Analgesic effects of adenosine in Syndrome X are counteracted by theophylline: a double-blind placebo-controlled study

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ABSTRACT

It has been proposed that adenosine mediates ischaemic pain in humans. Patients with cardiac Syndrome X are hypersensitive to potential pain stimuli, including adenosine. On the other hand, recent findings suggest that low-dose adenosine infusion may have analgesic effects. Our aim was to test two hypotheses: (1) that the analgesic effect of adenosine is peripheral in origin, and (2) that part of the hypersensitivity to pain of patients with cardiac Syndrome X results from a disturbed mechanism of adenosine analgesia. A total of 12 female Syndrome X patients and eight healthy age-matched female controls were studied in a randomized, double-blind and placebo-controlled study. Adenosine (70 µg/min) or placebo was infused into the forearm via an intra-arterial catheter. After 15 min of infusion, a tourniquet on the upper arm was inflated to 225 mmHg to ensure arterial occlusion. The patient then carried out dynamic handgrip work at 60 Hz. Pain or discomfort in the forearm was estimated continuously according to the Borg CR-10 scale. After the first test, theophylline was infused for 10 min intravenously at a dose of 5 mg/kg body weight. The ischaemic forearm test was then repeated. On a second occasion, the procedure was repeated with the opposite treatment (adenosine/placebo). Only six of 12 Syndrome X patients completed the protocol because of pain during the catheterization procedure or an inability to establish an intra-arterial line. The time to onset of pain in the working, ischaemic forearm was greater for subjects treated with adenosine than for those treated with placebo, both in Syndrome X patients who tolerated catheterization (49 ± 27 s compared with 32 ± 18 s; P < 0.03) and in healthy controls (40 ± 19 s compared with 16 ± 8 s; P < 0.02). The time to maximum pain, limiting ischaemic work, was also greater with adenosine pretreatment both in Syndrome X patients (137 ± 28 s compared with 106 ± 28 s; P < 0.03) and in healthy controls (109 ± 31 compared with 82 ± 18 s; P < 0.01). After infusion of theophylline there was no difference between adenosine and placebo in either group. Intra-arterially infused adenosine had similar peripheral analgesic effects on experimentally induced muscular ischaemia in those female Syndrome X patients who tolerated intra-arterial catheterization and in healthy controls. Thus adenosine analgesia is counteracted by theophylline, suggesting that the effect is mediated by membrane-bound peripheral adenosine receptors.

INTRODUCTION

Adenosine has been proposed as a mediator of ischaemic pain in humans [1–3]. Intravenous and intracoronary bolus infusions of adenosine elicit angina pectoris-like pain in healthy volunteers and in patients with angina pectoris. In the latter group, the pain is indistinguishable from their habitual anginal pain, but the ECG does not show ischaemic changes [1,4,5]. Syndrome X is defined as the presence of effort-
induced angina pectoris, chest pain and ST-depressions during an exercise test, but normal coronary angiograms. The pathophysiological mechanisms behind the appearance of pain in this syndrome remain unclear [6–9]. In Syndrome X patients, anginal chest pain appears to develop without signs of myocardial ischaemia [10]. Furthermore, Syndrome X patients show hypersensitivity, with development of pain after mechanical cardiac stimulation [11] and after pharmacological stimuli such as adrenaline [12]. Intravenous bolus injections of adenosine induce anginal chest pain in Syndrome X patients at lower doses than in patients with known ischaemic heart disease or in healthy volunteers [13].

Adenosine is a neuromodulator that has both excitatory and inhibitory effects. Whether the effect is excitatory or inhibitory depends on the activation of different receptor subtypes and their differing effects on different targets. Such neuromodulatory effects on cardiac ganglia have been shown in experimental animal models [14–16]. Studies in animals have suggested an analgesic effect of adenosine both when given as spinal or intrathecal infusions and after peripheral application [17,18]. Studies in humans have shown that the continuous intravenous infusion of a low concentration of adenosine reduces the requirements for narcotic gases and analgesics peroperatively [19,20]. We have previously studied patients with angina pectoris and with verified coronary artery disease, and we reported a significant decrease in chest pain during exercise after low-dose adenosine infusion [21]. Our study also confirmed earlier observations of increased heat pain thresholds after adenosine infusion [22,23]. Thus, adenosine can act both as an algesic and as an analgesic agent. An intriguing hypothesis is that the balance between the algesic and analgesic effects of adenosine is disturbed in Syndrome X, with less analgesic effects resulting in hypersensitivity and pain.

