Propacetamol augments inhibition of platelet function by diclofenac in volunteers

E. Munsterhjelm1*, T. T. Niemi1, M. T. Syrjälä2, O. Ylikorkala3 and P. H. Rosenberg1

Department of Anaesthesiology and Intensive Care Medicine, 2Department of Clinical Chemistry and 3Department of Obstetrics and Gynaecology, Helsinki University Hospital, Finland

*Corresponding author: PO Box 340 (P-floor), FIN-00029 HUS, Finland. E-mail: edward.munsterhjelm@hus.fi

Background. Acetaminophen (paracetamol) enhances the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs). Acetaminophen is a weak inhibitor of cyclooxygenase (COX), and its combination with an NSAID may augment COX inhibition-related side effects.

Methods. Ten healthy male volunteers (21–30 yr) were given diclofenac 1.1 mg kg⁻¹ alone, a combination of propacetamol 30 mg kg⁻¹ (which is hydrolysed to 50% acetaminophen) and diclofenac 1.1 mg kg⁻¹ or placebo intravenously in a double blind, crossover study. Platelet function was assessed at 5 min, 90 min and 22–24 h by photometric aggregometry, platelet function analyser (PFA-100™) and by measuring the release of thromboxane B₂ (TxB₂). Analgesia was assessed with the cold pressor test.

Results. Platelet aggregation induced with arachidonic acid was fully inhibited by both diclofenac alone and the combination at the end of the 30-min drug infusion. Propacetamol augmented the inhibition by diclofenac at 90 min (P=0.014). At 22–24 h, platelet function had fully recovered. TxB₂ release was inhibited by the combination of propacetamol and diclofenac at 90 min in comparison with diclofenac alone (P=0.027). PFA-100™ detected no difference in platelet function between these two groups. No analgesic effect was detected with the cold pressor test.

Conclusions. The combination of propacetamol and diclofenac inhibits platelet function more than diclofenac alone. This should be considered when assessing the risk of surgical bleeding.

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The use of non-steroidal anti-inflammatory drugs (NSAIDs) in postoperative pain management is well documented.¹ Acetaminophen is also an effective analgesic.² Acetaminophen and NSAIDs are often given in combination, to increase the analgesic effect.² ³ The mechanism of action of NSAIDs is considered to be inhibition of cyclooxygenase (COX), the key enzyme in prostaglandin formation.⁴ The inhibition of COX-1 is the basis of their anti-aggregatory effect on platelets, as production of pro-aggregatory thromboxane A₂ is blocked.⁵ The analgesic effect of NSAIDs, on the other hand, is mediated through inhibition of inducible COX-2.⁵ The analgesic effect of acetaminophen is probably mediated through central COX-inhibition.⁶ COX-3, a recently characterized variant of the COX enzyme, is mainly expressed in the central nervous system and appears to be more readily inhibited by acetaminophen than are COX-1 and COX-2.⁷ However, acetaminophen has also peripheral COX-1 inhibiting⁸ and anti-aggregatory properties.⁷ We therefore hypothesized that acetaminophen augments the inhibitory effect of NSAIDs on platelet aggregation through increased inhibition of platelet COX-1.

Methods

The protocol was approved by the local Medical Ethics committee and the National Agency for Medicines. Ten healthy, non-smoking male volunteers aged 21–30 yr were investigated in this double-blinded, randomized, placebo-
controlled crossover study. Written informed consent was obtained. The volunteers were not allowed to use acetylsalicylic acid for 10 days and no other drugs for 1 week before each experiment.

**Experimental procedures**

After 3 h of fasting, a 30-min infusion of either diclofenac (Voltaren®, Novartis, Finland) 1.1 mg kg⁻¹, a combination of diclofenac 1.1 mg kg⁻¹ and propacetamol (Pro-Dafalgan®, Bristol-Myers Squibb, France) 30 mg kg⁻¹ or placebo was given intravenously on three different occasions in random order with at least a 1-week interval between the experiments. Propacetamol is a pro-drug that, given intravenously, is rapidly hydrolysed into 50% acetaminophen and 50% diethylglycine by plasma esterases. Diclofenac and propacetamol were diluted in 100 ml or 250 ml of normal saline, respectively. The solutions were blinded, and the code broken when all experiments had been performed. A dorsal vein on the hand was cannulated with a 20-gauge cannula (Venflon™, Becton Dickinson, UK). During the test the volunteers were resting.

