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RESEARCH PAPER

# The interaction of ibuprofen and diclofenac with aspirin in healthy volunteers

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**Background and purpose:** Aspirin reduces the risk of myocardial infarction and stroke by inhibiting thromboxane production in platelets. This inhibition can be competitively antagonized by some non-steroidal anti-inflammatory drugs (NSAIDs).
**Experimental approach:** By measuring thromboxane $B_2$ production in healthy volunteers, we investigated whether ibuprofen (800 mg three times daily for 7 days) or diclofenac (50 mg three times daily for 7 days) taken concurrently with aspirin 80 mg (once daily for 7 days) influenced the inhibitory effect of aspirin. The effects were compared with aspirin 30 mg (once daily for 7 days), which is the lowest dose of aspirin with a proven thromboprophylactic effect.
**Key results:** The median percentage inhibition of thromboxane $B_2$ levels by 30 mg or 80 mg aspirin was 90.3% (range 83.1–96.0%) and 98.0% (range 96.8–99.2%) respectively. The inhibition by concurrent administration of slow release diclofenac and 80 mg aspirin was 98.1% (range 97.2–98.9%), indicating no interference between aspirin and diclofenac. The inhibition decreased significantly by concurrent administration of immediate release ibuprofen and 80 mg aspirin (86.6%; range 77.6–95.1%) to a level less than 30 mg aspirin.
**Conclusions and implications:** As alternatives are easily available, NSAIDs such as diclofenac should be preferred to ibuprofen for combined use with aspirin.
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**Keywords:** aspirin; ibuprofen; diclofenac; thromboprophylaxis; interaction

**Abbreviations:** COX, cyclo-oxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; TX, thromboxane

## Introduction

As aspirin has been shown to reduce the risk of myocardial infarction and stroke (Patrono *et al.*, 2001; Antithrombotic Trialists' Collaboration, 2002), it is widely used in cardiology and neurology. The pharmacological action is based on acetylation of a serine residue at position 529 of the platelet enzyme cyclo-oxygenase (COX)-1, which causes a blockade of the catalytic site (Catella-Lawson *et al.*, 2001; Patrono *et al.*, 2001). Thereby, thromboxane (TX)$A_2$ cannot be formed in platelets from its substrate arachidonic acid.

Thromboxane $A_2$ is a potent vasoconstrictor and platelet aggregating agent. As it has a short half-life, it is normally measured as its more stable hydration product, $TXB_2$. After acetylation, anucleated platelets cannot regenerate COX-1 and hence, the inhibition is irreversible. Thus, renewal of platelet $TXA_2$ production totally depends on the synthesis of new platelets. In this way, adequate inhibition of $TXA_2$ production can be maintained by aspirin administered once daily, in spite of its short half-life of 20 min (Patrono *et al.*, 2001).

In contrast to aspirin, most other non-steroidal anti-inflammatory drugs (NSAIDs) antagonize $TXA_2$ formation in a reversible, competitive manner. As long as an NSAID is present in the COX-1 channel, it obstructs the access of aspirin to the serine residue and thus the irreversible inactivation of platelet COX-1 (Loll *et al.*, 1995; Catella-Lawson *et al.*, 2001). If the NSAID remains in the COX-1 channel until most of the aspirin has been metabolized, the synthesis of $TXA_2$ may resume after subsequent removal of the NSAID. In this way, NSAIDs may impair the thromboprophylactic action of aspirin. Whether this occurs depends on the affinity of the NSAID for the COX-1 enzyme. Catella-Lawson *et al.* (Catella-Lawson *et al.*, 2001) showed an interaction of ibuprofen with aspirin in healthy volunteers, but no such effect with diclofenac. In that study, a single daily dose of ibuprofen interfered with the irreversible platelet COX-1 inhibition

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induced by immediate release aspirin when ibuprofen was administered 2 h before aspirin, but there was no interference when it was administered 22 h before, that is, 2 h after aspirin. When ibuprofen was administered three times daily, the interference also occurred when enteric-coated aspirin was administered 2 h before the ibuprofen, that is, 12 h after the previous intake of ibuprofen. In order to mimic a typical daily dosing regime, in the present study we investigated the interaction of ibuprofen and diclofenac (three times daily) with immediate release aspirin (once daily) administered concurrently in healthy volunteers.