In the present investigation, our aim was to study a peripheral site of analgesic action of adenosine with an experimental design that eliminated confounding systemic factors. This was done by studying the effects on experimentally induced ischaemia of the regional intra-arterial infusion of low-dose adenosine before and after systemic theophylline infusion in a double-blind, randomized, placebo-controlled study. We also aimed to study whether adenosine administered peripherally to Syndrome X patients would result in altered pain perception.

Methods

Subjects

Two groups were studied. The first group consisted of 12 female patients aged 50–64 years (age range 58 ± 5 years; weight 69 ± 9 kg; height 161 ± 7 cm) with angina-like, effort-induced chest pain (Canadian Cardiovascular Society functional class II), normal coronary angiograms, normal left ventricular function (as assessed by ventriculography) and abnormal exercise stress test results (chest pain and downslope or rectilinear ST-depression > 0.1 mV 60 ms after the J-point in more than two leads). No patient had a history of hypertension, valvular disease or other systemic illnesses. Only six out of 12 patients completed the protocol.

A group of eight healthy female volunteers, weight- and age-matched to the patient group, acted as controls. These volunteers were free of medication. The patients and controls did not smoke, and did not take beverages containing caffeine or xanthine on the day of the experiment.

The study was approved by the local Ethics Committee, and conformed with the principles outlined in the Declaration of Helsinki. All experiments were performed in a fully equipped laboratory of clinical physiology. The background and procedure were thoroughly explained to the patients and volunteers, and informed written consent was obtained from each subject before the study began.

Protocol

This was a randomized, double-blind, placebo-controlled, crossover study (Figure 1). Each subject was tested twice. The tests were performed at the same time of day in a fully equipped clinical physiology laboratory.

The experiment started with the establishment of an intravenous line and, on the contralateral forearm, an intra-arterial catheter. The catheter was established using the Seldinger technique and using a local anaesthetic applied subcutaneously a couple of minutes before the catheter was positioned. The subject rested in a semi-reclining position. Heart rate and 12-lead electrocardiography (Mingograph; Siemens Elema, Stockholm, Sweden) were monitored continuously.

Thereafter, a continuous intra-arterial infusion of adenosine or placebo (saline) was started, with an infusion rate for adenosine of 70 µg min⁻¹ kg⁻¹ forearm. The forearm weight was calculated on the basis of water immersion to be approx. 1 kg. The dose of adenosine was chosen based on previous studies [21,23] which suggested
that it should be sufficient to elicit analgesia without provoking discomfort. This infusion continued for 15 min.

After 15 min of intra-arterial infusion of adenosine or placebo, a tourniquet was applied to the upper arm above the intra-arterial catheter and the infusion was continued. A pressure of 225 mmHg was then applied using an inflated cuff to ensure arterial occlusion. The subject was then told to carry out hand contractions on a dynamic handgrip apparatus placed in a position that allowed work to be performed in a semi-reclining position. The subject was instructed to carry out one contraction per s (60 Hz). During the contractions, the subject was told to report pain or discomfort in the forearm according to the Borg Category Ratio scale (Borg CR-10). The test was terminated and the tourniquet deflated when the pain intensity reached the unacceptable level of Borg 10, or when the subject was unable to carry out any further contractions. When the tourniquet was deflated, the subject was told to report the disappearance of pain.

After the complete disappearance of pain, the patient rested for 5 min and then the non-specific adenosine antagonist theophylline was infused intravenously into the contralateral arm for 10 min. The dose given was 5 mg of theophylline/kg body weight, which has been shown to result in therapeutic plasma concentrations [1]. After the theophylline infusion was completed, the tourniquet on the contralateral upper arm was inflated to 225 mmHg and the ischaemic handgrip contraction test was repeated using the same procedure. After this, the catheter was withdrawn.

