Aminophylline inhibits adaptation to ischaemia during angioplasty

Role of adenosine in ischaemic preconditioning

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The ability of brief periods of ischaemia to protect the heart from subsequent ischaemia has been termed ‘ischaemic preconditioning’. In order to assess the role of adenosine receptor stimulation in this phenomenon we studied the ischaemic preconditioning effect during angioplasty in 10 control patients and in 10 patients pre-treated with 5 mg kg⁻¹ aminophylline, an adenosine receptor antagonist.

The ischaemic response was assessed by analysis of the intracoronary electrocardiogram every 10 s during three consecutive inflations of 90 s with a reperfusion time of 180 s. The severity of transmural local ischaemia was expressed as the magnitude of the ST segment shift in relation to the time during each inflation. The control patients showed an improved tolerance to myocardial ischaemia: ST segment shift decreased from 1.42 ± 0.49 mV at the end of the first inflation to 1.03 ± 0.44 mV at the end of the third inflation (P<0.001). However, in patients pre-treated with aminophylline, the ischaemic response was not significantly different during three inflations.

Conclusion Aminophylline inhibits ischaemic preconditioning, as assessed by analysis of the intracoronary ST segment changes during angioplasty. This suggests that ischaemic preconditioning is mediated by adenosine receptor stimulation in humans.

Key Words: Ischaemic preconditioning, angioplasty, aminophylline, adenosine.

Introduction

Brief periods of ischaemia and reperfusion make the heart more resistant to subsequent more prolonged episodes of ischaemia; this phenomenon has been termed ‘ischaemic preconditioning’ and has been recognized in a variety of animal species. The basic mechanism of this improved ischaemic response is the subject of intensive investigation. Although several mechanisms seem to be involved in preconditioning (e.g. alterations in energy metabolism, stress proteins, collateral circulation) there is strong experimental evidence that stimulation of the adenosine receptor by endogenous adenosine, which is released in increasing amounts by the ischaemic myocardium, may play a major role in the increased ischaemic tolerance afforded by preconditioning. However this mechanism may not be involved in all animal species as was suggested by Li et al.

Recently the phenomenon of ischaemic preconditioning has also been investigated in patients during percutaneous transluminal coronary angioplasty (PTCA). PTCA indeed offers a unique opportunity to study the myocardial ischaemic response during repetitive transient coronary occlusions. Several studies have demonstrated that during angioplasty an improved tolerance to myocardial ischaemia occurs as is evidenced by reduction of clinical, electrocardiographic (ST segment deviation) and haemodynamic changes during successive balloon inflations. An important question remains as to whether stimulation of the adenosine receptor is also involved in the phenomenon of ischaemic preconditioning in humans.

We therefore studied the ischaemic response, as assessed by analysis of intracoronary ST segment changes, during three successive balloon occlusions in a group of control patients and in a group pre-treated with aminophylline, an adenosine receptor antagonist.
Methods

Study group

A total of 33 patients, referred for elective angioplasty with stable angina pectoris, were prospectively included in this study. Inclusion criteria were: (1) angioplasty of a non-infarct related stenotic vessel with angiographically normal regional contractility and with normal (TIMI 3) coronary flow before the first inflation and during each reperfusion; (2) absence of angiographically visible collateral circulation; (3) ST segment shift ≥0.5 mV during the first inflation, minimizing the possible contribution of collaterals to the ischaemic response during balloon inflation; (4) stable intracoronary electrocardiographic signal during three consecutive balloon inflations allowing continuous recording of ST segment changes.

Exclusion criteria were the inability to keep the balloon inflated for 90 s because of haemodynamic or electrical instability and the intake of drugs interfering with adenosine metabolism (e.g. dipyridamole and aminophylline) 24 h before the angioplasty procedure. Other cardiovascular medications were not discontinued before the PTCA procedure. In two patients the protocol was interrupted early because of haemodynamic instability. A total of five patients showed incomplete blood flow restoration (TIMI≤3) with incomplete normalization of the ST segment after the first inflation and were therefore excluded from the study. In six patients, ST segments could not be analysed adequately because of unstable and/or incomplete intracoronary ECG recording during the procedure. Thus, a total of 20 patients (15 men and five women) with a mean age of 58 ± 9 years (range 42 to 73) were eligible for further data analysis.

