibitors, does not interfere with platelet function and thromboxane levels, in contrast with naproxen (a non-selective COX inhibitor).

KEY WORDS: Platelet aggregation, Thromboxane, Bleeding time, Non-steroidal anti-inflammatory drugs, Rheumatoid arthritis, Meloxicam, Naproxen.

World-wide, non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs. In 1971, Sir John Vane discovered the central role of cyclo-oxygenase (COX) in their mode of action [1]. COX is responsible for the synthesis of prostaglandins from arachidonic acid. In 1990, Needleman *et al.* [2] demonstrated the existence of two isoforms of the COX enzyme. COX-1 helps to maintain normal organ function and is considered a 'housekeeping' enzyme. As such, prostaglandins formed by COX-1 protect the gastric mucosa, stimulate platelet aggregation and support renal function. In contrast, COX-2 is expressed during inflammation and cell damage [1, 3, 4]. The prostaglandins synthesized in these conditions accelerate the inflammatory process. Consequently, the

ideal anti-inflammatory agent should block COX-2, but not COX-1.

Many studies have focused on the more favourable gastrointestinal tolerance of COX-2-specific and -selective inhibitors compared with the classic, non-selective NSAIDs [4]. However, data concerning the side-effects on platelet aggregation are scarce. Classical NSAIDs have definite effects on platelet aggregation through inhibition of COX-1 [5], the unique iso-enzyme for the formation of thromboxane. In accordance with the results of former studies in healthy volunteers [6, 7], we recently demonstrated in patients with rheumatoid arthritis (RA), that nabumetone (a more selective COX-2 inhibitor) did not interfere with platelet aggregation to the same extent as naproxen (a non-specific NSAID) [8]. In this study, thromboxane concentrations were not analysed. Other clinical studies with patients, obviously different from healthy volunteers, are lacking.

The goal of the present study was to compare the effects of another selective COX-2 inhibitor (meloxicam)

Submitted 18 April 2001; revised version accepted 19 October 2001.

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with naproxen on platelet function, thromboxane levels, and bleeding time in patients with RA.

Patients and methods

Out-patients, between 18 and 75 yr old, suffering from RA according to the criteria of the American College of Rheumatology [9] were selected. To be included, a normal renal function (creatinine clearance >75 ml/min, according to the Cockcroft and Gault formula [10]), a normal platelet count ($>150 \times 10^9/l$) and no known allergy to NSAIDs were mandatory. The use of acetylsalicylic acid or any other medication influencing platelet function was allowed. Steroids were limited to a stable dose not exceeding the equivalent of prednisolone 10 mg daily. All patients had to give informed consent. The local ethics committee approved the study.

This randomized cross-over study comprised four 2-week periods (see Fig. 1). In the second and fourth periods, both following a 2-week washout of all NSAIDs, study medication was prescribed. The patients were randomized to either naproxen 500 mg bid or meloxicam 7.5 mg bid in the second period. The alternative NSAID was prescribed in the fourth period. After each period a platelet count, platelet aggregation studies, bleeding time tests, and thromboxane measurements were performed; in addition, activated partial thromboplastin time (aPTT) and prothrombin time (PT) measurements were carried out at the start of the study.

Blood was obtained by venipuncture without veno-occlusion in the morning (1 h after NSAID intake in the second and fourth periods). It was collected in 5-ml vacutainer tubes with citrate (0.129 M) or ethylene diamine tetra acetic acid (EDTA; 0.0045 M). Platelet counts were measured on a Technicon H1 (Bayer, Leverkusen, Germany) automated cell counter. aPTT and PT were measured on a STA coagulometer (Roche, Mannheim, Germany). Bleeding time tests were performed as described by Ivy *et al.* [11]. Plasma samples for thromboxane-B2 measurements were obtained by two times centrifugation of EDTA blood at 1850 g for 10 min at room temperature, and stored at -80°C until determination. Thromboxane-B2 was determined using the radioimmunoassay method of NEN-Life Science Products (Shelton, CT, USA).