The entire procedure was repeated after an interval of 2 weeks, using whichever agent had not been infused in the first test (adenosine or placebo).

**Statistics**

Two-factor analysis of variance (ANOVA) with repeated measures was used to test for significant differences between the two groups and treatments. Results are presented as means ± S.D. The criterion of significance was set to $P < 0.05$.

**RESULTS**

The time to onset of pain (Table 1 and Figure 2) increased after administration of adenosine, both in Syndrome X patients (+108%) and in healthy controls (+136%), with no significant difference between the groups. After administration of theophylline there were no differences between the effects of placebo and adenosine in either group (Figure 3).

![Figure 2 Time to onset of pain in each Syndrome X patient (left) and each control subject (right) after placebo infusion and after adenosine (ADO) infusion](image)

![Figure 3 Time to onset of pain after administration of theophylline in each Syndrome X patient (left) and each control subject (right) after placebo infusion and after adenosine (ADO) infusion](image)

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Syndrome X patients</th>
<th>Healthy controls</th>
<th>Intergroup difference</th>
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<tr>
<td></td>
<td>Placebo</td>
<td>Adenosine</td>
<td>$P$ value</td>
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<td>To onset of pain</td>
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<tr>
<td>Before theophylline</td>
<td>32 ± 10</td>
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<tr>
<td>Before theophylline</td>
<td>106 ± 28</td>
<td>137 ± 28</td>
<td>&lt; 0.03</td>
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<tr>
<td>After theophylline</td>
<td>96 ± 27</td>
<td>103 ± 10</td>
<td>NS</td>
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Figure 4 Time to maximum pain in each Syndrome X patient (left) and each control subject (right) after placebo infusion and after adenosine (ADO) infusion

Figure 5 Time to maximum pain after administration of theophylline in each Syndrome X patient (left) and each control subject (right) after placebo infusion and after adenosine (ADO) infusion

The time to maximum pain showed similar characteristics: it increased after administration of adenosine both in patients with Syndrome X (34%) and in healthy controls (35%), with no significant difference between the groups (Figure 4). After theophylline infusion, the time to maximum pain after adenosine infusion was no greater than that after treatment with placebo in the two groups (Figure 5). The maximum pain intensity was 6 ± 2 according to the Borg CR-10 scale, and this value was the same in the two groups.

Heart rate was unchanged during adenosine infusion. On theophylline infusion, the heart rate increased from 63 ± 10 beats/min to 77 ± 15 beats/min (P < 0.05). There were no differences between the groups. Heart rate increased in both groups during handgrip contractions, from 65 ± 12 beats/min at rest to 86 ± 19 beats/min after work (P < 0.03). No ECG changes were observed during infusions or handgrip contractions.

In the Syndrome X group, 12 patients were tested but only six completed the protocol. It was impossible to establish an intra-arterial line in four patients (two patients on day 1 and two patients on day 2) because of severe, unbearable pain in the forearm during catheterization. Despite local anaesthetic, arterial spasm was seen in two patients, in one case combined with an experience of unbearable forearm pain. One control subject reported discomfort in the forearm after intra-arterial puncture. Two Syndrome X patients experienced palpitations and discomfort in the chest after theophylline infusion. No control subject or Syndrome X patient reported discomfort or pain during the 15 min adenosine infusion prior to ischaemia. All the control subjects who were tested completed the protocol.

DISCUSSION

This is the first double-blind, placebo-controlled study that shows regional analgesic effects of adenosine on muscular ischaemia. A local low-concentration infusion of adenosine had similar analgesic effects on forearm ischaemia in patients with Syndrome X who tolerated intra-arterial catheterization as in healthy control subjects. This effect was counteracted by the administration of the non-specific adenosine receptor antagonist theophylline, which suggests a peripheral site of action of adenosine-mediated analgesia occurring at membrane-bound adenosine receptors [21].

Obviously, it is possible that the patients who tolerated intra-arterial catheterization may constitute a subgroup of Syndrome X patients who are less prone to arterial spasm and vascular pain. Therefore the results of the present study cannot be generally applied to the entire population of Syndrome X patients, but to those 50% of patients that it was possible to study using the present design. Thus adenosine-induced peripheral antinociception is normal in patients with Syndrome X who can tolerate the procedure of intra-arterial catheterization. This is consistent with a dissociation between cutaneous and visceral pain sensitivity in Syndrome X, and with the findings of Cannon et al. [11].

