The Effects of Adenine and Dimethyl Sulfoxide on the Mouse Pancreas

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The authors have studied the effects of dimethyl sulfoxide (DMSO) on the plasma α-amylase activity in mice that sustained a pancreatic injury induced by an oral administration of adenine.

In mice given a 5% solution of DMSO as drinking water for 3 d prior to the administration of adenine (175 mg/kg), and also drank this DMSO solution until the end of the experiment, hyperemia of the pancreas was observed and the level of plasma α-amylase activity became significantly higher than the level seen in the control mice. A pathological examination also revealed vacuolation and zymogenic degranulation. Further, the plasma α-amylase activity level increased only in mice given this 5% DMSO solution, and no increase was noted in mice given a 3% or a 1% DMSO solution for drinking water. Further, the pancreatic lipid peroxide level of mice given this 5% DMSO solution was significantly higher than the level seen in the control group.

Based on the above results and associated data, it is thought that an oral administration of adenine can induce a pancreatic injury in the mouse, and that this injury is sustained with the assistance of DMSO.

Keywords — adenine; dimethyl sulfoxide; α-amylase; pancreas; HE staining; vacuolation; zymogen degranulation; lipid peroxide

Introduction

In humans, adenine, a purine found in nucleotides, is changed through adenine phosphoribosyl transferase into adenine nucleotide and is utilized in the body.1) It also has been reported that adenine is able to prevent the cytotoxic activity of the 6-thiopurines when incubated with human leukemia cell lines.2) However, when adenine was orally administered to the mouse,3) the rat4,5) and intravenously administered in the dog,6) a renal injury occurred. Further, reports have speculated that oxygen-free radicals may play an important role in the cause of such injuries.7,8)

Therefore, to test if oxygen free radicals may influence a renal injury, dimethyl sulfoxide (DMSO), reported to be one of the radical scavengers,9) was given to the mice that had received an oral administration of adenine, and it was found that the induced renal injury did not heal, and that hyperemia of the pancreas occurred. These results have led us to investigate the effect of adenine and DMSO on the functions of the mouse pancreas.

Materials and Methods

Used were male ddY mice (6 weeks old), obtained from Japan SLC Co. Ltd. (Shizuoka, Japan), that were kept for one week prior to the experiments, during which time they were given a standard mouse feed (MF, Oriental Yeast Co. Ltd., Tokyo). The adenine and DMSO were purchased from Wako Pure Chemical Industries Ltd. (Tokyo). Six mice were used for each experimental group.

1) The Test Protocol of the DMSO-Administered Mouse Group — The mice of this group received a 5% DMSO solution as drinking water and an oral administration of 175 mg/kg of adenine. The DMSO drinking period for 4 separate sets of mice in this group were 1, 2, 3, and 5 d, respectively, prior to the adenine administration and until the finish of the experiment. A DMSO control group (DMSO group) also received a 5% DMSO drinking water for 3 d, and then administered distilled water instead of adenine. Similarly, an adenine control group (adenine group) received tap water for drinking and administered adenine. A control group received tap water for drinking and distilled water instead of adenine.
2) The Effect of Different Concentrations of DMSO — DMSO at solution strengths of 1, 3, or 5% were separately given to the groups of mice as drinking water for 3 d before the 175 mg/kg of adenine administration and until the finish of the experiment.

3) The Effect of the Adenine Dose — 43.8, 87.5, 175.0, or 700.0 mg/kg of adenine was orally administered into the separate groups of mice receiving a 5% DMSO solution as drinking water.

Twenty-four hours after the adenine administration, mice from each group were anesthetized and sacrificed, after which blood samples were collected. Each pancreas then was fixed in 10% formalin, from which specimen slices were made, embedded in paraffin, and later stained with hematoxylin and eosin (HE).

The lipid peroxide level of a pancreatic homogenate was measured by the method of Okawa et al. 10)

Using clinical "Monotest" kits (Boehringer Mannheim, Germany), the α-amylase and lipase activities were also measured. Further, using clinical "NESCAUTO" kits (Nippon Shoji Ltd., Osaka), the lactate dehydrogenase (LDH) activity, sugar, the plasma urea nitrogen (BUN), and the creatinine levels were assayed by an autoanalyzer (HITACHI 705, Hitachi Co., Tokyo).

All results are given in means ± S.E.M., and differences among groups were determined by a one-way analysis of variance. Furthermore, a comparison of the means among the groups was evaluated by using the method of Dunnett.

