Electrophysiological and Biochemical Effects of Exposure to 2,5-Hexanediol on Peripheral Nerve in Experimental Diabetic Rats

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Abstract: Electrophysiological and Biochemical Effects of Exposure to 2,5-Hexanediol on Peripheral Nerve in Experimental Diabetic Rats: Takashi Kimura, et al. Department of Public Health and Hygiene, Oita Medical University—Both 2,5-hexanediol (2,5-HD) and diabetes mellitus (DM) cause peripheral neuropathy. Workers with asymptomatic DM could possibly be exposed to n-hexane converted to 2,5-HD in liver. To clarify 2,5-HD influences on peripheral nerves in DM, electrophysiological and biochemical changes in DM rats were compared in 2,5-HD treated and untreated groups. Four groups of rats were studied: the Control group consisted of non-diabetic rats treated with a placebo; the HD group, of non-diabetic rats treated with 2,5-HD; the DM group, of diabetic rats treated with 2,5-HD; the DM+HD group, of diabetic rats treated with 2,5-HD. 2,5-HD was administered at 100 mg/kg/day, five days a week for 8 weeks. The motor nerve conduction velocity (MCV) and motor distal latency (DL) in the rat's tails and glucose, fructose, sorbitol and myo-inositol levels in the sciatic nerves were measured. The MCV in the DM+HD group was significantly reduced from the 4th week compared with those in the other groups. 2,5-HD had no influence on the levels of glucose, fructose, sorbitol and myo-inositol in either the diabetic or non-diabetic group. These results indicated that exposure to 2,5-HD hastened the onset of peripheral neuropathy in experimental diabetic rats. This study indicates that 2,5-HD in combination with DM enhances the neurotoxicity. But the mechanisms of the neurotoxic interactions between 2,5-HD and DM are still unknown. It can be hypothesized that workers with hyperglycemia can suffer from neuropathy due to exposure to n-hexane earlier than those without hyperglycemia.

Key words: Neurotoxicity, Diabetes mellitus, 2,5-Hexanediol, Motor nerve conduction velocity, Motor distal latency, Glucose, Sorbitol, Fructose, Myo-inositol, Rat

The compound 2,5-hexanediol (2,5-HD) is the neurotoxic metabolite of n-hexane, which is widely used as a solvent in industry and chemistry. 2,5-HD is known to produce peripheral neuropathy1,2, which is indicated by progressive distal numbness in the limbs in humans. In severe cases, decreased motor nerve conduction velocity (MCV) is observed3,4. Indeed, the prevalence of patients with diabetes mellitus (DM) is very high5, and many people with hyperglycemia work in industry. DM causes peripheral neuropathy as one of its chronic complications6,7, the signs of which are distal tingling paresthesia, pain and dysesthesia in the limbs8,9. Nevertheless, DM patients often show no symptoms in the beginning of this neuropathy, though decreased MCV is observed earlier than the onset of other symptoms. DM produces decreased MCV by increasing glucose levels and hyperactivity of the polyol pathway in peripheral nerve axons and Schwann cells10,11. Within the polyol pathway, glucose is reduced to sorbitol by aldose reductase coupled with the oxidation of NADPH to NADP+ in the first step in the pathway. Sorbitol is oxidized to fructose coupled with the reduction of NAD+ to NADH by sorbitol dehydrogenase in the second step in the pathway. Thus, the increase in polyol pathway activity results in the increase in sorbitol and fructose, and leads to a reduction in myo-inositol levels in peripheral nerves12. Peripheral neuropathy induced by 2,5-HD and that observed in DM represent toxic and metabolic neuropathy, respectively. It is considered that industrial workers with asymptomatic DM will be exposed to n-hexane. This study was designed to investigate the effect of exposure to a major metabolite of n-hexane, 2,5-HD, on peripheral nerves in experimental diabetic rats. We measured MCV and the motor distal...
The measurements of MCV and DL (5 cm) were carried out under general anesthesia. All rats were intraperitoneally injected with sodium amobarbital (60 mg/kg body wt). To minimize the effects of body temperature differences on conduction velocity, the tail of each rat was warmed in a liquid paraffin bath maintained at 37°C. The methods used to measure MCV and DL have been described in detail by Miyoshi and Misumi. MCV and DL were measured with an evoked electromyograph (Neupak II plus, Nihon-Koden, Japan).