Patients with Syndrome X experience disabling anginal pain, but show no signs of myocardial ischaemia. These patients do not appear to be a homogeneous group with regard to pathophysiology, but it has been suggested that they have a low pain threshold and hypersensitivity to normally painless stimuli [11–13]. Furthermore, Syndrome X patients show a low threshold for chest pain induced by bolus injections of adenosine [13].

Jonzon et al. [24] showed that pain was decreased during the ischaemic forearm test after administration of the adenosine receptor blocker theophylline. This pain was therefore thought to be caused by ischaemically released adenosine acting at adenosine receptors. In contrast with this, our study suggests that pretreatment for 15 min with a low dose of exogenous adenosine decreases the subjects’ sensitivity to endogenously released adenosine acting at adenosine receptors during a short burst of ischaemia, such as that provoked by the ischaemic forearm test. This could be due to a preconditioning of the algesic adenosine receptors, followed
by their desensitization. This would result in a decrease in the algesic effects of adenosine. However, adenosine receptor activation has complex effects, and influences, for example, nociceptive transmission and inflammation, and the design of the present study does not allow definite conclusions in this regard.

Biaggioni et al. [25] reported increased sympathetic tone in healthy humans after a short intravenous infusion of low-to-moderate adenosine levels (20–80 μg min⁻¹ kg⁻¹). This increase in sympathetic activity could not be entirely explained by baroreceptor unloading, and was therefore thought to be an effect of direct afferent nerve stimulation. Furthermore, exogenous adenosine activates forearm afferent nerves to produce reflex sympathetic activation, and it has been proposed that adenosine is a metabolic trigger of the exercise pressor reflex in humans [26]. Doses of adenosine similar to that used in the present study have been shown by Rongen et al. [27] to produce systemic sympathetic activation, which proves that the doses are effective locally.

An increase in sympathetic nerve activity might lead to an increased pain threshold [28,29], and may be linked to the activation of endogenous peripheral pain-inhibiting systems of an opioid and non-opioid nature [30]. Interestingly, Sylven et al. [31] reported that infusion of β-endorphin counteracted adenosine-provoked angina-pectoris-like pain, while infusion of Met-enkephalin did not. Theoretically, therefore, the analgesic effect of adenosine could depend on an altered pain threshold caused by increased sympathetic activity. However, in Syndrome X, a systemic adrenaline infusion causes chest pain similar to the patients’ habitual angina, which suggests a lower pain threshold with significantly increased sympathetic tone, at least in the short term [12].

The anti-nociceptive effects of adenosine agonists have been studied at the spinal level. It has been found that peripherally activated adenosine receptors can reduce inflammation-related nociception in mice [17,32]. One possible mechanism is that adenosine activates central descending pain-inhibitory mechanisms. If this is true, it supports the suggestion that opioid analgesia works through the release of adenosine in the spinal cord [33–36]. However, it has not been shown that adenosine passes the blood/brain barrier [25]. The elimination time for adenosine is less than 10 s, which supports the hypothesis that it does not pass the blood/brain barrier. We administered a dose of 70 μg of adenosine min⁻¹kg⁻¹ forearm, which means that, for a body weight of approx. 70 kg, the maximum systemic administration was 1 μg min⁻¹kg⁻¹. This dose could not possibly have any systemic effects, and consequently no effects beyond the blood/brain barrier.

Thus the mechanism of pain in Syndrome X does not appear to involve an impaired peripheral local anaesthetic effect of adenosine. A limitation of the present study, however, is that 50% of our patients were unable to complete the protocol due to unbearable pain during catheterization or an inability to establish an intra-arterial line, and therefore the results of the study may not be generally applicable to the entire Syndrome X population.

In conclusion, intra-arterially infused adenosine has similar peripheral analgesic effects on experimentally induced muscular ischaemia in both female Syndrome X patients and healthy controls. The adenosine analgesia is counteracted by theophylline, which suggests that the effect is mediated by membrane-bound peripheral adenosine receptors.

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