In neurology, 30 mg once daily is the lowest dose of aspirin with proven thromboprophylactic action (The Dutch TIA Trial Study Group, 1991); hence, we also examined whether a possible interaction of an NSAID with the usual dose of 80 mg aspirin once daily results in less inhibition of $TXB_2$ production than with 30 mg aspirin taken once daily. If this occurs, the thromboprophylactic action of 80 mg aspirin once daily can no longer be taken for granted in daily use.

## Methods

*Study subjects and study design*

Twelve healthy Caucasian volunteers (eight men and four women, median age 42 years, range 26–58 years) were enrolled in the study. All subjects gave written informed consent before participating and the study was approved by the Dutch regional Ethical Review Board, Arnhem-Nijmegen, the Netherlands. All study participants refrained from smoking and alcohol for at least 24 h before enrolment. No concomitant medication was taken during the course of the study, with the exception of oral contraceptives. The study was designed as an open-label, three-sequence, crossover, single/multiple-dose trial (see Figure 1). The volunteers were randomly assigned to either immediate release aspirin 30 or 80 mg (Pharmachemie, the Netherlands) once daily at 15:00 h, slow release diclofenac 50 mg three times daily (Pharmachemie, the Netherlands) or ibuprofen 800 mg three times daily (2 tablets of 400 mg, Pharmachemie, the Netherlands) both at 15:00, 23:00 and 7:00 h (Figure 1). After a washout period of 14 to 42 days, the volunteers proceeded with another dosing regime according to randomization.

Venous blood samples were collected immediately before the administration of the study medication (baseline), 24 h after the last intake of aspirin 30 mg and aspirin 80 mg, 8 h after the last intake of the single treatments of diclofenac or ibuprofen, and 24 h after the last intake of aspirin 80 mg in combination with ibuprofen or diclofenac, which is 8 h after the last intake of the NSAID (see Figure 1). Every blood sample was drawn with the use of a 21-gauge cannula inserted into the antecubital vein of each subject. A 10 mL blood sample was collected in a plain non-siliconized Vacutainer dry tube for TXB2 measurements and 3 mL blood sample was collected in a tube with $K_3$EDTA (Becton Dickinson BV, Leiden, the Netherlands) as anticoagulant for platelet measurements. Platelet levels remained within the reference range in all blood samples taken (data not shown).

*$TXB_2$ measurements and statistical analysis*

Thromboxane $B_2$ in serum was determined in each blood sample by enzyme immuno-assay (Cayman Chemical Company, Ann Arbor, USA) (Pradelles *et al.*, 1985). After collection, blood samples were immediately incubated at 37°C to stimulate platelet TX production during coagulation. After 1 h, blood samples were centrifuged at 4200× *g* for 10 min at 4°C. Specimens were subsequently stored until assayed according to the manufacturer's guidelines.

Data for $TXB_2$ are expressed as median with range. Paired variables were analysed using the Wilcoxon signed rank test. A $P$ value <0.05 was considered significant. All statistical computations were performed using 'Analyse it' for Microsoft Excel 2003.

## Results

Median baseline $TXB_2$ concentrations before aspirin or NSAID (999 nmol·$L^{-1}$ range 495–2775) were in agreement with previous reports (Van Kraaij *et al.*, 2002; Hovestad-Witterland *et al.*, 2003). Aspirin (30 mg) reduced $TXB_2$ concentrations by 90.3% (range 83.1–96.0%) compared with baseline, whereas aspirin (80 mg) reduced $TXB_2$ concentrations by 98.0% (range 96.8–99.2%). Diclofenac reduced $TXB_2$ concentrations significantly less than ibuprofen, but with more individual variation (30.3%; range 20.3–76% vs. 83.4%; range 69.7–97.7%). Diclofenac in combination with aspirin (80 mg) reduced $TXB_2$ to the same degree as aspirin (80 mg) alone (98.1%; range 97.2–98.9%), whereas the combination of ibuprofen with aspirin (80 mg) reduced the $TXB_2$ concentration less than

