A comparison of the effects of nabumetone vs meloxicam on serum thromboxane B₂ and platelet function in healthy volunteers

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Aims To compare the effects of nabumetone and meloxicam, two cyclo-oxygenase-2 (COX-2) preferential nonsteroidal anti-inflammatory drugs (NSAIDs), on platelet COX-1 activity and platelet function.

Methods Twelve healthy volunteers (3 male, 9 female, median age 22 years) participated in an open, randomized, cross-over trial of nabumetone 1000 mg twice daily vs meloxicam 7.5 mg twice daily during 1 week with 2 weeks wash-out. After a second 2 week wash-out period, one dose of indomethacin 50 mg was given as a positive control to check for NSAID induced inhibition of platelet function. COX-1 inhibition was measured as percentage inhibition of serum TXB₂ generation in clotting whole blood, and as closure time with use of the platelet function analyser PFA-100. Data are reported as median with range. Paired variables were analysed using Wilcoxon's signed rank test.

Results TXB₂ levels decreased significantly after all three medications, but percentage inhibition after nabumetone and indomethacin (88% and 97%, respectively) was significantly higher than after meloxicam (63%) ($P < 0.05$). Closure times increased significantly after administration of all three medications ($P < 0.05$). Increases in closure time after administration did not differ between nabumetone and meloxicam (24% and 14%, respectively), but were significantly larger after indomethacin administration (63%) ($P < 0.01$).

Conclusions In the maximum registered dosage, nabumetone inhibits thromboxane production much more than meloxicam, signifying less COX-2 selectivity of the former. However, both nabumetone and meloxicam cause only minor impairment in platelet function in comparison with indomethacin and the difference between them is not significant.

Keywords: cyclo-oxygenase inhibition, non-steroidal anti-inflammatory drugs, platelet function, COX-2 selectivity, thromboxane B₂ synthesis

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) demonstrate both pain and inflammation inhibiting properties as well as several adverse effects such as inhibition of platelet function, gastro-intestinal damage and renal impairment. Whereas the therapeutic anti-inflammatory effects are mainly caused by inhibition of the iso-enzyme

cyclo-oxygenase-2 (COX-2), the side-effects mentioned above are believed to result from inhibition of the iso-enzyme COX-1. Therefore, it has been suggested that COX-2 selective NSAIDs would cause less side-effects compared with nonselective NSAIDs [1]. In the Netherlands, two available NSAIDs are considered COX-2 preferential, i.e. meloxicam and nabumetone. This implies, that these NSAIDs are more selective to COX-2 than other older generation NSAIDs, but to a much lesser degree than newer COX-2 selective agents such as celecoxib and rofecoxib. Nevertheless, it has been suggested that COX-2 preferential NSAIDs such as meloxicam and nabumetone display decreased toxicity compared with nonselective NSAIDs [2].

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In the present study, we describe a direct comparison of the COX-1 effects of two COX-2 preferential NSAIDs, meloxicam and nabumetone in healthy volunteers. The production of thromboxane B₂ (TXB₂), the stable metabolite of thromboxane A₂, during whole blood clotting is used as an index of platelet COX-1 activity *ex vivo* [3]. COX-1 effects are highly dependent on the experimental model applied. In our opinion, the information most relevant for clinical practice is the degree of COX-1 inhibition that can be obtained during administration of the maximum registered dosage of a certain selective NSAID. Therefore, we investigated the effects of meloxicam and nabumetone when used in daily doses of 15 mg and 2000 mg, respectively. We compared both medications with indomethacin, which is a preferential COX-1 inhibitor. Measurement of serum TXB₂ production does not necessarily reflect the degree of impairment of platelet function [4]. Therefore, we also measured impairment of platelet function following nabumetone and meloxicam with the Platelet Function Analyser (PFA-100).

Methods

Twelve healthy nonsmoking Caucasian volunteers (3 men, 9 women, median age 22 years, range 20–37 years) entered the study. All subjects gave written informed consent before participating in the study. The study was approved by the Ethics Committee of the Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands. All study participants refrained from smoking and alcohol for at least 1 week before enrolment and during the entire study. None of the subjects was using concomitant medications during the study. The study was designed as an open, two-armed, randomized crossover trial. Volunteers were randomly assigned to either meloxicam 7.5 mg (Movicox[®], Boehringer Ingelheim BV, Alkmaar, The Netherlands) or nabumetone 1000 mg (Mebutan[®], SmithKline Beecham Farma, Rijswijk, The Netherlands), both twice daily at 08.00 h and 18.00 h after a meal, both for a period of 7 days. After a washout period of 14 days, the volunteers used the alternative regimen. After a second washout period of 14 days, a control experiment was performed, in which subjects were given one dose of 50 mg indomethacin (Indocid[®], Merck Sharp and Dohme, Haarlem, The Netherlands).

