Dose-dependent Inhibition of Platelet Function by Acetaminophen in Healthy Volunteers

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Background: Acetaminophen (paracetamol) is widely used for postoperative analgesia. Its mechanism of action is inhibition of prostaglandin synthesis in the central nervous system, and acetaminophen is traditionally not considered to influence platelet function. The authors studied the dose-dependent inhibition of platelet function by acetaminophen in healthy volunteers.

Methods: Thirteen healthy male volunteers (aged 19–26 yr) were given placebo or 15, 22.5, or 30 mg/kg acetaminophen intravenously in a double-blind, crossover study. Ten and 90 min after infusion, platelet function was assessed by photometric aggreometry and by measuring release of thromboxane B2, analgesia by cold pressor test, and plasma acetaminophen concentrations by high-performance liquid chromatography.

Results: When triggered with 500 μl arachidonic acid, median platelet aggregation (area under the curve) was 25.7, 22.8, 4.1, or 3.6 × 10^3 area units (P < 0.001) 10 min after placebo or 15, 22.5, or 30 mg/kg acetaminophen, respectively. An increasing concentration of arachidonic acid attenuated the antiaggregatory effect. After 90 min, platelet function was recovering. Release of thromboxane B2 was also dose-dependently inhibited by acetaminophen. Although plasma concentration of acetaminophen increased linearly with the dose, no analgesic effect was detected in the cold pressor test.

Conclusions: Acetaminophen, which is a weak inhibitor of platelet cyclooxygenase 1, has a dose-dependent antiaggregatory effect. This property may become clinically significant in patients with intrinsic or drug-induced impairment of hemostasis.

CYCLOOXYGENASE (COX), the key enzyme in prostaglandin formation, is an important pharmacological target. The antithrombotic effect of acetylsalicylic acid is caused by irreversible inhibition of COX-1, constitutively expressed in platelets, whereas the analgesic effect of nonsteroidal antiinflammatory drugs (NSAIDs) is mediated through inhibition of COX-2, induced during inflammation. The main mechanism of action of acetaminophen is inhibition of prostaglandin synthesis in the central nervous system, the recently characterized COX-3 being a possible target. However, acetaminophen has also peripheral COX-1 inhibiting properties.

Normal platelet function is dependent on the production of proaggregatory thromboxane A2 (TxA2) through COX-1, and acetaminophen has been shown to inhibit platelet function both in vitro and in high intravenous doses in vivo. Acetaminophen is widely used for postoperative analgesia, although the optimal dose is debatable. In pediatric patients, no analgesic ceiling effect was detected when acetaminophen was administered rectally in doses up to 60 mg/kg. However, high doses of acetaminophen may alter platelet function through peripheral COX-1 inhibition. Because proper platelet function is essential for adequate intraoperative and postoperative hemostasis, we studied the dose-dependent effect of acetaminophen on platelet function in healthy volunteers. We also measured the analgesic effect of acetaminophen with the cold pressor test as a painful stimulus.

Materials and Methods

The protocol was approved by the Ethics Committee for Studies in Healthy Subjects and Primary Care in the Hospital District of Helsinki and Uusimaa (Helsinki, Finland) and by the National Agency for Medicines in Finland. Fifteen healthy, nonsmoking men aged between 19 and 26 yr volunteered in this double-blinded, randomized, placebo-controlled, crossover study. Written informed consent was obtained from each subject before the study. Normal plasma alanine transaminase and aspartate aminotransferase activities were a prerequisite for participation. Two volunteers withdrew their consent before completing the study. The use of acetylsalicylic acid was forbidden for 10 days and that of other drugs affecting platelet function was forbidden for 1 week before each experiment.

Experimental Procedures

Every volunteer participated in four experiments with at least a 1-week interval. After 3 h of fasting, 15, 22.5, or 30 mg/kg acetaminophen (Perfalgan®; Bristol-Myers Squibb, New York, NY) or placebo (0.9% NaCl; Braun, Kronberg, Germany) was given as a 10-min intravenous infusion through a 20-gauge cannula (Venflon; Becton Dickinson, Franklin Lakes, NJ) in a dorsal vein of the hand. The infusions were blinded and administered in random order. The code was not broken until all experiments had been performed.