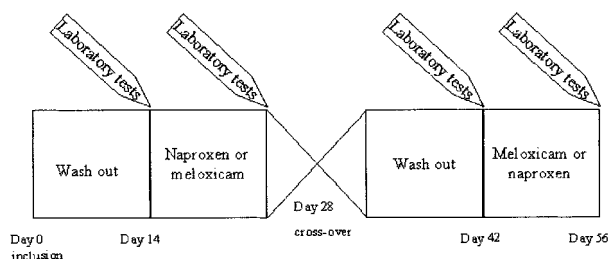


FIG. 1. Study design.

For the platelet aggregation studies, platelet-rich plasma (PRP) was obtained by centrifugation of citrated blood at 200 g for 10 min and platelet-poor plasma (PPP) was obtained by centrifugation at 1850 g for 15 min at room temperature. The platelet concentration in the PRP was diluted to approximately $250 \times 10^9/l$ using PPP. The platelet aggregation studies were performed according to Born's method [12] at 37°C using a PAP-4 aggregometer (Meyvis, Bio Data Corp, USA). During the experiment, optical density was continuously recorded. The 0 and 100% aggregation levels were set with PRP and PPP, respectively. When a stable baseline was obtained with 450 μl of PRP, 50 μl of one of the aggregation-inducing agents was added. The final concentrations in the reaction vessel were: 2.5 and 5.0 μM adenosine diphosphate (ADP), 1.0 and 5.0 μM epinephrine, 1.0 and 4.0 mg/l collagen, 0.6 and 1.2 g/l ristocetin and 500 mg/l arachidonic acid. The maximum height and slope of the aggregation curves were determined (see Fig. 2) and the type of curve was described: biphasic, monophasic, or non-sustained.

Differences in bleeding time, thromboxane concentration, maximum platelet aggregation and the slope of the aggregation curve between baseline and day 14 of the use of either study NSAID were calculated. The differences after 2 weeks of intake of naproxen were compared with those after 2 weeks of intake of meloxicam by using non-parametric paired tests. The results of the various aggregation tests were correlated with the results of the thromboxane measurements and bleeding time tests by means of the Spearman test.

Results

The patient group consisted of five males and five females aged 31–64 yr (mean 55 yr). One patient used steroids (10 mg prednisolone daily). Seven patients were on stable disease-modifying anti-rheumatic drug therapy: one had methotrexate, four sulphasalazine, one aurothioglucose, and one azathioprine combined with hydroxychloroquine.

At baseline, after the first washout, platelet counts, aPTT, PT and bleeding time tests were normal.

In general, the influence of naproxen on platelet aggregation exceeded the influence of meloxicam, except

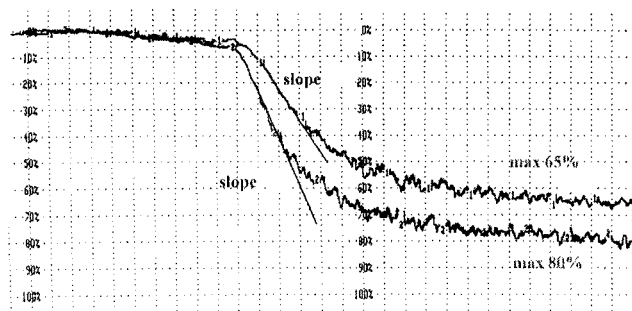


FIG. 2. An example of a platelet aggregation curve.