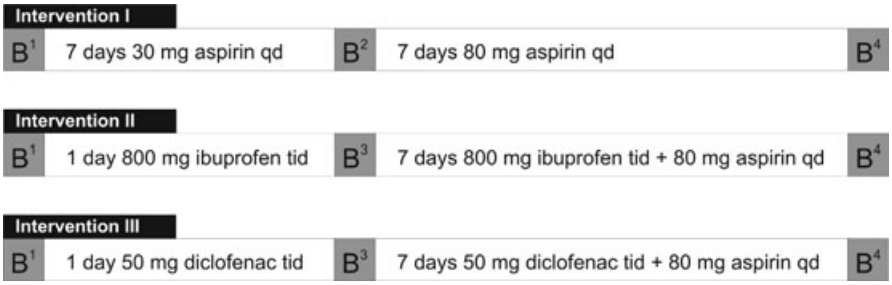


**Figure 1** The three different intervention groups and blood withdrawal time points. $B^1$: pretreament blood collection. $B^2$: blood collection at steady state 24 h after last intake of aspirin 30 mg. $B^3$: blood collection at steady state 8 h after last intake of non-steroidal anti-inflammatory drug (NSAID). $B^4$: blood collection at steady state 24 h after last intake of aspirin 80 mg and 8 h after last intake of NSAID.

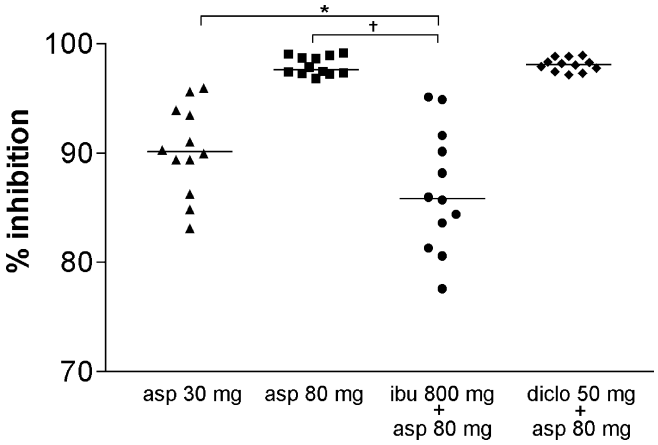


**Figure 2** The percentage inhibition of thromboxane $B_2$ formation due to aspirin 30 mg (asp 30 mg), aspirin 80 mg (asp 80 mg), the combination of ibuprofen 800 mg with aspirin 80 mg (ibu 800 mg + asp 80 mg) and the combination of diclofenac 50 mg with aspirin 80 mg (diclo 50 mg + asp 80 mg): †$P = 0.0005$, *$P = 0.04$.

aspirin (30 mg) once daily (86.6%; range 77.6–95.1%) ($P = 0.04$; Figure 2).

## Discussion and conclusions

In this study, the measurement of $TXB_2$ formed during the clotting of whole blood was used as a sensitive index of COX-1 activity in platelets (Patrono *et al.*, 1985) and the procedure may be used to evaluate any possible interference with the thromboprophylactic effect of aspirin. Platelet function or aggregation tests were not used, as we have previously shown that aspirin causes a marked increase in variation in measurements of platelet function in healthy volunteers (Klein Gunnewiek *et al.*, 2005). Furthermore, platelet function tests are global tests, which are influenced by several factors that lead to platelet activation and aggregation. The antithrombotic effect and possible aspirin resistance might be measured with these tests. The interaction of NSAIDs with the thromboprophylactic effect of aspirin, however, is only caused by obstruction of the COX-1 channel and therefore we believe that the measurement of COX-1 activity should be the method of choice in studies of such interactions in healthy volunteers.