Methods

Animals and treatment

Fifty six 8-week-old male Wistar rats weighing 275.0 ± 1.7 g (mean±SE), were randomly allocated to diabetic and non-diabetic (normal) groups. The rats allocated to the diabetic group were given a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg body wt, dissolved in citrate buffer pH 4.5). Two weeks after the STZ injection, blood samples were collected from the tail vein and the glucose levels in blood were determined. The rats with a non-fasting blood glucose level >15 mmol/l (270 mg/dl) in the diabetic group were selected. One rat in which the blood glucose level was ≤15 mmol/l was excluded from this study. The remaining 27 rats were randomly subdivided into two groups. One of them was injected subcutaneously in the back with 2,5-HD 100 mg/kg/day (dissolved in 0.2 ml of saline), five days a week for 8 weeks (DM + HD group). The other was injected subcutaneously with a placebo, 0.2 ml of saline (0.9%), in the same manner (DM group). In addition, the non-diabetic (normal) group was also randomly subdivided into two groups. One of them was injected subcutaneously with 2,5-HD 100 mg/kg/day (dissolved in 0.2 ml of saline) in the same manner (HD group). The other was injected subcutaneously with a placebo, 0.2 ml of saline (0.9%), in the same manner (Control group). All rats were housed individually in an air conditioned lab maintained on a 12:12 h light-dark cycle and allowed to take food (CE-2, Clea Company, Japan) and water ad libitum. MCV was measured every 2 weeks from the start of exposure to 2,5-HD or the placebo to the end of treatment. Body weight was measured every week. After the last MCV measurement was taken, blood glucose was determined again. After 1 week, all the rats were anesthetized with ethyl ether and sacrificed by exsanguination from the aorta. Bilateral sciatic nerves were removed, weighed and frozen at −80°C until assay. Blood glucose was analyzed with a TIDEX system (Bayer-Sankyo, Japan) by the hexokinase method. The experiments were performed in accordance with the guidelines for animal experimentation of Oita Medical University.

Electrophysiology

The measurements of MCV and DL (5 cm) were carried out under general anesthesia. All rats were intraperitoneally injected with sodium amobarbital (60 mg/kg body wt). To minimize the effects of body temperature differences on conduction velocity, the tail of each rat was warmed in a liquid paraffin bath maintained at 37°C. The methods used to measure MCV and DL have been described in detail by Miyoshi and Misumi. MCV and DL were measured with an evoked electromyograph (Neupak II plus, Nihon-Koden, Japan).

Nerve sugars and myo-inositol

The frozen right sciatic nerve was placed in a glass homogenizer. Thirty µl of methyl-α-mannopyranoside as internal standard and 1 ml of distilled water were added. The homogenizer was kept in boiling water for 20 min and the nerve was then homogenized. The mixture was cooled and deproteinized with 0.2 ml of 0.19 mol/l zinc sulfate and 0.2 ml of 0.2 mol/l barium hydroxide. The sample was centrifuged at 2,600 r.p.m. for 10 min and the supernatant was freeze-dried. The lyophilisate was silylated under a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane (10:2:1, v/v; 0.5 ml). After 24 h incubation at room temperature, distilled water (2 ml) and cyclohexane (0.2 ml) were added and the sample vortex mixed. After centrifugation at 2,600 r.p.m. for 2 min, the cyclohexane phase was aspirated for chromatography. Two µl of the extract was injected with a microsyringe directly into a GC-15A gas chromatograph (Shimadzu, Japan) equipped with a flame ion detector. A Hicap-CBP 1 (50 m, i.d. 0.25 mm) fused silica capillary column was used for detection. The capillary column temperature was 180°C with helium as the carrier gas. Both the injection port and detector temperature were 200°C. Chromatograph peaks were measured electronically with a C-R4A chromatopac (Shimadzu, Japan). A standard mixture containing glucose, sorbitol, fructose, myo-inositol and methyl-α-mannopyranoside was assayed and standard curves for individual sugars were constructed. Concentrations of the various sugars in the nerve samples were calculated with these standard curves.

Drugs and other chemicals

Sodium amobarbital was purchased from Nihon-Shinyaku (Kyoto, Japan). The other reagents used in this study were purchased from Sigma (St. Louis, USA) or Wako (Osaka, Japan).

