Influence of Aspirin Ingestion on In-Vitro Formation and Lysis of Platelet-Fibrin-Thrombi

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Influence of Aspirin Ingestion on In-Vitro Formation and Lysis of Platelet-Fibrin-Thrombi

Ch. Beythien, A. Schuermann, W. Terres, Th. Meinertz

Introduction: Aspirin is successfully used in primary and secondary prevention of myocardial infarction and in combination with plasminogen activators in lysis. Since the optimal dose is still unknown, we developed an in-vitro model to investigate dose dependent influence of aspirin ingestion on formation of combined platelet-fibrin-thrombi and lysis, in-vitro thromboxane A2-synthesis, thrombus histology, and platelet aggregation parameters.

Methods: Thrombi were induced by adding CaCl2 (final concentration 10 mmol/l) and collagen (1 mg/ml) to platelet rich plasma (250 platelets per ml) of healthy non-smoking volunteers. In a cross-over design, doses of none, 20, 50, 100 or 500 mg of aspirin per day were administered for one week. After thrombus aging for 10 (TA10) or 30 min (TA30) thrombolysis over 30 min with urokinase (100, 500 or 2000 IU/ml) was performed.

Results: Depending on increasing aspirin doses, thrombus formation time was prolonged from 292 to 354 seconds; no aspirin vs 500 mg (p < 0.001). Initial thrombus weight of TA10 decreased from 18.5 ± 1.4 to 14.7 ± 1.7 mg (p < 0.001). This was also true for TA30; 15.1 ± 1.1 reduced to 12.4 ± 1.3 mg (p > 0.001). After 30 min of lysis with 2000 IU urokinase final thrombus weight of TA10 was lower than of TA30; no aspirin 5.2 ± 1.3 vs 500 mg 5.5 ± 0.9 mg (p < 0.01) and 7.0 ± 1.8 vs 6.3 ± 1.0 mg (p < 0.05), respectively. Lysis efficacy of urokinase 500 and 2000 IU showed no significant differences, 100 IU/ml had minor lysis efficacy. Inhibition of in-vitro thromboxane synthesis increased from 0 to 99.7 % (p < 0.001). Aggregation rate decreased from 114 ± 20 to 60 ± 12 %/min, extent of aggregation decreased from 86 ± 5 to 62 ± 11 %, and lag time increased from 49 ± 14 to 68 ± 23 sec (p < 0.0001 each).

Conclusion: Corresponding to increasing aspirin doses, thrombus formation time was prolonged, initial thrombus weight was reduced, and lysis was less effective. Urokinase 100 IU/ml induced only minor thrombus weight reduction, 500 and 2000 IU/ml showed comparable results. Lysis of TA30 was less efficacious. Aggregation rate decreased, extent of aggregation decreased, and lag time increased. In our in-vitro model the optimal dose for aspirin was between 50 and 100 mg per day.

Key words: aspirin dosage, thrombolysis, platelets, platelet aggregation

A cute thrombus formation is the common cause of unstable angina, myocardial infarction and sudden ischaemic death [1]. Platelet adhesion and aggregation at ruptured atherosclerotic plaques followed by fibrin formation produce an occlusive or nearly occlusive thrombus in a coronary artery [2]. Thrombolysis is a well-established method to treat myocardial infarctions. The combination of thrombolytic agents with aspirin improves the efficacy of lysis in vivo and in vitro [3, 4]. Aspirin inhibits platelets by irreversible acetylation of platelet cyclooxygenase-isomerization 1 [5–7] and so suppresses thromboxane A2-formation [8, 9]. It is used in primary [10] and secondary prevention of myocardial infarction and stroke [11]. However, the optimal aspirin dose still remains unknown. To further evaluate a dose dependent efficacy we introduced an in-vivo model to test the influence of aspirin ingestion on formation and lysis of in-vitro induced platelet-fibrin-thrombi. In addition, the influence on in-vitro thromboxane A2-synthesis, thrombus histology, and platelet aggregation parameters were investigated.

Methods
Volunteers were randomly selected, apparently healthy non-smokers, who had taken none, 20, 50, 100 or 500 mg aspirin per day in a cross-over design for one week. There were at least two weeks without medication between each series. The sequence of dose application was randomized. After short venous occlusion, blood was collected from a large antecubital vein through a needle of 1.1 mm internal diameter into a plastic syringe. Nine parts of blood were mixed with one part of 3.13 % sodium citrate. The blood was centrifuged at 300 g for 10 minutes. After removal of the platelet rich plasma the remaining plasma was centrifuged at 2500 g for ten minutes. Platelet rich plasma and platelet poor plasma were mixed to obtain a final platelet count of 250/ml. For aggregation tests and thrombus production an aggregometer (APACT, Labor GBmbH, Hamburg, Germany) was used. Thrombus formation was induced by adding CaCl2 (final concentration 10 mmol/l) and collagen in glucose buffer (final concentration 1 mg/l, Hormon Chemie Munchen, Germany). Depending on aspirin dose, thrombus formation occurred after 292 to 354 sec around the stirrer in the cuvette. Time until thrombus generation was recorded. Fibrinogen was measured pre and post thrombus formation (fibrinogen kinetic test, Boehringer Mannheim, Germany). To measure platelet aggregation aliquots of 500 µl citrated platelet rich plasma were incubated for 5 min at 37 °C with a rotating stirrer at 1200 rpm. Aggregation was induced by adding collagen in glucose buffer (final concentration 1 mg/l). Initial light transmission of platelet rich plasma was set at 0 %, platelet poor plasma plus added substances was set at 100 %. Maximal rate, maximal extent of platelet aggregation, and lag time were assessed.