Results

Figure 1 shows the external appearance of the

![Fig. 1. Effect of Adenine on the Mouse Pancreas](image)

A 5% solution of DMSO was first administered as drinking water for 3 d, after which each took a dose of adenine at a different strength and administered it to separate mouse groups. After 24 h, the pancreas was removed. (a) control, (b) pancreas that received 175 mg/kg of adenine, (c) pancreas that received 350 mg/kg of adenine, (d) pancreas that received 700 mg/kg of adenine.
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Table I. Effect of DMSO on the Plasma Parameters

<table>
<thead>
<tr>
<th></th>
<th>α-Amylase (U/ml)</th>
<th>Sugar (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>LDH (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9.60 ± 0.70</td>
<td>199 ± 9</td>
<td>26.9 ± 1.1</td>
<td>0.26 ± 0.02</td>
<td>241 ± 13</td>
</tr>
<tr>
<td>DMSO group</td>
<td>6.13 ± 0.73</td>
<td>178 ± 7</td>
<td>20.7 ± 0.8</td>
<td>0.18 ± 0.004</td>
<td>209 ± 11</td>
</tr>
<tr>
<td>Adenine group</td>
<td>9.53 ± 0.51</td>
<td>201 ± 11</td>
<td>52.0 ± 9.8b</td>
<td>0.40 ± 0.05b</td>
<td>659 ± 127a</td>
</tr>
<tr>
<td>1-DA group</td>
<td>9.65 ± 1.00</td>
<td>208 ± 15</td>
<td>80.7 ± 20.8b</td>
<td>0.65 ± 0.13b</td>
<td>699 ± 135a</td>
</tr>
<tr>
<td>2-DA group</td>
<td>15.68 ± 4.09</td>
<td>251 ± 14</td>
<td>104.6 ± 36.3b</td>
<td>0.75 ± 0.10b</td>
<td>545 ± 16b</td>
</tr>
<tr>
<td>3-DA group</td>
<td>14.81 ± 2.53a</td>
<td>251 ± 46</td>
<td>113.5 ± 11.1b</td>
<td>0.47 ± 0.05b</td>
<td>809 ± 91b</td>
</tr>
<tr>
<td>5-DA group</td>
<td>23.95 ± 3.69b</td>
<td>277 ± 60</td>
<td>101.8 ± 7.0b</td>
<td>0.63 ± 0.09b</td>
<td>697 ± 50b</td>
</tr>
</tbody>
</table>

A 5% DMSO solution was given as drinking water for varying periods and 175 mg/kg of adenine was administered. After 24 h, plasma samples were collected.

a) p < 0.05, b) p < 0.01 vs control group. Mean ± S.E.M. of 6 mice.

pancreas of DMSO-drunk mice that had been given a different strength dose of adenine. Hyperemia of the pancreas was observed when adenine at strengths of 175 or 350 mg/kg were administered. A pancreas isolated from the 700 mg/kg adenine-administered group appeared whitish and sclerotic.

Table I shows the effects on the plasma when a 5% solution of DMSO was given as drinking water. The level of the plasma α-amylase activity in the control group was 9.60 ± 0.70 U/ml (mean ± S.E.M.), and the levels seen in the DMSO group (6.13 ± 0.73 U/ml) and the Adenine group (9.53 ± 0.51 U/ml) did not change. When this DMSO drinking water was given for 3 d (3-DA group) and for 5 d (5-DA group) before the adenine administration, the level of the plasma α-amylase activity were 14.81 ± 2.53 U/ml (3-DA group) and 23.95 ± 3.69 U/ml (5-DA group), and these levels became significantly higher than the level seen in the control group (3-DA group, p < 0.05; 5-DA group, p < 0.01).

Further, in all adenine-administered groups, the BUN and creatinine levels and LDH activity increased, but no differences were observed in these levels between the Adenine group and the other adenine-administered groups.

Figure 2 shows the effect of different concentrations of DMSO. When a 5% DMSO solution was given as drinking water for 3 d prior to the adenine administration and for 24 h after, the level of the α-amylase activity was 15.57 ± 3.42 U/ml and became significantly higher than the level seen in the control group (7.27 ± 0.29 U/ml) (p < 0.05). However, in mice that were given either a 3% or a 1% DMSO drinking solution over the same protocol period, no change was seen in the level of the α-amylase activity (3% DMSO group, 9.56 ± 1.07 U/ml; 1% DMSO group, 7.31 ± 0.44 U/ml).

Figure 3 shows the effect of different concentrations of adenine on the α-amylase activity.

![Fig. 2. Effect of Different DMSO Concentrations on the Plasma α-Amylase Activity](image)

Solutions with different DMSO concentrations were given as drinking water for 3 d, after which 175 mg/kg of adenine was administered. After 24 h, plasma samples were collected. For a comparison, the control group received tap water for drinking and distilled water instead of adenine. The DMSO mouse group received a solution of 5% DMSO for drinking water and distilled water instead of adenine. Mean ± S.E.M. of 6 mice. a) p < 0.05 vs control group.
When an adenine dose of 175 mg/kg was orally administered, the α-amylase activity became significantly higher than the level seen in the control group ($p<0.05$).