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were centrifuged at 3,000 g. The coefficient of variation was 17.7% (n = 11005). The curve of aggregometry was recorded. Aggregation was allowed to proceed for 300 s; after that, concentrations are known to cause platelet aggregation.

Diego, CA). Based on previous experience, these concentrations of 500, 750, or 1,000 M; arachidonic acid to a final concentration of 1.5 or 3 M. Reagents were purchased from Sigma-Aldrich (St. Louis, MO) and Calbiochem (San Diego, CA). Based on previous experience, these concentrations are known to cause platelet aggregation. Aggregation was allowed to proceed for 300 s; after that, plasma for thromboxane B2 (TxB2) determination was prepared as described previously.6 The area under the curve of aggeregmetry was recorded.

Thromboxane B2 Concentration. Thromboxane B2 is the stable metabolite of TxA2, released during aggregation. TxB2 concentrations in platelet-rich plasma triggered with 3 M ADP or 1 mM arachidonic acid were determined with a radioimmunoassay.15 The interassay coefficient of variation was 17.7% (n = 15).

Acetaminophen Concentration. Blood samples were centrifuged at 3,000g for 10 min, and plasma was stored at −20°C. Acetaminophen concentration was determined using high-performance liquid chromatography.16 The limit of quantification was 0.1 mg/l, and the day-to-day coefficients of variation were 7.4% at 16.8 mg/l and 4.2% at 36.8 mg/l (n = 6).

Statistical Analysis
The sample size needed was estimated in advance as described in the statistical literature.17 The study was designed to discover a difference in platelet aggregation between each acetaminophen group and placebo greater than 1 SD, with a power of 80% (α error = 5%, Bonferroni correction was applied). The sample size needed was n = 11. A difference smaller than 1 SD was considered of minor clinical significance. Data distribution was tested with the Kolmogorov-Smirnov test, and non-parametric statistics were used for nonnormally distributed data. Nonnormally distributed data are presented as medians and 25th/75th percentiles; normally distributed data are presented as mean values and 95% confidence intervals. The difference between all groups was analyzed with the Friedman test (repeated-measures analysis of variance on ranks), and when a significant difference was encountered, each acetaminophen group was further compared with placebo using the Wilcoxon matched pairs signed rank sum test and applying the Bonferroni correction. Statistical testing was with SigmaStat for Windows Version 2.03 (SPSS Inc., Chicago, IL). Confidence intervals were calculated using the appropriate t distribution.

Results
Thirteen volunteers completed the study according to the protocol, all of whom showed normal platelet function before drug infusion. Plasma acetaminophen concentrations increased linearly with dose, and exceeded 17.0 mg/l at 10-min sampling after all doses used (table 1). Ninety minutes after infusion, plasma acetaminophen concentrations decreased, but they remained significantly above 10 mg/l after 22.5 and 30 mg/kg acetaminophen. No acetaminophen was detected before infusion in any of the volunteers.

Effect of Acetaminophen on Platelets
Acetaminophen dose-dependently inhibited platelet aggregation triggered with arachidonic acid, ADP, or
epinephrine (table 2). Ten minutes after infusion, 15 mg/kg acetaminophen caused a significant inhibition of platelet aggregation triggered with arachidonic acid. Inhibition was most pronounced with 500 \( \mu M \) arachidonic acid. An increasing concentration of arachidonic acid counteracted the inhibition; with 1,000 \( \mu M \) arachidonic acid, it was minimal, although still statistically significant. Aggregation triggered with ADP or epinephrine was less sensitive to inhibition by acetaminophen; at 10 min, a significant inhibition was achieved only with 30 mg/kg acetaminophen. The inhibition was reversible; aggregation in response to all triggers was recovering at 90 min after infusion, reflecting the decreasing plasma concentration of acetaminophen in the last sample.

**Cold Pressor Test**

Pain threshold in the cold pressor test showed a large variation and was not increased by acetaminophen (fig. 1). Time until sensation of strong pain was also highly variable and not significantly influenced by acetaminophen (data not shown).