Venous blood samples were drawn from an antecubital vein before the infusion and at 5 min, 90 min and 22–24 h thereafter. A 20-gauge needle (PrecisionGlide™, Becton Dickinson, UK) was used and the samples were collected into polypropylene tubes (Vacuette®, Greiner bio-one, Austria) containing 3.2% buffered citrate, giving a volume ratio of 1:10.

**Cold pressor test**

Immediately after every blood sample, except that at 22–24 h, a cold pressor test was performed. The volunteer immersed his non-dominant hand, halfway to the elbow, into an ice bath and estimated the pain intensity on a 10 cm visual analogue scale at 30 and 60 s. The volunteer was instructed to withdraw his hand earlier, if the pain became unbearable. There was at least a 60 min interval between the cold pressor test and the next blood sample.

**Laboratory tests**

Platelet count and haemoglobin concentration were determined with a Sysmex K-1000 blood cell counter (Sysmex Corporation, Kobe, Japan).

**Platelet aggregation**

Platelet aggregation was measured with a four channel photometric aggregometer (Packs-4, Helena Laboratories, USA) based on the method of Born. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifuging at +20°C at 160 g for 9 min and at 3000 g for 5 min, respectively. Platelet count in PRP was adjusted to 300×10⁶ litre⁻¹ ± 10% by diluting with autologous PPP. During continuous stirring (1000 r.p.m.) at 37°C, 270 μl PRP was analysed. Aggregation was induced by adding 30 μl of an agonist, adenosine diphosphate (ADP), to a final concentration of 3 μM, arachidonic acid to a final concentration of 1 nM, collagen to a final concentration of 50 μg/ml, and thrombin receptor activating peptide (TRAP, SFLLRN) to a final concentration of 10 μM. The reagents were purchased from Sigma-Aldrich (St Louis, USA) and Bachem (Weil am Rhein, Germany). The concentrations were chosen to ensure a high probability of aggregation based on previous experience. The aggregation was allowed to proceed for 300 s, after which plasma for thromboxane B₂ (TxB₂) determination was prepared as described earlier. The area under the curve of the aggregometry was recorded, and 300 s% (300 s×10%) was subtracted to compensate for the 10% dilution of PRP when the agonist was added.

**Thromboxane B₂ concentration**

TxB₂ is the stable metabolite of thromboxane A₂ (TxA₂), released during aggregation. The TxB₂ concentration in PRP after aggregation induced with ADP or arachidonic acid was determined with a radioimmunoassay as described earlier. The assay sensitivity was 80 pg/ml, and the intra-assay coefficient of variation was 17% (n=9).

**PFA-100™**

With the platelet function analyser (PFA-100™, Dade Behring, USA) we determined closure times on duplicate samples of 900 μl citrated whole blood. After collection, all samples were incubated at room temperature for 30 min to 2 h. Cartridges containing collagen/epinephrine or collagen/ADP membranes were used. The upper detection limit of the closure time is 300 s. When exceeded, the result was considered 300 s to allow statistical analysis.

All results are expressed as percentage of pre-infusion value.

**Statistics**

The sample size needed was estimated in advance as described in the statistical literature. The study was designed to discover a difference in platelet aggregation between the diclofenac group and the combination group greater than 1 SD, with a power of 80% (α-error=5%). The theoretical sample size needed was n=7.85. A difference smaller than 1 SD was considered of minor clinical significance. The difference between the groups was analysed with the Friedman repeated measures analysis of variance on ranks test. When a significant difference was detected, the diclofenac and the combination groups were further compared with the Wilcoxon signed rank test.

