Acetylsalicylic acid may protect the patient by increasing fibrin gel porosity. Is withdrawing of treatment harmful to the patient?

K. Fatah*, H. Beving†, A. Albåge‡, T. Ivert‡ and M. Blombäck*

Departments of *Laboratory Medicine, †Experimental Surgery and ‡Thoracic Surgery, Karolinska Hospital/Karolinska Institute, Stockholm, Sweden

The effect of acetylsalicylic acid in preventing cardiovascular complications is ascribed to acetylation of the enzyme cyclo-oxygenase thereby inhibiting prostaglandin synthesis. Acetylsalicylic acid, however, also acetylates fibrinogen. In the present pilot study, we investigated the permeability, i.e. porosity, of the fibrin gel in male patients with stable angina pectoris treated with this drug, before and at 1 and 2 weeks after withdrawal. Ten patients were treated with 75 mg and eight with 160 mg. The results were compared to those in seven untreated healthy controls. Bleeding times were longer during treatment and were reduced after withdrawal indicating patient compliance. Fibrin gels were more porous during treatment although there were large inter-individual variations in porosity. One week after withdrawal, the porosity was reduced by 30-41%, i.e. the network became tighter (75 mg group $P=0.001$; 160 mg group $P=0.002$). The tightness was more pronounced after withdrawal than in the untreated controls. In conclusion, the protective effect of acetylsalicylic acid may be ascribed to its effect not only on platelets but also on fibrinogen. The withdrawal of acetylsalicylic acid may cause a markedly reduced fibrin gel porosity that we assume is disadvantageous in patients with cardiovascular disease.

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Key Words: Fibrin gel network, acetylsalicylic acid, cardiovascular disease.

Introduction

Prophylactic treatment with acetylsalicylic acid has become an established therapy for primary (high-risk patients) and secondary (high- and low-risk patients) prevention of myocardial infarction and other arterial thromboembolic events in patients with cardiovascular disease. The beneficial effect of acetylsalicylic acid is ascribed to acetylation of a serine residue in the platelet enzyme cyclo-oxygenase, resulting in reduced levels of thromboxane A$_2$, a platelet-aggregating and vasoconstrictive prostaglandin. However, other plasma proteins are also acetylated, such as albumin, in which a lysine residue is acetylated and it has also been shown in vitro experiments that lysine residues in fibrinogen are acetylated. The fact that free amino groups of lysine in fibrinogen are involved in Factor XIII-induced cross-binding, when fibrin is stabilized, and in the binding to fibrinogen of proteins in the fibrinolytic system, implies that fibrinogen with acetylated lysine residues may have altered behaviour of importance for blood coagulation and fibrinolysis. Several studies have indicated that acetylsalicylic acid, in vitro and in vivo in normal subjects, increases fibrinolytic capacity.

We have previously shown that male patients with an acute myocardial infarction before the age of 45 years have less porous fibrin gel networks than healthy matched controls. The prophylactic effect of acetylsalicylic acid in the clinic and the above described effects on fibrinogen, prompted us to evaluate the fibrin gel structure in patients during treatment with the drug and after its withdrawal. For this purpose, we studied male patients with stable angina pectoris, in whom acetylsalicylic acid treatment was discontinued 2 weeks before elective coronary artery bypass surgery.

Methods

The study was approved by the Ethics Committee of the Karolinska Hospital.
Table 1  Bleeding times in the patients when treated with 75 mg or 160 mg acetylsalicylic acid (ASA) and after withdrawal

<table>
<thead>
<tr>
<th>Patients</th>
<th>During treatment</th>
<th>One week after withdrawal</th>
<th>Two weeks after withdrawal</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>75 mg ASA</td>
<td>8</td>
<td>347</td>
<td>117</td>
<td>230–565</td>
</tr>
<tr>
<td>160 mg ASA</td>
<td>8</td>
<td>254</td>
<td>72</td>
<td>125–370</td>
</tr>
</tbody>
</table>