After 10 min or 30 min of aging, thrombi were weighed the first time by removing the microstirrer with surrounding thrombi by a magnetic microstick from the cuvette, simultaneously light transmission of plasma was assessed photometrically. At a thrombus age of 10 or 30 min, lysis over 30 min was started. Thrombolysis was initiated by adding 50 ml urokinase (final concentration 100, 500 or 2000 IU/ml, Medac, Hamburg, Germany), and 50 ml of isotonic NaCl was added for control investigation. For histological examination, 10 min old thrombi generated in platelet rich plasma
The efficacy of lysis was dependent on the dosage of aspirin and urokinase. After 30 min of lysis with 2000 IU urokinase the final thrombus weight at 500 mg of aspirin was higher than in the control experiments without aspirin; 54.0 % versus 64.5 % of the initial weight (p < 0.01). There were no differences between 100 and 500 mg of aspirin. The lysis efficacy of urokinase 500 and 2000 IU showed no significant differences. 100 IU/ml urokinase was less effective. The weight reduction was only 8 % in average vs control without urokinase (Tab. 3).

Thrombus age was another parameter influencing thrombus weight and lysis efficacy. The initial weight of TA10 thrombi without aspirin medication was higher than of TA30 thrombi, 18.5 ± 1.4 vs 15.1 ± 1.1 mg (p < 0.001). The lysis efficacy of 2000 IU/ml urokinase was more pronounced; final thrombus weight of TA10 5.2 ± 1.3 vs 7.0 ± 1.8 mg (p < 0.001). In the experiments with aspirin medication, initial weight of TA10 thrombi was significantly higher, weight after lysis significantly lower than TA30 thrombi; weight reduction under lysis with 2000 IU/ml urokinase in average was 59.2 % vs 47.2 % (p < 0.001). The influence of aspirin on thrombolysis of TA10 starts with 50 mg (p < 0.05 vs no aspirin), and of TA30 with 100 mg (p < 0.05 vs no aspirin).

Fibrinogen values were between 117 and 306 mg/dl. No significant dependence could be demonstrated on aspirin intake or dosage. After thrombus formation, no fibrinogen was detectable in the plasma. The inhibition of in-vitro thromboxane synthesis was also dose dependent. Under the influence of 50 mg of aspirin the inhibition was 96.7 % and reached its optimum with 99.7 % at 500 mg aspirin. 20 mg aspirin resulted in a reduction of only 55.5 % (Fig. 1).

Aggregation rate decreased from 114 ± 20 to 60 ± 12 %/min (p < 0.0001). There was no further reduction from 100 to 500 mg. Extent of aggregation decreased from 86 ± 5 to 62 ± 11 %. Lag time increased from 49 ± 14 to 68 ± 23 sec (p < 0.0001). There were no differences between 50, 100, and 500 mg.

Histological examination revealed thrombi with one or more smaller cores of platelet aggregates with small amounts of fibrin inside the cores. The cores were surrounded by large portions of fibrin and platelet fragments. Plasma contents were found within the fibrin masses. With elevating doses of aspirin ingestion, plasma contents decreased and thrombus density increased macro- and microscopically (Figs. 2, 3).

Discussion

Acute myocardial infarctions are mostly caused by occlusion of coronary arteries due to thrombus formation at fissured or ruptured atherosclerotic plaques [1]. In this in-vitro model the thrombi produced were similar to those found in patients deceased from myocardial infarction or unstable angina [12]. Histological examination showed one or more smaller cores of platelet aggregates with small amounts of fibrin in between. Cores were surrounded by large portions of fibrin. Aspirin did not influence fibrinogen measurements, thus the thrombus weight could not be influenced by this parameter.