All blood samples were obtained after a resting period of 10 min. Baseline venous blood samples were taken on day 0 and after each washout period. Profile venous blood samples were obtained 2 h after the last meloxicam and last nabumetone doses, and also 2 h after the single indomethacin dose. Every blood sample was drawn with use of Vacutainer tubes (Becton Dickinson BV, Leiden, The Netherlands) and a 21-gauge cannula inserted into the

antecubital vein of each subject. The compression band was released after insertion, and the first 3 ml blood obtained were not used for measurement. In each blood sample, we measured serum TXB₂ levels and *ex vivo* bleeding time. TXB₂ concentrations in serum were determined by enzymeimmuno-assay (Cayman Chemical Company, Ann Arbor, USA) [5]. Samples were obtained in 10 ml nonsiliconized Vacutainer dry tubes and immediately incubated at 37° C to stimulate platelet thromboxane production during coagulation. After 1 h, blood samples were centrifuged at 4200 *g* for 10 min at 4° C. Specimens were subsequently stored at –70° C until assayed according to the manufacturers procedures. *Ex vivo* bleeding time was determined with use of the PFA-100 (Platelet Function Analyser, Dade NV, Amersfoort, The Netherlands). This instrument has been designed as an alternative for the bleeding time test because the bleeding time is inherently variable, time consuming and patient unfriendly [6]. The PFA-100 aspirates a blood sample under constant vacuum from a sample reservoir through a capillary and a microscopic aperture cut into a membrane, coated with collagen and epinephrin. This biochemical stimulus together with a high shear rate generates platelet adhesion, activation, and aggregation under standardized flow conditions, slowly building up a stable platelet plug closing the aperture. The time required to obtain full occlusion of the aperture is reported as *ex vivo* bleeding time or 'closure time'. For this test, blood was collected in Vacutainer tubes containing 0.35 ml of 0.129 mol l^{–1} (3.8%) buffered sodium citrate, pH 5.2, with a draw volume of 3.15 ml. We used a citrate concentration of 0.129 mol l^{–1} (3.8%) because a previous study demonstrated a clearly enhanced sensitivity of PAF-100 testing for acetylsalicylic acid induced platelet dysfunction when blood samples were collected with 0.129 mol l^{–1} rather than 0.106 mol l^{–1} sodium citrate [7]. All tests were performed at room temperature within 1 h. Results were expressed in seconds with a maximum measurable value of 300 s.

Data are reported as median with range. The data were combined regardless of the treatment sequence, comparing post-treatment with pretreatment measurements. Analysis of paired variables was performed with Wilcoxon's signed rank test. A *P* value <0.05 was considered significant. All statistical computations were performed using the SPSS statistical package (SPSS 9.0, Chicago, Illinois, USA).

Results

All volunteers completed the study. Five subjects experienced mild nausea, all after taking nabumetone,

but they were able to complete the study with blood samples taken at all occasions.

Serum thromboxane B_2 concentration

Baseline TXB_2 concentrations before nabumetone (628 nmol l^{-1} , range 85–1361), meloxicam (769 nmol l^{-1} , range 184–1163), and indomethacin (634 nmol l^{-1} , range 99–953) did not differ significantly. TXB_2 concentrations decreased significantly after all three medications. Percentage inhibition of TXB_2 concentrations after nabumetone (88%) and indomethacin (97%) was significantly higher than inhibition after meloxicam (63%) ($P < 0.05$) (Figure 1).

Closure time, PFA-100 method

The mean baseline closure time measurements before nabumetone (123 s, range 78–185), meloxicam (119 s, range 86–183), and indomethacin (131 s, range 69–191) were not significantly different. After a single indomethacin dose, closure time increased significantly by 63%, and was prolonged to 300 s in 6 of 12 patients ($P < 0.01$). After treatment with nabumetone and meloxicam, closure time increased by 24% and 14%, respectively ($P < 0.05$). However, these increases were significantly smaller than the increase in closure time after indomethacin ($P < 0.01$). There was no significant

difference between nabumetone and meloxicam with regard to prolongation of closure time (Figure 2).