Statistical analysis

All statistical analyses were performed by means of SPSS for Macintosh version 6 (SPSS Japan, Tokyo, Japan). Data were expressed as the means ± SE. Differences among the groups were compared by one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test to assign differences to individual groups, when overall significance (p<0.05) was attained. When the distribution of data was not Gaussian, the Mann-Whitney U test was used.
Results

Body weight

The changes in body weight for all groups are shown in Fig. 1. The increase in body weight in the diabetic groups (DM and DM+HD groups) was significantly reduced from the start of 2,5-HD treatment (i.e. two weeks after STZ injection) compared with the non-diabetic groups (Control and HD groups). The body weight in the DM+HD group tended to be lower than in the DM group, and a significant difference between the DM+HD and DM groups was observed only from the 3rd to the 5th week. The body weight in the HD group compared with the Control group was not significantly different during the experiment period.

Motor nerve conduction velocity and distal latency

MCV values for all groups are shown in Fig. 2. MCV in the DM+HD group decreased significantly from the 4th week compared with those in the Control, DM and HD groups. The increasing rates of MCV with age in the DM and HD groups became low from the 6th week compared with the Control group (not significant). MCV in the DM group was significantly reduced at the 8th week compared with the Control group. MCV in the HD group was not significantly different compared with the Control group. DL values for the distal part (5 cm) of tail in rats for all groups are shown in Fig. 3. DL in the DM+HD group was significantly prolonged from the 4th week compared with those in the Control, DM and HD groups.

Blood glucose

The changes in blood glucose for all groups are shown in Table 1. A significant increase in blood glucose levels was observed in the diabetic groups (DM and DM+HD groups) compared with those in the non-diabetic groups (Control and HD groups). The differences between the Control and HD groups, and between the DM and DM+HD groups in blood glucose were not significant, which indicates that exposure to 2,5-HD did not influence the blood glucose levels in this study.

Nerve sugars and myo-inositol

Glucose, sorbitol, fructose and myo-inositol levels in the sciatic nerves for all groups are shown in Table 2. Glucose, sorbitol and fructose concentrations in the sciatic nerves were significantly increased in the diabetic groups (DM and DM+HD groups) compared with those in the non-diabetic groups (Control and HD groups). There were no significant differences in glucose, sorbitol and fructose concentrations between the Control and HD groups.
groups and also between the DM and DM + HD groups. Myo-inositol concentrations in the sciatic nerves were significantly reduced in the diabetic groups (DM and DM + HD groups) compared with those in the non-diabetic groups (Control and HD groups). There were no significant differences in myo-inositol concentrations between the Control and HD groups and also between the DM and DM + HD groups.

### Table 1. Variations in blood glucose levels during the experiment

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Group code</th>
<th>n</th>
<th>Glucose (mmol/l)</th>
<th>Before</th>
<th>8 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic rats</td>
<td>Control</td>
<td>14</td>
<td>4.7 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>HD</td>
<td>14</td>
<td>5.3 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>2,5-HD-treated</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>DM</td>
<td>13</td>
<td>16.8 ± 1.3</td>
<td>22.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>DM + HD</td>
<td>14</td>
<td>17.9 ± 1.4</td>
<td>21.8 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>2,5-HD-treated</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE. *p<0.05.

### Table 2. Glucose, sorbitol, fructose and myo-inositol levels in the sciatic nerve

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Group code</th>
<th>n</th>
<th>Glucose (mmol/mg wet wt)</th>
<th>Sorbitol (mmol/mg wet wt)</th>
<th>Fructose (mmol/mg wet wt)</th>
<th>Myo-inositol (mmol/mg wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic rats</td>
<td>Control</td>
<td>14</td>
<td>1.17 ± 0.21</td>
<td>0.51 ± 0.03</td>
<td>0.49 ± 0.04</td>
<td>1.42 ± 0.11</td>
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<tr>
<td>Non-treated</td>
<td>HD</td>
<td>14</td>
<td>0.99 ± 0.20</td>
<td>0.50 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>1.55 ± 0.06</td>
</tr>
<tr>
<td>2,5-HD-treated</td>
<td>*</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>DM</td>
<td>13</td>
<td>8.18 ± 1.38</td>
<td>0.70 ± 0.03</td>
<td>2.82 ± 0.14</td>
<td>1.06 ± 0.07</td>
</tr>
<tr>
<td>Non-treated</td>
<td>DM + HD</td>
<td>14</td>
<td>7.03 ± 0.72</td>
<td>0.68 ± 0.04</td>
<td>2.55 ± 0.19</td>
<td>0.93 ± 0.06</td>
</tr>
<tr>
<td>2,5-HD-treated</td>
<td>*</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

All values are means ± SE. *p<0.05.