The study population was randomly allocated to a control group or to a group pre-treated with an intravenous administration of 5 mg kg⁻¹ aminophylline 24 h before the angioplasty procedure. Early drop-outs were equally divided over the two study groups, so that the final study population consisted of two study groups of 10 patients. The clinical and angiographic characteristics of both groups were similar: there were no significant differences with regard to sex, age, localization and severity of coronary artery stenosis, anti-anginal therapy, arterial blood pressure and plasma cholesterol levels (Table 1). Anatomical severity of the coronary artery lesions was assessed quantitatively using the automated edge-detection analysis program of DG300 digital coronary angiography system (General Electric, Buc, France). Informed consent was obtained from all study patients.

Experimental protocol

Angioplasty was performed using the Judkins technique and a movable guidewire system. All patients were premedicated with a combination of a narcotic analgesic (fentanyl) and a neuroleptic (droperidol). Intravenous heparin (10 000 IU) was administered after introduction of an 8F sheath in the femoral artery. A continuous infusion of intravenous isosorbide dinitrate (0.5 μg kg⁻¹ min⁻¹) and of dextran (100 cc h⁻¹) was started 30 min before procedure. Aminophylline 5 mg kg⁻¹ 20 min⁻¹ was given intravenously 30 min before the procedure in 10 patients.

The angioplasty protocol consisted of three consecutive balloon inflations of 90 s duration, separated by a reperfusion interval of 180 s. The PTCA procedure was completed in accordance with standard clinical and angiographic judgement. All procedures were successfully performed and no complications were encountered.

Myocardial ischaemia was assessed by analysis of ST segment changes on a continuous recording of the intracoronary electrocardiogram (ECG). The intracoronary ECG was obtained with the distal end of the guidewire placed in the centre of the ischaemic zone by connecting lead V₂ of the standard ECG to the proximal end of the guidewire with a sterile clip. Special attention was given to the position of the guidewire in order to obtain a stable signal during the three consecutive inflations. Surface and intracoronary ECGs were recorded simultaneously with a paper speed of 10 mm s⁻¹ using a standard 12 lead recorder (Siemens Cathcor). Calibration was carried out before the procedure (10 mm = 1 mV), and ST segment measurements after the procedure by a cardiologist who was blinded to the study protocol.

The shift of the ST segment was measured 50 ms after the J point, using the PR interval as the isoelectric point of reference. Measurements were carried out every 10 s during each inflation. To assess the reliability of ST segment analysis by one observer, a total of 60 intracoronary QRS-T complexes at different stages of inflation protocol were selected at random from the two study groups and the ST segment of these QRS-T complexes was analysed by two independent cardiologists. A very close correlation (r²=0.94, slope=1.0±0.04) was found between both measurements, with an absolute mean difference of 0.10±0.08 mV.

Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>Aminophylline (n=10)</th>
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<tbody>
<tr>
<td>Males/females</td>
<td>7/3</td>
<td>8/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58±10</td>
<td>58±9</td>
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<tr>
<td>Dilated vessel, n</td>
<td></td>
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<tr>
<td>LAD-LCx-RCA</td>
<td>5-3-2</td>
<td>5-3-2</td>
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<tr>
<td>Sex, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Beta-adrenergic blocking agents</td>
<td></td>
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<tr>
<td>Calcium channel antagonists</td>
<td></td>
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<tr>
<td>Plasma cholesterol (mg 100 ml⁻¹)</td>
<td>235±45</td>
<td>225±30</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>119±15</td>
<td>116±21</td>
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LAD = left anterior descending artery; LCx = left circumflex artery; MLD = minimal luminal diameter; RCA = right coronary artery.
Table 2  Haemodynamic variables

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<th>Controls (n=10)</th>
<th>Aminophylline (n=10)</th>
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<tbody>
<tr>
<td></td>
<td>HR (beats . min⁻¹)</td>
<td>MAP (mmHg)</td>
</tr>
<tr>
<td><strong>Inflation I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t=0 s</td>
<td>69 ± 10</td>
<td>94 ± 9</td>
</tr>
<tr>
<td>t=90 s</td>
<td>68 ± 10</td>
<td>102 ± 14</td>
</tr>
<tr>
<td><strong>Inflation II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t=0 s</td>
<td>65 ± 10</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>t=90 s</td>
<td>64 ± 8</td>
<td>101 ± 18</td>
</tr>
<tr>
<td><strong>Inflation III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t=0 s</td>
<td>66 ± 13</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>t=90 s</td>
<td>70 ± 12</td>
<td>102 ± 17</td>
</tr>
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HR = heart rate; MAP = mean arterial pressure; RPP = rate pressure product (heart rate x systolic arterial pressure).

Both the time to onset and the severity of regional transmural ischaemia were assessed. The time from the start of the inflation to the appearance of 0.2 mV ST segment shift (Δ 0.2) was used as a marker of the onset of ischaemia. The severity of transmural ischaemia was expressed as the magnitude (mV) of the ST segment shift in relation to the time during inflation. Blood pressure and heart rate were continuously recorded. Rate-pressure product (heart rate x systolic arterial pressure) was used as a marker of myocardial oxygen consumption.