TABLE 1. Differences in platelet aggregation after naproxen *vs* meloxicam, compared by using non-parametric paired tests

| | Mean difference after naproxen | Mean difference after meloxicam | <i>P</i> value |
|---------------------------|-----------------------------------|------------------------------------|----------------|
| ADP 2.5 μ M | -17.6 | +5.4 | 0.02 |
| ADP 5.0 μ M | -12.6 | -2.9 | 0.04 |
| Epinephrine 1.0 μ M | -42.7 | -4.8 | 0.01 |
| Epinephrine 5.0 μ M | -37.9 | -1.7 | 0.01 |
| Collagen 1.0 mg/l | -49.9 | -3.1 | 0.01 |
| Collagen 4.0 mg/l | -22.5 | -3.1 | 0.04 |
| Arachidonic acid 500 mg/l | -55.0 | +1.6 | 0.01 |
| Ristocetin 0.6 g/l | +1.5 | +0.6 | 0.59 |
| Ristocetin 1.2 g/l | -1.3 | +9.6 | 0.48 |

when aggregation was induced by ristocetin (Table 1). Similar results were obtained considering the changes in the slope of the aggregation curve after induction by epinephrine 5.0 μ M, collagen and arachidonic acid ($P = 0.01$; data not shown). Disappearance of secondary aggregation, as observed by a monophasic (instead of biphasic) type of aggregation curve in tests with epinephrine, was more often demonstrated after the use of naproxen than after meloxicam (in 5/10 and 1/10 patients, respectively). Disaggregation, as observed by a non-sustained type of aggregation curve in tests with ADP, occurred more frequently after the use of naproxen than after meloxicam (in 7/10 and 1/10 patients, respectively).

The change in bleeding time after naproxen was significantly greater than after meloxicam (+1.35 *vs* +0.3 min; $P = 0.02$).

Baseline thromboxane-B2 levels were 228 (130–310) pg/ml, changing to 246 (150–350) pg/ml after 2 weeks of meloxicam and to 117 (70–140) pg/ml after 2 weeks of naproxen ($P = 0.41$ *vs* $P = 0.01$, respectively, for comparison with baseline values).

The results of the aggregation tests (except when induced by ristocetin) correlated highly with the thromboxane measurements: correlation coefficients (r) between 0.59 and 0.80, with $P < 0.01$. The changes in bleeding time correlated with those in thromboxane levels ($r = -0.48$; $P < 0.05$) and with the changes in platelet aggregation, when stimulated by epinephrine (both concentrations; $r = -0.49$ and -0.52 ; $P < 0.05$) and arachidonic acid ($r = -0.56$; $P < 0.01$).

Discussion

Platelet aggregation is stimulated by thromboxane. NSAIDs, by blocking the COX-1 enzyme, inhibit thromboxane production and thus interfere with normal platelet aggregation. Although this effect is often used in therapy (as is the case with acetylsalicylic acid in cardiovascular diseases), it can also be an unwanted side-effect. The importance of precise knowledge regarding platelet inhibition of NSAIDs is illustrated by the possible increase of cardiovascular disease in patients using selective COX-2 inhibitors [13, 14].

Naproxen and meloxicam have comparable plasma half-lives (15 and 20 h, respectively) and both reach steady-state plasma levels in about 75–100 h, i.e. 4 days. Hence, the influence of the time of NSAID intake on the various laboratory tests (performed after 14 days) can be considered minimal.

Platelet aggregation can be studied by using inductors like ADP, epinephrine, collagen, arachidonic acid and ristocetin; all, except the latter (which is used as a control test for other bleeding conditions), are dependent on COX-1. So, with COX-1 inhibition (e.g. by NSAIDs), platelet aggregation will progress slower (with a less steep slope of the curve), and reach a smaller maximum (and sometimes even disaggregation); moreover, secondary aggregation will not take place. The data of this study unequivocally show significant differences in the inhibition of the COX-1-dependent tests after using meloxicam as compared with naproxen.

Two weeks of naproxen significantly reduced thromboxane-B2 levels, whereas 2 weeks of meloxicam left them unchanged. These differences in thromboxane-B2 levels correlated well with the results of the platelet aggregation tests, which has never been reported. Hitherto, only our own, former, study investigated the changes in platelet aggregation after the use of non-selective *vs* COX-2-selective NSAIDs in patients, but without thromboxane measurements [8]; all other studies were carried out in healthy volunteers [6, 15–19].

