Alprazolam decreases isoproterenol induced myocardial damage in the rat

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ABSTRACT  Myocardial trauma and damage were induced by two doses of isoproterenol 40 mg·kg⁻¹ in rats. Isoproterenol injections alone caused the expected cardiotoxicity. Two doses of alprazolam 0.5 mg·kg⁻¹ decreased the severity of electrocardiographic changes after isoproterenol injection and the amount of myocardial tissue damage. The frequency of ischaemic ST-T changes, flat or inverted T waves, Q wave appearance, and heart block was significantly reduced in the alprazolam group. The alprazolam treated group had a significantly smaller proportion of infarcted tissue in the ventricular section (2.3%) and in the apical sections (1.8%) than the vehicle injected rats (6.5% and 14.7% respectively). There was no difference in wet heart weight or in heart rate between the alprazolam group and the control group. These results show that alprazolam has a cardioprotective effect in rats treated with cardiac damaging doses of isoproterenol.

Materials and methods

Male Sprague-Dawley rats weighing 280-380 g were housed individually with free access to water and standard laboratory rat chow. We measured electrocardiographic (ECG) changes, heart rate, proportion of infarcted cardiac tissue as visualised by triphenyl tetrazolium chloride (TTC) staining, and wet weight of hearts. ECG leads were implanted subcutaneously under ketamine/acepromazine anaesthesia. After a 24 h recovery period shown to be sufficient in previous experiments, rats were allocated to a control group (n=10) and an alprazolam treatment group (n=10) by randomisation. Both groups received intraperitoneal injections of isoproterenol (40 mg·kg⁻¹ in saline solution) on two consecutive days. This regimen induces a reliable pattern of myocardial damage with minimal effects on other organ systems. Six hours after the first isoproterenol injection and 2 h before the second isoproterenol injection rats received a subcutaneous injection of either alprazolam (Upjohn: 0.5 mg·kg⁻¹ in saline/ethanol/polyethylene glycol 300 buffer) or vehicle (control). One minute tracings of ECG and heart rate data were obtained before (0) alprazolam or vehicle injection and at time intervals of 1, 5, 15, 30, 60, and 90 min after injection. An additional ECG
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**TABLE 1** Number of ectopic beats and severity of ECG changes in control and alprazolam treated rats after injections of isoproterenol, alprazolam, or buffer. Values are mean(SEM)

<table>
<thead>
<tr>
<th>Drug (day)</th>
<th>Ectopic beats (per min)</th>
<th>ECG changes (severity score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alprazolam</td>
</tr>
<tr>
<td>Isoprenaline (1)</td>
<td>1.9(0.85)</td>
<td>3.2(1.67)</td>
</tr>
<tr>
<td>Alprazolam (1)</td>
<td>1.3(0.45)</td>
<td>2.2(0.79)</td>
</tr>
<tr>
<td>Alprazolam (2)</td>
<td>0.6(0.31)</td>
<td>0.9(0.69)</td>
</tr>
<tr>
<td>Isoprenaline (2)</td>
<td>2.8(0.99)</td>
<td>0.9(0.31)</td>
</tr>
</tbody>
</table>

*p<0.05 between groups at this injection.

Severity score was calculated by multiplying the universal grade of I (ischaemic ST-T changes), 2 (flat or inverted T waves), 3 (Q wave), or 4 (heart block) by the number of time periods in which change is noted.

tracing was obtained before the animals were killed. Hearts were removed, rinsed gently in saline, blotted dry, and weighed. The hearts were then sliced into atrial, ventricular, and apical sections in preparation for TTC staining. After staining and formalin fixation, the heart sections were further sliced and examined for evidence of infarction. Areas of infarct were excised and weighed on a Mettler balance to determine the proportion of infarcted tissue in the apical, atrial, and ventricular sections. The total proportion of cardiac tissue affected was also determined.

The severity of ECG changes was graded on a severity scale of 1-4 (table 1). A severity score consisting of ischaemic ST-T changes (score 1), flat or inverted T waves (score 2), Q wave appearance (score 3), and heart block (score 4) was computed for each animal at each injection time by multiplying the severity grade (1-4) by the number of time periods in which these changes were noted.

Statistical analysis of the data was performed using a one way ANOVA, a two way ANOVA with repeated measures, and unpaired t tests as appropriate.