**Results**

All volunteers showed normal platelet function with the methods used. However, aggregation induced with collagen displayed a high intra-assay variation, and therefore the data with collagen were excluded from further analysis.
Immediate effect of analgesics

Both diclofenac and the combination of diclofenac and propacetamol had an immediate inhibitory effect on platelet aggregation induced with arachidonic acid and ADP (Fig. 1, Table 1). Platelet aggregation induced with TRAP showed results comparable with ADP (data not shown). TxB₂ release during aggregation induced with ADP or arachidonic acid was reduced by both diclofenac and the combination (Table 2). The closure time with PFA-100 was prolonged (Table 3). We did not detect any effect of propacetamol on this immediate inhibition of platelet function by diclofenac with any of the methods used.

90 min after infusion

Reversibility of the inhibition of platelet aggregation by diclofenac was evident 90 min after the infusion (Table 1). In combination with propacetamol, this reversibility was almost fully prevented. When induced with arachidonic acid, the difference between diclofenac and its combination

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**Table 1** Platelet aggregation induced with ADP. All data are percentage of pre-infusion value, median (25th/75th percentile). Each volunteer (n=10) was given placebo, diclofenac, and a combination of diclofenac and propacetamol. Statistical tests are: Friedman repeated measures analysis of variance on ranks test (all groups) and Wilcoxon signed rank test (diclofenac vs combination).

<table>
<thead>
<tr>
<th>Time after drug administration</th>
<th>Drugs</th>
<th>P-value (all groups)</th>
<th>P-value (diclofenac vs combination)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Diclofenac</td>
<td>Combination</td>
</tr>
<tr>
<td>5 min</td>
<td>99.3 (96.6/101.2)</td>
<td>83.4 (66.8/87.9)</td>
<td>82.8 (73.3/88.7)</td>
</tr>
<tr>
<td>90 min</td>
<td>98.6 (97.0/99.5)</td>
<td>93.9 (83.1/95.9)</td>
<td>82.7 (69.7/94.4)</td>
</tr>
<tr>
<td>22–24 h</td>
<td>101.3 (97.8/103.2)</td>
<td>102.8 (98.2/104.9)</td>
<td>101.2 (99.9/103.7)</td>
</tr>
</tbody>
</table>

**Table 2** TxB₂ release from activated platelets. All data are percentage of pre-infusion value, median (25th/75th percentile). Each volunteer (n=10) was given placebo, diclofenac, and a combination of diclofenac and propacetamol. Statistical tests are: Friedman repeated measures analysis of variance on ranks test (all groups) and Wilcoxon signed rank test (diclofenac vs combination).

<table>
<thead>
<tr>
<th>Activating agent</th>
<th>Time after drug administration</th>
<th>Drugs</th>
<th>P-value (all groups)</th>
<th>P-value (diclofenac vs combination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid</td>
<td>5 min</td>
<td>97.6 (88.8/107.4)</td>
<td>1.6 (0.9/2.1)</td>
<td>1.3 (0.9/1.8)</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>104.0 (81.5/107.9)</td>
<td>44.1 (19.3/71.9)</td>
<td>10.6 (3.8/27.1)*</td>
</tr>
<tr>
<td></td>
<td>22–24 h</td>
<td>98.3 (81.7/111.8)</td>
<td>92.6 (79.5/102.7)</td>
<td>87.5 (70.9/104.0)</td>
</tr>
<tr>
<td>ADP</td>
<td>5 min</td>
<td>104.1 (89.6/111.6)</td>
<td>3.3 (2.8/4.6)</td>
<td>2.4 (1.8/4.8)</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>84.9 (70.1/105.7)</td>
<td>14.2 (9.7/42.4)</td>
<td>7.3 (3.9/9.2)</td>
</tr>
<tr>
<td></td>
<td>22–24 h</td>
<td>95.1 (87.5/104.4)</td>
<td>83.9 (75.7/92.2)</td>
<td>100.1 (89.9/135.8)</td>
</tr>
</tbody>
</table>
with propacetamol was statistically significant (Fig. 1). TxB2 release was significantly reduced in the combination group compared with diclofenac alone (Table 2). Platelet function measured with PFA-100 failed to detect any difference between the groups (Table 3).