**Statistical analysis**

All data are reported as mean ± 1 standard deviation. Comparison of haemodynamic variables and ischaemic responses between the three inflations was performed using one factor analysis of variance (ANOVA) for repeated measurements with a post hoc Scheffe’s F-test to evaluate differences. Paired Student’s t-tests were used to compare haemodynamic variables at the beginning and the end of an inflation. An unpaired t-test (two tailed) was used to determine differences between both groups regarding age, stenosis severity, plasma cholesterol levels and haemodynamic parameters. Differences among both groups for discrete variables were assessed by Chi-square test. A P value of <0.05 was considered as significant.

**Results**

**Haemodynamic findings**

Heart rate, arterial blood pressure and rate-pressure product measured at the start and the end of the repetitive balloon inflations were similar in both study groups (Table 2). Within each group, the haemodynamic parameters did not change significantly during each and between the three consecutive balloon inflations.

**Ischaemic response during repetitive coronary occlusions**

Myocardial ischaemia developed more slowly during the third inflation in the control group (Fig. 1). The time from the start of the inflation to the appearance of 0.2 mV ST segment shift (Δ 0.2) increased in the control group significantly from 11 ± 5 s during the first inflation up to 23 ± 8 s during the third inflation (P<0.001). However, in the group pre-treated with aminophylline, the rate of onset of myocardial ischaemia (Δ 0.2) was not significantly prolonged during the third inflation: 17 ± 6 s vs 13 ± 5 s during the first inflation (Fig. 1).

Myocardial ischaemia, expressed as the amplitude of the ST segment shift measured every 10 s on the intracoronary ECG, was significantly less severe during the third inflation in the control group (Fig. 2). The ST segment shift 90 s after the start of coronary occlusion decreased from 1.42 ± 0.49 mV during the first inflation to 1.03 ± 0.44 mV during the third inflation (P<0.001).

![Figure 1](https://academic.oup.com/eurheartj/article-abstract/17/4/539/539651/fig1)
However, in the group pre-treated with aminophylline, the ischaemic response remained relatively unchanged (Fig. 2). The ST segment shift 90 s after the start of coronary occlusion was not significantly different during the third inflation (1.43 ± 0.83 mV) from the first inflation (1.32 ± 0.83 mV).

**Discussion**

Ischaemic preconditioning has been the focus of many studies, mostly using infarct size or arrhythmias as the endpoint for assessing the cardioprotective effect of this phenomenon. In the present study the process of ischaemic preconditioning was investigated during coronary angioplasty in humans by evaluating the myocardial ischaemic response during successive balloon occlusions. We could demonstrate that aminophylline, an adenosine receptor antagonist, inhibits the adaptation to myocardial ischaemia during angioplasty, which suggests that adenosine receptor stimulation is involved in the process of ischaemic preconditioning in humans.

The present study confirmed the finding of other angioplasty studies that also showed a reduction in the ST segment shift during successive balloon inflations after the first balloon inflation. In the present study, an improved tolerance to myocardial ischaemia was observed during the third inflation, whereas in most other angioplasty studies the reduction in ST segment shift occurred during the second inflation. Differences in methodology may account for this finding: in the present study the inflation time was kept constant at 90 s, whereas in most other studies it was maintained for a minimum of 120 s. It should be borne in mind that while in experimental models longer periods of ischaemia induced stronger cardioprotective responses, the cardiac adaptation to ischaemia after a single balloon inflation of 90 s was probably too marginal to be detected.

The principal aim of the present study was to evaluate the role of adenosine receptor activation in the process of ischaemic preconditioning in humans. Although the basic mechanism of ischaemic preconditioning is not yet fully understood, a key feature and probable final pathway of this improved ischaemic response is a reduction in ischaemic myocardial energy demand which is manifested by slower utilization of high energy phosphates and slower accumulation of products of anaerobic glycolysis.