Patients

Eighteen male patients with stable angina pectoris, were treated with Trombyl (Pharmacia, Uppsala, Sweden) containing acetylsalicylic acid and magnesium oxide, which accelerates dissolution. Ten patients, mean age 67 (range 55–77) years, received 75 mg of acetylsalicylic acid daily and eight, mean age 67 (range 42–76) years, received 160 mg daily. The mean body weight was 82 (range 63–90) kg for the 75 mg group and 78 (range 58–94) kg for the 160 mg group. Eight patients, four in each group, had a history of myocardial infarction. Two in the 75 mg group, and three in the 160 mg group had hypertension. Two in each group had hyperlipidaemia. One in each group was an active smoker. Angiography had demonstrated a significant obstruction in one (n=2), two (n=1) or three (n=12) coronary artery branches or left main coronary stenosis (n=3). The patients were accepted for elective coronary artery bypass surgery and, according to our present protocol, Trombyl was discontinued 2 weeks before the operation to minimize perioperative blood loss. The patients were investigated when they were on treatment, i.e. 2 weeks before surgery (treatment was withdrawn after sampling), 1 week after withdrawal, and 2 weeks after withdrawal, i.e. on the day before the operation.

Controls

Seven healthy men were enrolled as untreated controls. Their mean age was 63 (range 45–77) years.

Blood sampling, bleeding time and fibrin gel measurements

Blood samples were drawn in the morning, the subjects having fat-fasted since the evening before. Nine parts of blood were drawn into vacuum tubes (Becton Dickinson Vacutainer, Meylan, Cedex, France) containing one part buffered trisodium citrate (0.129 mol.1⁻¹), pH 7.4. The tubes were centrifuged and the plasma was withdrawn and aliquoted into plastic tubes and frozen at −70°C pending further analyses.

Bleeding times were measured in eight patients in the 75 mg group and in seven patients in the 160 mg group with a Simplate II device, according to the manufacturer’s instructions (Organon Teknika, Durham, NC, U.S.A.). During the procedure, the sphygmomanometer cuff was inflated to 40 mmHg. Fibrinogen concentration was analysed by a syneresis method. Fibrin gel permeability or porosity (Ks) was determined by flow measurements. Briefly, CaCl₂ and thrombin are added in rapid succession to a dialyzed plasma sample containing aprotinin in plastic cuvettes, forming a gel network. On top of this gel, a specially constructed stopper is inserted. Five different pressures are used to percolate a buffer through the gel and the flow (ml/hour) is then measured. The flow through the gels depends on the porosity, which is expressed as the permeability coefficient (Ks) of the network.

Statistical methods

Differences between the levels during treatment and after 1 and 2 weeks withdrawal, respectively, were analysed with paired t-test and analysis of variance was employed to identify differences between the three groups.

Results

The clinical characteristics of the two patient groups were similar. During treatment with acetylsalicylic acid, bleeding times were longer than in the untreated control group (Table 1). In the 75 mg group, four out of eight patients, and in the 160 mg group five out of seven patients had bleeding times longer than 275 s (the longest bleeding time in the control group) during treatment. In the 75 mg group, the mean times were reduced by 27 and 41%, 1 and 2 weeks (P=0.004 and P=0.007) after withdrawal, respectively. The corresponding reductions in the 160 mg group were 24 and 35% (P=0.005 and P=0.009, respectively). Bleeding times did not differ significantly between the controls and the two groups after withdrawal, and they were similar in the two patient groups during treatment and after withdrawal.
Table 2  Fibrinogen concentrations in the patients when treated with 75 mg or 160 mg acetylsalicylic acid (ASA) and after withdrawal