**Table 3. Thromboxane B\(_2\) Release from Activated Platelets**

<table>
<thead>
<tr>
<th>Aggregation Trigger</th>
<th>Time after Drug Administration</th>
<th>0 mg/kg</th>
<th>15 mg/kg</th>
<th>22.5 mg/kg</th>
<th>30 mg/kg</th>
<th>( P ) Value (All Groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mm arachidonic acid</td>
<td>Pre</td>
<td>1,201 (1,111/1,491)</td>
<td>1,227 (1,058/1,406)</td>
<td>1,060 (826/1,496)</td>
<td>1,193 (1,119/1,379)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>1,432 (1,182/1,526)</td>
<td>1,012 (8,20/1,188)</td>
<td>748 (573/979)</td>
<td>923 (739/1,000)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>1,262 (1,190/1,458)</td>
<td>1,301 (1,026/1,419)</td>
<td>968 (653/1,222)</td>
<td>1,181 (987/1,260)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 ( \mu M ) ADP</td>
<td>Pre</td>
<td>24.7 (20.9/41.1)</td>
<td>24.9 (13.7/39.5)</td>
<td>21.5 (19.3/36.1)</td>
<td>35.0 (22.2/44.5)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>28.5 (18.1/40.0)</td>
<td>18.5 (9.5/24.2)</td>
<td>10.0 (6.3/20.8)</td>
<td>6.6 (3.2/11.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>30.8 (20.2/39.7)</td>
<td>22.6 (16.2/29.2)</td>
<td>15.8 (12.6/26.0)</td>
<td>12.7 (10.0/28.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are concentrations (\( \mu g/l \)) reported as medians (25th/75th percentiles). Each volunteer (n = 13) was given placebo and 15, 22.5, and 30 mg/kg acetaminophen. Sampling was before drug administration (Pre) and 10 and 90 min after infusion. Statistical tests are the Friedman test (all groups) and Wilcoxon matched pairs signed rank sum test with Bonferroni correction (each acetaminophen dose vs. placebo): * \( P < 0.05 \), † \( P < 0.01 \), ‡ \( P < 0.005 \).

ADP = adenosine diphosphate.
Acetaminophen has shown negative results.\textsuperscript{7,8} In the current study, intravenous doses (approximately 1 g) of oral acetaminophen has a clear inhibitory effect in large doses\textsuperscript{6} to prostaglandin G\textsubscript{2} in the cyclooxygenase reaction,\textsuperscript{20} the mechanism by which the reactions in the catalytic center of COX. It has been suggested that acetaminophen, like other phenolic compounds, can act as a reducing agent and quench a tyrosyl radical necessary for propagation of the cyclooxygenase reaction.\textsuperscript{21} In our study, the antiaggregatory effect of acetaminophen was inversely related to the concentration of arachidonic acid used as a trigger. This suggests a competition either between acetaminophen and arachidonic acid for entering the cyclooxygenase reaction or between acetaminophen and prostaglandin G\textsubscript{2} for entering the peroxidase reaction. Previous data from vascular endothelial cells point toward the latter alternative.\textsuperscript{22}

When ADP or epinephrine was used to trigger aggregation, the inhibitory effect of acetaminophen was much less pronounced. In contrast to aggregation induced with arachidonic acid, no clear difference was detected when the concentration of ADP was increased. This is probably because aggregation triggered with ADP is mainly independent of COX activity and TxA\textsubscript{2} release.\textsuperscript{23} ADP and epinephrine bind directly to their own receptors on the surface of the platelet. Two ADP-binding receptors, P2Y\textsubscript{1} and P2Y\textsubscript{12}, have been isolated,\textsuperscript{24} whereas only one adrenergic receptor, \(\alpha_{2A}\), seems to be active in platelets.\textsuperscript{25}

In previous studies, the conclusion was drawn that acetaminophen does not inhibit platelet function.\textsuperscript{7,8} Our results contradict this conclusion. We suggest two main reasons for this contradiction. First, ADP, epinephrine, and collagen were used to trigger aggregation in those studies.\textsuperscript{7,8} When we triggered platelet aggregation with ADP or epinephrine, only the highest dose of intravenous acetaminophen, 30 mg/kg, corresponding to a total dose of approximately 2 g, caused a significant inhibition. Second, a lower peak plasma acetaminophen concentration is achieved with oral than with intravenous administration.\textsuperscript{26} Because approximately 1 g oral acetaminophen was used in the previous studies, no inhibition could have been detected under those circumstances.