**Results**

After isoproterenol injection, the heart rate increased rapidly and appreciably from about 340 to 500 beats·min⁻¹ and remained increased for about 6 h. The heart rate then decreased and reached about 380 beats·min⁻¹ before the second injection. Again an injection of isoproterenol increased heart rate quickly to 580 beats·min⁻¹ and the rate remained increased for 6 h but decreased to 340 beats·min⁻¹ 24 h later. No significant difference was noted in heart rate between the vehicle and the alprazolam treated group after any injection of isoproterenol or before killing of the animals.

There was no significant difference between the control group and the alprazolam treated group in the number of ectopic beats occurring per minute after any injection time (table 1). There was no significant difference between the alprazolam group and the control group in severity score after the first isoproterenol injection on day 1. However, after the second isoproterenol injection on day 2, the alprazolam group showed fewer ECG changes and had a significantly (p<0.05) lower severity score than did the control group.

There was no significant difference between the wet heart weights of the control and the alprazolam groups (table 2). There was no significant difference in proportion of atrial tissue infarcted between the control group and the alprazolam group. However, the alprazolam group had a significantly smaller proportion of infarcted tissue in the ventricular sections (p<0.05) and apical sections (p<0.001) and a smaller total proportion of tissue affected (p<0.025) than the control group.

**Discussion**

Isoproterenol (or a degradation product of isoproterenol) produced a pronounced increase in heart rate which far outlasted the half life of isoproterenol and caused the expected cardiac toxicity in our control group such as ECG abnormalities and myocardial necrosis. The group treated with isoproterenol and alprazolam showed significantly less cardiotoxicity. There was a substantial decrease in the severity of ECG changes and of the proportion of infarcted tissue.

It is of interest to speculate how alprazolam reduced...
isoproterenol induced cardiac damage. It is possible but unlikely that alprazolam administration interferes directly with the action of isoproterenol on the heart (for example, blockade of isoproterenol action) since there was no difference after isoproterenol injection in heart rates between the control group and the alprazolam treated group. However, alprazolam might have indirectly reduced the effects of isoproterenol. A growing body of evidence exists suggesting such cardioprotective effects of alprazolam. Alprazolam administration reduces significantly adrenaline and noradrenaline concentrations during stress in both rats and humans. Alprazolam significantly reduces ventricular arrhythmias in dogs undergoing coronary artery ligation. Alprazolam administration decreases anginal symptoms in patients whose angina has been stabilised with propranolol without changing stroke volume, cardiac output, heart rate, or blood pressure. Patients treated with antianxiety drugs suffer fewer subsequent myocardial infarctions than do patients receiving a placebo. Our studies also show that the anxiolytic alprazolam significantly decreases ECG abnormalities and actual cardiac necrosis during cardiac insult with isoproterenol.

There are two possible mechanisms of action for this protective effect of benzodiazepine administration against cardiac damage. A reduction in catecholamine concentrations, in particular plasma adrenaline concentrations by alprazolam administration during isoproterenol stress, may have decreased sympathetic receptor stimulation, which is an important factor in the development of ventricular arrhythmias. This is supported by at least two previous studies that have shown that benzodiazepine administration has antiarrhythmic effects in dogs undergoing coronary artery ligation. A second mechanism may involve peripheral benzodiazepine receptors in cardiac muscle. Diazepam decreases the action potential duration and decreases the contractility of guinea pig papillary muscle by modulating calcium channel fluxes. Classical calcium channel blockers such as verapamil and diltiazem have similar actions and are thought to prevent tissue damage by preventing an intracellular buildup of calcium. Peripheral benzodiazepine receptor antagonists inhibit the action of calcium channel blockers. If benzodiazepines can be shown to act as a type of atypical calcium channel blockers then the protective effects of alprazolam against myocardial infarction as shown in this study could be partially attributed to this mechanism.

The efficacy of alprazolam administration in the prevention of pathological ECG changes and cardiac tissue infarction caused by isoproterenol indicates that there may indeed be clinical benefit obtained by using alprazolam in the prevention or treatment, or both, of myocardial infarction.

We thank Mr D Bower and Dr D Liu for technical assistance and Upjohn Company, Kalamazoo, MI, for their financial support of this study.

References