<table>
<thead>
<tr>
<th>Patients</th>
<th>During treatment</th>
<th>One week after withdrawal</th>
<th>Two weeks after withdrawal</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Mean SD Range Reduction P</td>
<td>4.2 0.70 3.10-5.30</td>
<td>4.0 0.47 3.40-5.01 5% 0.38</td>
<td>4.1 0.58 3.36-5.15 2% 0.64</td>
<td></td>
</tr>
<tr>
<td>75 mg ASA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>160 mg ASA</td>
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The longer bleeding times during treatment than after withdrawal indicated that the acetylsalicylic acid had been absorbed when the patients were treated and that they had discontinued treatment, as instructed. The increased fibrinogen concentrations observed in the patients agree with earlier reports that patients with cardiovascular complications have higher fibrinogen levels. The findings that during treatment with acetylsalicylic acid, the fibrin gel was more porous and that, after withdrawal, the porosity was less, i.e., the network tighter, suggests that the treatment has positive effects. In many patients it was tighter after withdrawal than in the controls, especially in the 75 mg group. Three patients on 75 mg had a low porosity even when on treatment. It might be that these patients ought to have had a higher dose. The low porosity in these patients and the low porosity observed after withdrawal, support our earlier findings that patients prone to cardiovascular events have a tighter and more rigid fibrin gel network than healthy controls. As acetylsalicylic acid treatment makes the gel more porous, the sudden withdrawal of this treatment, resulting in a much less porous gel, may be hazardous in such patients. The effect seemed to vary between individuals. During treatment some had a looser gel than others. Also after withdrawal there was a large interindividual difference; some had a tighter network than others.

Discussion

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The 'positive' effect on the fibrin gel network seems to be abolished in most patients 1 week after withdrawal (see Fig. 1). The half-life of fibrinogen varies greatly between different subjects\textsuperscript{14} and therefore the effects following withdrawal may vary. The mean half-life of fibrinogen is about 4.5 days\textsuperscript{14}, and thus there might still be an effect by acetylsalicylic acid up to 3 weeks after withdrawal. Some of the differences may be due to different absorption of the drug and/or different degrees of acetylation of the fibrinogen molecule.

A more porous fibrin gel during treatment probably makes the acetylated fibrin more prone to fibrinolysis and less likely to aggregate to fibrin monomers as shown in vitro and in vivo, in normal subjects especially\textsuperscript{15-17}. A tighter gel, with low porosity and smaller fibrin fibres, is less sensitive to fibrinolysis\textsuperscript{15,16}. In a recent study using some samples of the present patient material we found that the fibrinolytic potential decreases with the porosity, confirming the results of the above authors\textsuperscript{17}.

Our observations require additional investigations. Whether there is a greater 'positive' effect after the administration of acetylsalicylic acid in a specific dosage and whether there is a rebound effect after withdrawal in some individuals remains to be proven. Investigations should also aim at finding whether the drug produces its optimal effects on fibrinogen and on the cyclo-oxygenase pathway simultaneously. One week after withdrawal there seems to be a negative effect on both the fibrin gel network and on the cyclo-oxygenase pathway (the latter being a recent finding by Beving \textit{et al.}, personal communication) — this needs to be confirmed. The negative effects may explain why we, at our clinic, have observed fatal and non-fatal myocardial infarctions and/or aggravation of angina pectoris in some patients shortly before elective coronary bypass operations, when treatment had been withdrawn 2 weeks before operation. Blocking of the cyclo-oxygenase pathway by acetylsalicylic acid will, in principle, last as long as the life span of the platelets, i.e. 8–11 days\textsuperscript{18} and most of the effect on fibrinogen is also gone by that time.

Our findings that acetylsalicylic acid produces a more porous fibrin gel in addition to its known effect on platelet aggregation may explain at least some of the surprising results in the so-called ISIS-2 trial, in which treatment with streptokinase alone or acetylsalicylic acid alone produced a highly significant reduction in mortality in patients having suspected acute myocardial infarctions compared with a placebo group\textsuperscript{19}.

The question of how to find an optimal dosage for the protective effect by acetylsalicylic acid both on the fibrin gel porosity, including the potential of fibrinolytic degradation, and on the inhibition capacity on the cyclo-oxygenase pathway for use in prophylactic treatment will certainly be debated, as will the problem of the influence of the two mechanisms on the risk of bleeding.

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\textbf{Figure 1} Fibrin gel porosity expressed as its permeability coefficient ($K_s$) during and after withdrawal of acetylsalicylic acid (ASA) in the patients and in the untreated controls. Filled symbols: patients initially treated with 75 mg and 160 mg ASA. Open symbols: untreated controls. Dotted lines: range of $K_s$ levels in controls.
References