Initial thrombus weight was dependent on dose of aspirin ingestion. With higher dosages, weight was reduced by diminished plasma contents. A possible reason for this phenomenon may be the delayed lag time and an increased thrombus formation time. Time until thrombus generation was significantly extended under the influence of aspirin. The inhibition of thrombin generation by acetylating prothrombin and/or

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**Table 1. Influence of aspirin dosage on thrombus generation time**

<table>
<thead>
<tr>
<th>Aspirin dosage</th>
<th>Thrombus generation time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>4 min 51 sec ± 47 sec</td>
</tr>
</tbody>
</table>

**Table 2. Influence of aspirin dosage on initial thrombus weight**

<table>
<thead>
<tr>
<th>Aspirin dosage</th>
<th>Initial thrombus weight 10 min after thrombus generation (n = 96)</th>
<th>Initial thrombus weight 30 min after thrombus generation (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18.5 ± 1.4 mg</td>
<td>15.1 ± 1.1 mg</td>
</tr>
<tr>
<td>20 mg ASS/die</td>
<td>16.9 ± 1.6 mg</td>
<td>14.3 ± 2.1 mg</td>
</tr>
<tr>
<td>50 mg ASS/die</td>
<td>15.6 ± 2.1 mg</td>
<td>13.4 ± 1.6 mg</td>
</tr>
<tr>
<td>100 mg ASS/die</td>
<td>15.5 ± 1.5 mg</td>
<td>12.9 ± 1.3 mg</td>
</tr>
<tr>
<td>500 mg ASS/die</td>
<td>14.7 ± 1.7 mg</td>
<td>12.4 ± 1.3 mg</td>
</tr>
</tbody>
</table>

**Table 3. Thrombus weight of TA10 after 30 min of lysis with urokinase (UK)**

<table>
<thead>
<tr>
<th>ASS-Dosage</th>
<th>Control</th>
<th>100 IU UK</th>
<th>500 IU UK</th>
<th>2000 IU UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.8 ± 1.2 mg</td>
<td>14.2 ± 1.4 mg</td>
<td>5.3 ± 0.9 mg</td>
<td>5.2 ± 1.3 mg</td>
</tr>
<tr>
<td>20 mg ASS</td>
<td>13.9 ± 1.6 mg</td>
<td>12.5 ± 1.4 mg</td>
<td>5.4 ± 0.8 mg</td>
<td>5.0 ± 1.4 mg</td>
</tr>
<tr>
<td>50 mg ASS</td>
<td>12.7 ± 1.1 mg</td>
<td>11.1 ± 1.3 mg</td>
<td>5.5 ± 1.0 mg</td>
<td>5.3 ± 1.3 mg</td>
</tr>
<tr>
<td>100 mg ASS</td>
<td>12.6 ± 1.1 mg</td>
<td>11.9 ± 1.0 mg</td>
<td>6.3 ± 1.6 mg</td>
<td>5.8 ± 1.3 mg</td>
</tr>
<tr>
<td>500 mg ASS</td>
<td>12.1 ± 0.9 mg</td>
<td>11.4 ± 1.2 mg</td>
<td>5.9 ± 1.1 mg</td>
<td>5.5 ± 0.9 mg</td>
</tr>
</tbody>
</table>
macromolecules of platelet membranes might be one reason [13, 14]. A reduced release of platelet factor 3 leading to a prolonged coagulation pathway may be another cause. The thrombus is more dense with minor plasma contents, thus plasminogen inside the thrombus is also reduced. Penetration of plasminogen activators into the thrombus is aggravated. The circumstance that the influence on TA30 starts only at 100 mg aspirin demonstrates that more density after thrombus retraction is a major component for lysis ability. With supposed body plasma volume of 2500 to 3000 ml, the used urokinase concentrations correspond to 250,000 to 6,000,000 IU per ml. The normal dose of 1,500,000 IU as a model the optimal dose was 500 IU urokinase per ml also. In other animal studies aspirin was also not able to accelerate thrombolysis of platelet-rich arterial thrombosis [15]. The same was found in a meta-analysis by Roux and colleagues [16].

The inhibition of \textit{in-vitro} induced thromboxane \(B_2\) synthesis correlated with this phenomenon of thrombus weight reduction. A reduction of more than 95\% of the thromboxane synthesis is necessary to induce a relevant inhibition of platelet function [8]. \textit{In-vivo}, a daily dose of 30–70 mg suppresses thromboxane synthesis nearly completely [17, 18]. Even 40 mg of aspirin effect inhibition of platelet aggregation. Lower doses need more time to decrease thromboxane synthesis [19, 20], higher dosages only cause quicker impact [21] but have no additional effects. Moreover, high doses suppress prostacyclin synthesis in vessel walls which could diminish desired effects. In addition, they more frequently induce gastro-intestinal ulcers.

In our \textit{in-vitro} model of combined platelet-fibrin-thrombi the optimal dose was between 50 and 100 mg aspirin per day. However, these \textit{in-vitro} results cannot directly implicate clinical consequences, but are a further contribution for aspirin dose finding.

\textbf{References}

3. DIS-2: Randomized trial of intravenous streptokinase, oral aspirin, both or neither among 17187 cases of suspected acute myocardial infarction. Lancet 1988; 349: 60.
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