Thromboxane A\textsubscript{2}, produced in platelets by COX-1 and thromboxane synthase, is unstable and decomposes rapidly into its stable metabolite TxB\textsubscript{2}. TxB\textsubscript{2} release triggered with arachidonic acid (1 mM) or ADP (3 \(\mu\)M) was also dose-dependently inhibited by acetaminophen. Although significant, TxB\textsubscript{2} release was considerably less inhibited by acetaminophen than by the traditional NSAID diclofenac in our previous study.\textsuperscript{14} Under similar conditions, median TxB\textsubscript{2} release in response to ADP shortly after an intravenous infusion of diclofenac (1.1 mg/kg) was only 3.3\% of preinfusion release, as compared with 24\% after acetaminophen (30 mg/kg) in the current study. Our results \textit{in vivo} therefore confirm previous observations \textit{in vitro} showing that acetaminophen is a weaker inhibitor of COX-1 than are conventional NSAIDs.\textsuperscript{5,27}

**Analgesic Effect**

The plasma concentration of acetaminophen required for optimal analgesia is not known. Antipyretic properties of acetaminophen are evident in the plasma concentration range of 10–20 g/l. This concentration or higher...
was observed 10 min after infusion with all doses tested, but after 90 min, plasma acetaminophen concentration remained significantly above 10 g/l only with doses higher than 15 mg/kg. Optimal analgesia may require higher concentrations than antipyresis in adults, but this topic is controversial. When acetaminophen was administrated rectally in children, a linearly increasing morphine-sparing effect was achieved with doses up to 60 mg/kg. Considering that the site of action of acetaminophen is mainly in the central nervous system, a high peak plasma concentration may be important. This could explain why 1 g intravenous acetaminophen has been found more effective in relieving pain than the same dose given orally. In an experimental study using transcutaneous electrical stimulation, 2 g intravenous acetaminophen was more effective than 1 g. Whether higher doses than 1 g intravenous acetaminophen are more effective also in a clinical setting is not known. In contrast to clinical observations, we detected no analgesic effect of acetaminophen with the cold pressor test, which provokes acute sharp pain. In a previous study, 1 g oral acetaminophen showed an analgesic effect in this pain model, but smaller doses were ineffective. Also in a previous study, we did not observe any analgesic effect in the cold pressor test using the combination of propacetamol (prodrug of acetaminophen) and diclofenac. In a recent study, acetaminophen was shown to reduce central hyperalgesia induced by electrical stimulation, further confirming the central mechanism of action of this drug. It is conceivable that the short duration of pain in the cold pressor test does not induce any central sensitization, and probably therefore no analgesic effect of acetaminophen was detected in this study.

Clinical Implications
Because acetaminophen inhibits TXA₂ synthesis less than traditional NSAIDs, surgical bleeding attributable to acetaminophen seems unlikely. A moderate inhibition of platelet aggregation peripherally could rather be beneficial. Low-dose aspirin, for example, has been shown to reduce the incidence of deep-vein thrombosis in patients undergoing surgery for hip fracture, as well as the incidence of death from pulmonary embolism. The situation would be different, however, if hemostasis is impaired by, for instance, drugs or massive hemorrhage. Acetaminophen has been suspected to increase the effect of oral warfarin, as demonstrated by a rise in the International Normalized Ratio. Impaired platelet function is a possible interaction that is not detected with standard tests of hemostasis. In conclusion, our results indicate that intravenous acetaminophen dose-dependently impairs platelet function for at least 90 min after its administration. Inhibition should be considered a typical routine dose in clinical practice. Large patient studies are needed to determine the clinical impact of acetaminophen-induced inhibition of platelet function.

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