**22–24 h after infusion**

On the next day, the inhibitory effect of both diclofenac and its combination with propacetamol had disappeared. Platelet aggregation, closure time with PFA-100 and TxB2 release had completely recovered (Fig. 1, Tables 1–3).

**Cold pressor test**

Pain induced with the cold pressor test showed a high degree of variation and was not reduced with the analgesics used (Fig. 2).

### Discussion

**Platelet function**

In the present study we demonstrated that propacetamol augments the inhibitory effect of diclofenac on platelet aggregation and TxB2 release. The anti-aggregatory effect of analgesic doses of diclofenac is well documented13 and it was confirmed in this study. We have shown previously that a large dose of propacetamol has a similar inhibitory effect on platelet aggregation and TxB2 release.9

When platelet aggregation was induced with arachidonic acid, the difference between diclofenac only and the combination of propacetamol and diclofenac was significant. Arachidonic acid is the physiologic substrate of COX, the key enzyme in prostaglandin formation. In platelets, COX-1 catalyses the conversion of arachidonic acid to TxA2, which binds to specific G-protein coupled receptors on the surface of the platelet.14 As COX is the rate-limiting step in this reaction, aggregation initiated with arachidonic acid is

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**Table 3** Platelet function measured as PFA-100 closure times. All data are percentage of pre-infusion value, median (25th/75th percentile). Each volunteer (n=10) was given placebo, diclofenac, and a combination of diclofenac and propacetamol. Statistical tests are: Friedman repeated measures analysis of variance on ranks test (all groups) and Wilcoxon signed rank test (diclofenac vs combination)

<table>
<thead>
<tr>
<th>Activating agent</th>
<th>Time after drug administration</th>
<th>Placebo</th>
<th>Diclofenac</th>
<th>Combination</th>
<th>P-value (all groups)</th>
<th>P-value (diclofenac vs combination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen/epinephrine</td>
<td>5 min</td>
<td>97.0 (82.3/108.4)</td>
<td>233.4 (197.8/246.9)</td>
<td>238.2 (219.8/259.7)</td>
<td>&lt;0.001</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>96.0 (77.5/103.4)</td>
<td>135.8 (123.7/141.0)</td>
<td>150.9 (129.0/172.9)</td>
<td>&lt;0.001</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>22–24 h</td>
<td>87.0 (82.5/103.8)</td>
<td>100.6 (89.6/108.0)</td>
<td>109.7 (95.5/120.1)</td>
<td>0.41</td>
<td>0.70</td>
</tr>
<tr>
<td>Collagen/ADP</td>
<td>5 min</td>
<td>93.3 (87.6/99.3)</td>
<td>102.8 (98.3/122.9)</td>
<td>102.5 (100.7/111.8)</td>
<td>0.002</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>91.5 (82.0/95.9)</td>
<td>91.6 (87.2/106.0)</td>
<td>98.6 (97.0/105.9)</td>
<td>0.082</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>22–24 h</td>
<td>96.2 (89.9/100.6)</td>
<td>99.6 (87.8/105.6)</td>
<td>104.5 (99.3/116.8)</td>
<td>0.15</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**Fig 2** Amount of pain induced with the cold pressor test after 30 s. The figure shows all individual estimations, before the infusion (pre) and at 5 and 90 min thereafter. Each volunteer (n=10) was given placebo, diclofenac and a combination of diclofenac and propacetamol. Statistics with the Friedman repeated measures analysis of variance on ranks test. *P=0.72, all groups; **P=0.71; ***P=0.38.
acid reflects the activity of COX. Traditional NSAIDs inhibit COX by binding to its active site and thereby blocking its catalytic activity.\(^5\)

When ADP and TRAP were used as agonists the anti-aggregatory effect of diclofenac and its combination with propacetamol were much less pronounced. This supports our hypothesis that the additive effect of propacetamol is mediated through inhibition of platelet COX. Both ADP and TRAP bind directly to receptors on the surface of the platelet. Two ADP-binding receptors, P2Y\(_1\) and P2Y\(_{12}\), have been isolated in platelets.\(^{15}\) TRAP activates the thrombin receptor, PAR-1.\(^{16}\) Activation of these receptors initiates intracellular signalling that activates the production of TxA\(_2\), although aggregation is not dependent on this pathway.\(^{17}\)