There is substantial experimental evidence that adenosine release, and activation of the adenosine receptor (mainly A1 adenosine receptor), is a proximate step in this process. Liu et al. showed that adenosine receptor blockade with 8-phenyltheophylline eliminated infarct size reduction due to ischaemic preconditioning and that intracoronary infusion of adenosine mimicked the cardioprotective effect afforded by ischaemic preconditioning, and Miura et al. demonstrated that dipyridamole significantly potentiated the infarct size limiting effect of preconditioning. Both studies were done on a rabbit model and supported the hypothesis that endogenous adenosine release during ischaemia contributes to ischaemic preconditioning. Other experimental studies have shown that the A1 adenosine receptor couples to the inhibitory guanine nucleotide-binding protein Gs, which in turn is linked to several effectors, including protein kinase C activation and ATP-sensitive K+ channel activation. It has been shown that these 'effectors' may induce increased ischaemic tolerance afforded by preconditioning. However, this pathway may not be involved in all species, as was suggested by Li et al. These authors studied the process of ischaemic...
preconditioning in rat hearts and found that adenosine was not responsible for the cardioprotective effects of ischaemic preconditioning.

In the present study, we demonstrated clearly that aminophylline inhibited the phenomenon of ischaemic preconditioning, as assessed by analysis of intracoronary ST segment changes during angioplasty. In fact the ischaemic response in the group pre-treated with aminophylline was not significantly different during three consecutive inflations. Since both study groups had similar clinical, angiographic and haemodynamic characteristics, the differences in ischaemic response between both study groups during repetitive coronary occlusions could be attributed to the action of aminophylline.

Aminophylline is a methylxanthine derivative with several cardiac properties: it blocks not only adenosine receptors on the cardiomocytes but also activates the alpha-adrenergic receptors through blockade of adenosine receptors on the presynaptic nerve terminals and inhibits cyclic nucleotide phosphodiesterase activity[16,17]. The last two properties are considered responsible for the increased myocardial contractility and myocardial oxygen demand during high doses of methylxanthines. At therapeutic concentrations, the effects of aminophylline are predominantly mediated by the non-selective blockade of adenosine receptors[17]. In the present study, aminophylline did not significantly influence the inotropic state of the heart since haemodynamic parameters, like heart rate, arterial pressure and rate-pressure product were not significantly different between the study groups. It appears, therefore, that the inhibitory effect of aminophylline on the observed phenomenon of ischaemic preconditioning during angioplasty results mainly from the blockade of adenosine receptors located on the ventricular myocytes. Although we did not measure adenosine concentrations during successive inflations, this finding strongly supports the hypothesis, based on animal studies, that endogenous adenosine, released by the myocardium during ischaemia, may be an important factor in the process of ischaemic preconditioning. Further indirect evidence of the importance of this pathway in humans comes from a recent study of Tomai et al. showing complete inhibition of ischaemic preconditioning during angioplasty by glibenclamide, a selective ATP-sensitive K⁺ channel, thereby suggesting that ischaemic preconditioning is mediated by activation of ATP-sensitive K⁺ channels in humans[18]. Mindful of the fact that ATP-sensitive K⁺ channel activation is linked with adenosine receptor activation in experimental models, the findings of Tomai et al. are in agreement with the conclusions of our study, that adenosine receptor stimulation is involved in the process of ischaemic preconditioning in humans[13,14].

However, it has been shown that intracoronary ECG registration is more sensitive and accurate in the detection of local myocardial ischaemia than multiple lead surface ECGs[19]. Evaluation of anginal pain severity during angioplasty could not be used as a parameter for ischaemia in our study since all patients were pre-treated with analgesics. Coronary blood flow and metabolic variables (e.g. lactate, adenosine) were not measured and deserve further investigation for a more complete interpretation of the results.

Since we used a non-selective adenosine receptor antagonist, our results do not provide an answer to the question which adenosine receptor (A₁, A₂ or A₃) is involved in ischaemic preconditioning in humans.

Reruitment of collateral circulation may contribute to the development of ischaemic preconditioning during coronary angioplasty, as was suggested by Cribier et al., but in the present study patients with overt visible collaterals were excluded and in order to minimize the possible contribution of non-visible collaterals, only patients with a ST segment shift ≥ 0.5 mV during the first inflation were included in the study.

**Clinical implications**

The results of the present study may have important implications on the use of methylxanthines in patients with coronary artery disease. Recent clinical studies have demonstrated that pre-infarct angina pectoris, probably through the effect of ischaemic preconditioning, confers a beneficial effect on in-hospital outcome after acute myocardial infarction[20,21]. In view of the results of the present study, we might presume that patients with acute myocardial infarction will not have the advantage of the cardioprotective effect of ischaemic preconditioning if they are under treatment with methylxanthines. Therefore, further investigation into the safety of methylxanthines in patients with acute ischaemic syndromes is warranted.

Finally, the present study confirms that the angioplasty model makes it possible to investigate the process of ischaemic preconditioning in humans. A better understanding of our own natural cardioprotective properties may ultimately lead to the development of more potent cardioprotective agents in the future.

We express our gratitude to the nurses and the technical staff of the department of invasive cardiology for their support.

**References**