At peak levels, the antagonistic effects of ibuprofen on the inhibition of COX-1 activity by aspirin has already been reported (Gengo *et al.*, 2008; Gladding *et al.*, 2008). In order to resemble daily dosing practice in the Netherlands, we administered aspirin as immediate release tablets concurrently with immediate release ibuprofen or slow release diclofenac. This dosing schedule mirrored that used by Renda *et al.* (2006), who co-administered ibuprofen and aspirin in patients with osteoarthritis and ischemic heart disease. The concurrent administration of the non-selective NSAID, ibuprofen, with aspirin (80 mg), counteracted the aspirin-induced COX-1 inhibition of $TXB_2$ in healthy volunteers to a level lower than the effect of aspirin (30 mg) once daily, thereby reducing any thromboprophylactic effect of aspirin. The Dutch TIA Trial Study Group (The Dutch TIA Trial Study Group, 1991) has shown that the thromboprophylactic effect of aspirin is questionable if the reduction of the platelet $TXB_2$ production falls below the reduction caused by aspirin (30 mg) once daily.

In accordance with its decreased affinity for COX-1, diclofenac did not affect the aspirin-induced COX-1 inhibition of platelet $TXB_2$ during concurrent administration of aspirin and diclofenac. This decrased affinity would also account for our finding that diclofenac taken alone reduced platelet $TXB_2$ concentration by only 30.3%, whereas ibuprofen alone resulted in 83.4% inhibition of platelet $TXB_2$. These observations have been reported earlier by Van Hecken *et al.* (2000), who showed increased COX-2 selectivity of diclofenac compared with ibuprofen.

It should be noted that our data using ibuprofen were obtained with the maximum registered dose of 800 mg three times daily. Using this dosing regime ibuprofen produces a greater inhibition of platelet $TXB_2$ than that produced by a lower dose regime (i.e. it compensates more for the NSAID-reduced action of aspirin). Therefore, the total inhibition of the $TXB_2$ may be even less if aspirin is combined with a lower dose of ibuprofen. This conclusion seems to be supported by the data of Catella-Lawson *et al.* (2001) who found an inhibitory effect on platelet $TXB_2$ of 67% with ibuprofen at a dose of 400 mg, whereas we found 86% with ibuprofen at a dose of 800 mg. However, in that study the last two doses of the NSAID were omitted (GA FitzGerald, personal communication). In that way the $TXB_2$ levels in the final serum sample are somewhat higher than those usually seen in common clinical practice, that is, in patients who continue to take their NSAIDs. We used immediate release aspirin instead of enteric-coated aspirin. The latter will decrease the interaction because the absorption of aspirin is faster from immediate release tablets and this reduces the possibility that the NSAIDs occupy the COX-1 channel before it is inactivated by aspirin.

Epidemiological studies (Curtis *et al.*, 2003; Kurth *et al.*, 2003; MacDonald and Wei, 2003) have reported conflicting data concerning the influence of ibuprofen on the cardioprotective effect of aspirin. These discrepancies may be due to methodological differences; such as differences in times of drug administration. The clinical relevance of the counteracting effect of ibuprofen on aspirin-induced $TXB_2$ inhibition is supported by the recent post-hoc analysis of the TARGET study (Farkouh *et al.*, 2007). High risk patients using ibuprofen in combination with aspirin had significantly more thrombotic cardiovascular events (2.14%) than patients using the selective COX-2 inhibitor lumiracoxib combined with aspirin (0.25%; $P < 0.03$), whereas no difference was observed in a subgroup using ibuprofen or lumiracoxib only (0.92% vs. 0.80% respectively).

Therefore, as alternatives are easily available, NSAIDs such as diclofenac should be preferred to ibuprofen for combined use with aspirin, as was recently proposed in the protocol for pain relief by the Dutch Family Practitioners Society.

## Conflict of interest

The authors state no conflict of interest.

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