Arsenic trioxide generates oxidative stress and islet cell toxicity in rabbit

S. Mukherjee, D. Das, S. Darbar, M. Mukherjee, A. S. Das and C. Mitra*

Department of Physiology, Presidency College, Kolkata 700 073, India

An oral exposure of rabbits to arsenic trioxide, 1.5 mg/kg body wt/day in a single dose for 30 consecutive days changed the normal features of oral glucose tolerance test (OGTT) to a diabetic form. Estimated glycated haemoglobin values supported OGTT results. Light microscopic studies have revealed significant reduction in the number of cells in the islets of treated animals compared to control. Also, increased serum amylase activity and production of nitrite and malondialdehyde were observed in the pancreas of treated animals. It appears that an alteration of the normal features of OGTT to diabetic form by an oral exposure to arsenic possibly could be a reason for islet cell damage caused by an enhanced activity of oxidative stress producing enzymes and/or by their products.

*For correspondence. (e-mail: chandan_mitrapresi@yahoo.com)
The percentile recovery was only 26, 33 and 38 respectively, at 90, 120 and 150 min. Results of glycated haemoglobin estimation are presented in Table 1. Results show that, compared to control, glycated haemoglobin level was significantly increased ($P < 0.001$) in all arsenic-treated animals. In the control it was $6.64 \pm 0.34$, which was within the expected values for non-diabetic condition, according to the kit specification. In arsenic-treated animals the level was $11.44 \pm 0.48$, which according to kit specifications showed poor glycemic control. Table 1 also shows the results of experiments for serum amylase activity of both control and arsenic-treated animals. Serum amylase activity is expressed in terms of Somogyi amylase unit/dl and, compared to control, in the arsenic-treated group it was found to increase significantly from $292 \pm 31$ to $421 \pm 26$ ($P < 0.001$).

Results of islet cell studies of both control and arsenic-treated groups of animals are shown in Figure 2a and b. Compared to the control, the number of cells in the islets of arsenic-exposed group were markedly low.

---

**Table 1.** Effect of oral exposure of arsenic trioxide ($As_2O_3$; 1.5 mg/kg body wt/day for 30 days) on HbA1c and serum amylase activity of rabbits

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Control</th>
<th>Arsenic-treated animals</th>
<th>Per cent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>$6.64 \pm 0.34$</td>
<td>$11.44 \pm 0.48^*$</td>
<td>72</td>
</tr>
<tr>
<td>Serum amylase</td>
<td>$292 \pm 31$</td>
<td>$421 \pm 26^*$</td>
<td>44</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE ($n = 5$); *$P < 0.001$. 

---
Table 2. Effect of oral exposure of arsenic trioxide (As$_2$O$_3$; 1.5 mg/kg body wt/day for 30 days) on NO and MDA production in pancreatic tissue extract of rabbits

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Control</th>
<th>Arsenic-treated animals</th>
<th>Per cent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO ($\mu$/mg wet tissue)</td>
<td>$0.677 \pm 0.03$</td>
<td>$1.212 \pm 0.09^*$</td>
<td>79</td>
</tr>
<tr>
<td>MDA (nmol/mg wet tissue)</td>
<td>$1.18 \pm 0.102$</td>
<td>$1.83 \pm 0.07^*$</td>
<td>55</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE ($n=5$); *$P<0.05$.

Table 2 shows the results of experiments for nitrite accumulation in the pancreatic tissue of both control and arsenic-treated animals. Nitrite accumulation, an indicator of nitric oxide synthesis, was expressed in terms of $\mu$/mg of tissue and, compared to control, in the arsenic-treated group it was found to increase significantly from $0.677 \pm 0.03$ to $1.212 \pm 0.09$ ($P<0.01$). Table 2 also shows the results of lipid peroxidation in pancreatic tissue of both control and arsenic-treated animals. Alteration in malondialdehyde (MDA) formation, an indicator of lipid peroxidation, was expressed in terms of nM/mg of tissue and, compared to control, in the arsenic-treated group it was found to increase significantly from $1.18 \pm 0.102$ to $1.83 \pm 0.07$ ($P<0.01$).

The present study revealed that oral exposure of arsenic trioxide possibly produces diabetes mellitus in rabbits, manifested by alteration of normal features of OGTT, viz. changes in peak glycemic response and a typical delay in recovery of normal blood glucose level (Figure 1). These changes thus corroborated well with the findings of earlier survey reports that arsenic might induce diabetes mellitus$^{1-3}$. Results of glycated haemoglobin (Table 1) further substantiated our observations of OGTT, as glycated haemoglobin reflects on a relatively more precise index of the degree of diabetes than glucose itself$^{13}$. This study further provided the experimental demonstration that in rabbits, chronic oral exposure to arsenic trioxide in situ caused significant decrease in population of pancreatic islet cells (Figure 2a and b), a typical histological feature which may be compared with development of insulin-dependent diabetes mellitus caused by beta-cell injury from specific toxic substances bypassing an autoimmune requirement$^{19}$. Our results of serum amylase activity (Table 1) further indicate that arsenic administration caused severe pancreatic damage$^{20}$, including possibly the damage of islet cells. Oxidative stress has been recognized as a major component in the chain of pathogenic events that cause late complications in diabetes mellitus$^{10}$. It is considered as a major contributor to vascular and neurogenic complications of patients with diabetes mellitus$^{21-26}$. It has also been reported that oxidative stress is involved in the cytotoxicity and genotoxicity of arsenic$^{8}$ and such arsenic-induced oxidative stress has been suggested to be due to the generation of NO, which can cause DNA damage and activate poly(ADP-ribose) polymerase$^8$, a major cause of islet cell damage in diabetes$^{27}$. In our study, the increase in production of NO in the arsenic-treated group compared to control was 79% (Table 2), suggesting that such high overproduction of NO possibly was pathological, as has been manifested by significant reduction in islet cell number.

Lipid peroxidation, compared to control, significantly increased in arsenic-treated animals in our study (Table 2), thus corroborating well with the earlier speculations that arsenite induces oxygen free radicals or promotes formation of lipid peroxides$^9$, and high levels of such lipid peroxidation products have been indicated in the development of diabetes$^{28-30}$. Thus, it may be proposed that chronic oral arsenic exposure possibly causes significant
damage to pancreas, particularly endocrine cellular components of pancreatic islets and thus may be responsible for transforming the normal physiological features of the OGTT curve to diabetic form.

In summary, we have demonstrated that rabbits on chronic oral exposure to arsenic, possibly develop an oxidative stress to cause deleterious effects on the endocrine pancreas. These effects could be, at least in part, the mechanism of action by which arsenic causes diabetes mellitus.


ACKNOWLEDGEMENTS. Financial assistance from the Department of Science and Technology, Government of West Bengal, India is gratefully acknowledged.

Received 21 July 2003; revised accepted 12 November 2003