The time frame of the additive effect of propacetamol and diclofenac fits well with a COX-mediated mechanism. Immediately after the infusion a high concentration of diclofenac fully inhibits COX and no additional effect of propacetamol could be detected. The anti-aggregatory effect of diclofenac was decreasing at 90 min because of its relatively short half-life in plasma, 1.1 h.\(^{18}\) At this stage the inhibitory effect on platelet aggregation was augmented by propacetamol. The corresponding time frame observed regarding the release of TxB\(_2\) further supports a COX-related mechanism. The TxA\(_2\), released through platelet COX-1 activity, rapidly decomposes into TxB\(_2\). At 90 min the TxB\(_2\) release, and therefore COX activity, was significantly inhibited by the presence of propacetamol as compared with diclofenac only. The inhibition of TxB\(_2\) release by the combination can be compared with the inhibitory effect of high-dose propacetamol reported earlier.\(^9\) The median TxB\(_2\) release was 48.4% of pre-infusion value after propacetamol (60 mg kg\(^{-1}\)) and 34.9% after ketorolac (0.4 mg kg\(^{-1}\)) from platelets activated with 8 \(\mu\)M ADP at 90 min after infusion.

A direct additive inhibitory effect on platelet COX by the drug combination is the most likely mechanism, a pharmacokinetic interaction through protein binding or metabolism seems less probable. In contrast to diclofenac, acetaminophen is not highly protein bound at therapeutic concentrations and the drugs have different metabolic pathways in the liver.\(^{19}\)

The platelet function analyser PFA-100\(^{TM}\) is an in vitro test for primary haemostasis, having fewer sources of errors than conventional Ivy’s bleeding time.\(^{20}\) The prolongation of collagen/epinephrine closure time demonstrated clearly the immediate inhibitory effect of both diclofenac and the combination of diclofenac and propacetamol. The drugs produced only a minor difference in the collagen/ADP closure time, which is in accordance with results obtained after ingestion of aspirin.\(^{21}\) However, no difference was seen between diclofenac and the combination, possibly because the maximal closure time is restricted to 300 s. In the present study photometric aggregometry was clearly more sensitive than PFA-100\(^{TM}\).

### Analgesic effect

In contrast to the clinical observations of a postoperative analgesic effect of both diclofenac\(^1\) and acetaminophen\(^2\) we detected no analgesic effect of the drugs with the cold pressor test as a noxious stimulus. This experimental pain model has been extensively applied in studies on both pain\(^22\) and analgesics.\(^{23}\) In a previous study, acetaminophen showed an analgesic effect in this pain model.\(^{24}\)

Although the major mechanism of action of NSAIDs is peripheral, there is evidence in favour of a central mechanism involving spinal COX-2 inhibition.\(^{25}\) This mechanism requires a noxious stimulus of longer duration to allow spinal COX-2 upregulation.\(^{25}\) The lack of an analgesic effect of diclofenac in our short-acting pain model is therefore logical. On the other hand, we did not detect any effect of the addition of acetaminophen either, which was unexpected considering previously published results.\(^{24}\)

### Clinical implications

The clinical implications of reduced platelet aggregation could be potentially both advantageous and hazardous. Low-dose aspirin has been shown to reduce the incidence of deep-vein thrombosis in patients undergoing surgery for hip fracture, and the incidence of death from pulmonary embolism.\(^{26}\) On the other hand, during the intra- and immediate postoperative period a reduced platelet function may cause bleeding. In a recent systemic review on NSAIDs and bleeding after tonsillectomy, an increased risk for re-operation after use of NSAIDs was found.\(^{27}\) Likewise, pretreatment with ibuprofen appears to increase perioperative blood loss during total hip replacement\(^{28}\) and low-dose aspirin has the same effect given before transurethral prostatectomy.\(^{29}\)

Our results indicate that adding propacetamol to a diclofenac treatment regime can augment the anti-aggregatory effect of this traditional NSAID. This should be taken into consideration when assessing the delicate balance between bleeding and thrombotic complications during and after surgery.

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