Figure 4 shows the lipid peroxide level of the mouse pancreas homogenate. When the mouse was given DMSO drinking solution, the reduction of pancreatic lipid peroxide level was observed (DMSO group, $0.69 \pm 0.02$ nmol malondialdehyde (MDA)/mg protein; control group, $0.85 \pm 0.05$ nmol MDA/mg protein, $p<0.05$). However, when adenine administered into mice given a 5% DMSO solution as drinking water, the production of lipid peroxide was $1.09 \pm 0.08$ nmol MDA/mg protein and was significantly higher than that level seen in the control group ($p<0.05$), but the level of the pancreas in the adenine-administered mice group without DMSO treatment did not change ($0.84 \pm 0.07$ nmol MDA/mg protein).

Figure 5 shows the HE staining pattern of a pancreas specimen. Vacuolation and zymogenic degranulation was seen to occur when 350 mg/kg of adenine was administered (b)[× 320].

Discussion

As has been observed in other experimental models, ischaemia, alcohol, fatty acids, and taurocholates can induce a pancreatic injury. It also has been recently reported that cerulein, a CCK analog, was able to induce acute pancreatitis. These experimental models, however, almost always involved surgical dissections, infusions, and/or repeated drug administrations.

When adenine was given to the rats over a long period, it was found that a chronic renal injury was induced. Further, with only a single dose of adenine we were able to induce a renal injury. Therefore, since it is also thought that active oxygen participates in an adenine-induced renal injury, we administered DMSO, expect-
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significantly change (Table I and Fig. 2). This result thereby suggested that DMSO and its metabolites did not directly act on the release of α-amylase. Further, the level of α-amylase activity in the Adenine group, which drank plain tap water, did not change. We also noted that the level of the α-amylase activity in the 3-DA and the 5-DA mice groups was significantly higher than the level of the control group ($p<0.05$) (Table I), and found that α-amylase activity level increased when the strength of the DMSO solution was 5%, but that the level remained unchanged when the DMSO concentration was less (Fig. 2). Additionally, when the adenine dose was 175 mg/kg or more in mice given a 5% DMSO solution, the α-amylase activity was significantly higher than the level seen in the control group (Fig. 3). These results suggest that the adenine deleteriously affected the mouse pancreas and that the DMSO assisted in sustaining this effect, although it is not clear whether the active form of adenine was certain. We then attempted to look into the mechanisms of how the adenine had induced the pancreatic injury.

As many reports describing the results in various experimental models have indicated that free radicals induce pancreatic injury, we began by measuring the pancreatic lipid peroxide levels of our mice (Fig. 4), and so it was found that the lipid peroxide level of the pancreas was significantly higher in the adenine mouse group given a DMSO solution for drinking water ($p<0.05$), although the level of a DMSO mice group without adenine administration reduced it from the level of the control mice group ($p<0.05$). Therefore, it is thought that free radicals are involved in causing this adenine-induced pancreatic injury and that DMSO sustained this deleterious effect.

Since various inflations can induce an increase in the α-amylase activity, it was thought that the isoamylase should be measured to determine whether the injury occurred in the pancreas. But as the antibody for the mouse pancreatic amylase could not be obtained, we were unable to isolate the mouse plasma amylase isozyme, since our electrophoresis method could only measure the status of the human amy-

![Fig. 5. HE Staining of Adenine-Administered Mouse Pancreas](image)

(a) control group, (b) the 350 mg/kg adenine mouse group.
lase isozyme. However, vacuolation and zymogen degranulation of the pancreas were observed in our pathological study of mouse groups that had received 350 mg/kg or more of adenine (Fig. 5), and had been given a 5% solution of DMSO as drinking water for 3 d prior to the administered adenine and until the end of the experiment. Therefore, we concluded that adenine had induced the pancreatic injury in these mice and that DMSO had sustained this condition.

Finally, the BUN and creatinine levels, which are indexes of a renal injury, showed an increase. Further, there was an increase in the LDH activity in all mice groups that had received adenine, irrespective of DMSO pretreatment. However, there was no change in the levels of BUN, creatinine and LDH activity in each adenine-administered group (Table I), so that our findings suggest that DMSO was not directly related to these changes.

In conclusion, it appears that the pancreatic injury occurred only when adenine and DMSO were used together and that free radicals are somehow involved in causing this injury, and that after this injury there was an increase in the level of the plasma α-amylase activity.

References