Bleeding time is influenced by platelet aggregation, but also by numerous other factors, such as the test method, skin depth, age, gender, underlying diseases and medication. As a result, studies on the influence of NSAIDs on bleeding time in healthy volunteers show conflicting results. In the present study, bleeding times increased significantly during the use of naproxen as compared with meloxicam. The differences in bleeding time correlated with the changes in both platelet aggregation and thromboxane concentration.

In conclusion, we have demonstrated that in RA patients, meloxicam, a representative of selective COX-2 inhibitors, has negligible effects on platelet function, as measured by bleeding time, aggregation studies and thromboxane-B2 measurements.

Acknowledgement

This study was supported by an unrestricted educational grant from Boehringer Ingelheim.

References

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature [New Biology]* 1971;231:232–5.
2. Fu JY, Masferrer JL, Seibert KN, Raz A, Needleman P. The induction and suppression of prostaglandin H₂ synthase (cyclooxygenase) in human monocytes. *J Biol Chem* 1990;265:16737–40.
3. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. *Inflamm Res* 1995; 44:1–10.

4. Hawkey CJ. COX-2 inhibitors. *Lancet* 1999;353:307–14.
5. Schafer AI. Effects of nonsteroidal antiinflammatory drugs on platelet function and systemic hemostasis. *J Clin Pharmacol* 1995;35:209–19.
6. Nunn B, Chamberlain PD. Effect of nabumetone (BRL 14777), a new anti-inflammatory drug, on human platelet reactivity *ex vivo*: comparison with naproxen. *J Pharm Pharmacol* 1982;34:576–9.
7. Stichtenoth DO, Wagner B, Frolich JC. Effects of meloxicam and indomethacin on cyclo-oxygenase pathways in healthy volunteers. *J Invest Med* 1997; 45:44–9.
8. Knijff-Dutmer EAJ, Martens A, vd Laar MAFJ. The effect of nabumetone versus naproxen on platelet aggregation in patients with rheumatoid arthritis. *Ann Rheum Dis* 1999;58:257–9.
9. Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
10. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31–41.
11. Ivy AC, Nelson AD, Bucher G. The standardisation of certain factors in the cutaneous 'venostasis' bleeding time technique. *J Lab Clin Med* 1941;26:1812–22.
12. Born GVR. Aggregation of blood platelets by adenosine and its reversal. *Nature* 1962;194:927–9.
13. Mukherjee D, Nissen SE, Toplo EJ. Risk of cardiovascular events associated with COX-2 inhibitors. *J Am Med Assoc* 2001;286:954–9.
14. Crofford LJ, Oates JC, McCune WJ *et al.* Thrombosis in patients with connective tissue diseases treated with specific cyclooxygenase 2 inhibitors. A report of four cases. *Arthritis Rheum* 2000;43:1891–6.
15. Freed MI, Audet PR, Zariffa N *et al.* Comparative effects of nabumetone, sulindac, and indomethacin on urinary prostaglandin excretion and platelet function in volunteers. *J Clin Pharmacol* 1994;34:1098–108.
16. Mengle-Gaw L, Hubbard RC, Karim A *et al.* A study of the platelet effects of SC-58635, a novel COX-2-selective inhibitor [abstract]. *Arthritis Rheum* 1997;40(Suppl.):93.
17. Jeremy JY, Mikhailidis DP, Barradas MA, Kirk RM, Dandona P. The effect of nabumetone and its principal metabolite on *in vitro* human gastric mucosal prostanoid synthesis and platelet function. *Br J Rheumatol* 1990; 29:116–9.
18. De Meijer A, Vollaard H, de Metz M, Verbruggen B, Thomas C, Novakova I. Meloxicam, 15 mg/day, spares platelet function in healthy volunteers. *Clin Pharmacol Ther* 1999;66:425–30.
19. Leese PT, Hubbard RC, Karim A, Isakson PC, Yu SS, Geis GS. Effects of celecoxib, a novel cyclooxygenase-2 inhibitor, on platelet function in healthy adults: a randomized, controlled trial. *J Clin Pharmacol* 2000;40:124–32.