Discussion

In the present study, we found that meloxicam 7.5 mg twice daily causes significantly less decrease in TXB_2 synthesis (66%) than nabumetone 1000 mg twice daily (88%). However, both medications caused only minor increases in closure time compared with indomethacin. The reductions of serum TXB_2 production after meloxicam and nabumetone were compatible with reductions previously reported [8–10], as was the 97% reduction of serum TXB_2 after indomethacin [8].

As in an earlier study with meloxicam [8], one of the volunteers showed a normal closure time (133 s) despite a nearly complete suppression of the TXB_2 production (6 nmol l^{-1}). In the same volunteer, the closure times also remained within normal ranges following nabumetone (118 s) although the TXB_2 level (9 nmol l^{-1}) demonstrated clear inhibition. This suggests the presence of resistance to impairment of platelet function by NSAIDs despite adequate suppression of the TXB_2 level. Others have also reported a normal closure time after aspirin treatment in a volunteer [6]. Therefore, our data clearly confirm that platelet function following NSAIDs cannot be regarded as an absolute reliable indicator of NSAID induced platelet cyclo-oxygenase inhibition.

Figure 1 Serum thromboxane B_2 levels in 12 healthy young volunteers. A, B, respectively, before and after 1 week nabumetone 1000 mg twice daily C, D, respectively, before and after 1 week meloxicam 7.5 mg twice daily E, F, respectively, before and after one dose of 50 mg indomethacin. Connected dots represent individual values. Vertical bars represent mean and s.d.

Figure 2 *Ex vivo* closure time by PFA-100 in 12 healthy young volunteers. A, B, respectively, before and after 1 week nabumetone 1000 mg twice daily C, D, respectively, before and after 1 week meloxicam 7.5 mg twice daily E, F, respectively, before and after one dose of 50 mg indomethacin. Connected dots represent individual values. Vertical bars represent mean and s.d.

Even a 88% inhibition of TXB₂ production by nabumetone did not result in a clear impairment of platelet function in this study. This may be explained by the findings of Reilly & Fitzgerald, who showed that significant reduction of urinary excretion of TXB₂ metabolites will not be achieved before a maximum *ex vivo* inhibition of thromboxane production (i.e. serum TXB₂ concentrations) by more than 95% [4]. This means that physiological thromboxane concentrations *in vivo* are much less susceptible to inhibition of platelet cyclo-oxygenase than the nonphysiological maximum thromboxane production in clotting blood *ex vivo*.

Our study has several important limitations. We investigated platelet function in a small group of healthy volunteers, whereas the clinical relevance of COX-2 selectivity lies mainly in the prevalence of renal and gastrointestinal side-effects. However, previous studies have shown a clear correlation between the relative potency of NSAIDs against COX-1 and adverse gastrointestinal effects and COX-2 specific inhibitors have demonstrated significantly less adverse effects compared to nonselective NSAIDs [11, 12]. The present study demonstrates that platelet COX-1 is inhibited much more following the maximum dose of nabumetone than following the maximum dose of meloxicam. This may suggest that nabumetone in antirheumatic dosage will cause more side-effects related to COX-1 suppression than meloxicam. We further found, that the higher COX-2 selectivity of meloxicam compared with nabumetone is not influencing impairment of platelet function. Nevertheless, future studies should investigate, whether the same is true for the risk of gastro-intestinal and renal impairment, which is the most important side-effect of NSAIDs.

Finally, the nonselective NSAIDs ibuprofen and indomethacin may interfere with the inhibition of TXB₂ production by low dose aspirin [13, 14]. This interaction does not occur between aspirin and the COX-2 selective NSAID rofecoxib [15]. Further investigations need to be performed into the degree of COX-2 selectivity that is necessary to avoid pharmacological interaction between NSAIDs and low dose aspirin. COX-2 selective NSAIDs might increase the risk of thrombotic complications by inhibiting the production of the antithrombotic prostaglandin PGI₂ (a potent vasodilator and inhibitor of platelet aggregation) but not the production of the prothrombotic TXA₂, a potent activator of platelet aggregation [16]. In the present study, meloxicam and especially nabumetone inhibited the production of TXB₂ substantially. However, this did not cause major inhibition of platelet function. This raises the suspicion that these COX-2 preferential NSAIDs may also increase the risk of thrombotic complications similar to COX-2 selective

NSAIDs, and future clinical studies should focus on the occurrence of such thrombotic complications in patients using these medications.

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