Discussion

2,5-HD, a metabolite of the industrial solvent n-hexane, is known to cause polyneuropathy. There are several hypotheses concerning the pathogenesis of its neurotoxicity, such as inhibition of glycolysis, pyrrole formation, crosslinking of neurofilaments, decreased phosphorylation of neurofilaments and diminished proteolysis of neurofilaments. Pathological findings in 2,5-HD neuropathy are characterized by giant axonal swellings above the nodes of Ranvier with accumulation of neurofilaments and secondary demyelination. Because degeneration starts from distal long and large axons and progresses toward proximal axons, it is called “dying-back” neuropathy. On the other hand, the pathogenesis of diabetic neuropathy is mainly represented by metabolic and ischemic-hypoxic vascular factors. Metabolic factors such as hyperglycemia can increase glucose, sorbitol and fructose levels and decrease myo-inositol levels in peripheral nerves. Ischemic-hypoxic factors such as endoneurial capillary abnormalities can decrease blood flow in nerves, increase endoneurial vascular resistance and decrease endoneurial oxygen tension. Histological features of diabetic neuropathy in humans include fiber loss, secondary demyelination and remyelination. Though neuropathy induced by 2,5-HD and observed in DM differ from each other pathogenetically and pathologically, both neuropathies are clinically similar. Therefore, when DM patients or experimental diabetic animals are exposed to 2,5-HD or its parent chemical n-hexane, a more severe distal axonopathy results. Misumi reported that MCV in rats exposed to 2,5-HD subcutaneously in the back at 200 mg/kg/day, 5 days a week, had decreased significantly by the 6th week. The total amount of 2,5-HD administered at that time was 2.4 g. In this study, rats were exposed to 2,5-HD at 100 mg/kg/day in the same manner and the total amount administered until the 8th week was 1.7 g, so that MCV and body weight in the HD group were not significantly different from those in the Control group. But when diabetic rats were treated with the same amount of 2,5-HD (DM + HD group), MCV decreased four or more weeks earlier than in the DM or HD groups. And as DL in the DM + HD group was also
significantly prolonged from the 4th week compared with the other groups, it is indicated that the peripheral nerve function of the rat's tail in the DM + HD group is damaged earlier than in the DM or HD groups as also found in the result of MCV measurements.

To clarify the mechanisms of early onset of decreased MCV in the DM + HD group, serum and nerve glucose levels and nerve sorbitol, fructose and myo-inositol levels which indicate the polyol pathway activity were measured. In experimental diabetic animals, it has been reported\(^6\),\(^32\) that decreased MCV is linked to myo-inositol depletion, so the changes in MCV caused by the administration of 2,5-HD in diabetic rats might be connected with changes in myo-inositol levels. But in this experiment, the administration of 2,5-HD had no effect on the levels of glucose in sera and nerves, or the levels of sorbitol, fructose and myo-inositol in nerves.

Secondly, the possibility of increased amounts of 2,5-HD in the body of DM patients or animals will be discussed. Severe diabetic nephropathy can result in renal failure, causing a decreased glomerular filtration rate (GFR). Consequently elimination of 2,5-HD from the body will be retarded by decreased renal clearance ability. But Hostetter et al.\(^13\),\(^33\) have reported that GFR in rats with moderate hyperglycemia was significantly higher than that in normal rats between 2 and 15 weeks after STZ administration. It is considered that the effect of the decreased GFR on 2,5-HD levels in blood would be small, even if GFR decreased, as the rate of excretion of 2,5-HD in the urine is only 1.5\(^6\). In view of the above mentioned facts, it could be supposed that no accumulation of 2,5-HD in the body due to diabetic nephropathy occurred during our experiment. But the amount of 2,5-HD in the urine or serum was not measured in this study, further studies are therefore required to clarify if the amount of 2,5-HD in the body will increase.

In conclusion, it was shown that exposure to 2,5-HD hastened the onset of peripheral neuropathy in experimental diabetic animals. But because the mechanisms of the neurotoxic interactions between 2,5-HD and DM are still not known, further investigations will be needed. But the results imply the possibility that industrial workers with hyperglycemia after exposure to n-hexane suffer from neuropathy earlier than those without hyperglycemia.

References