Influence of Different Doses of Methyl Ethyl Ketone on 2,5-Hexanedione Concentrations in the Sciatic Nerve, Serum, and Urine of Rats

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Abstract: Influence of Different Doses of Methyl Ethyl Ketone on 2,5-Hexanedione Concentrations in the Sciatic Nerve, Serum, and Urine of Rats: Takato YASUI et al. Department of Public Health and Hygiene, Oita Medical University—Rats were injected subcutaneously with 2,5-hexanedione (2,5-HD 2.6m mol/kg) alone (HD group) or with 2,5-HD and methyl ethyl ketone (MEK) (2.6 m mol/kg of each agent, HD&MEK group) or with 2,5-HD 2.6 m mol/kg and 5 times that dose (13.0 m mol/kg) of MEK (HD&5MEK group). The concentration of 2,5-HD in serum and in the sciatic nerve was determined 0.5, 1, 2, 4, 8, and 16 h after administration. Urinary 2,5-HD concentration was determined from the beginning of administration up to 16 h afterward. 1) The concentration of 2,5-HD in the serum, the sciatic nerve, and the urine was increased significantly (p<0.05) in the co-administered groups; the higher the MEK doses were, the greater was the increase. 2) The clearance of 2,5-HD from both the serum and the sciatic nerve was delayed in the co-administered groups. The highest concentration in serum and the sciatic nerve appeared at 1 and 2 h respectively. After administration, the biological half-life (t/2) of 2,5-HD from 1 to 8 h in serum was 6.5, 5.8 and 12.0 h for the HD, HD&MEK, and HD&5MEK groups respectively. From 8 to 16 h, the t/2 in serum was 1.2, 3.2 and 16.6 h for the HD, HD&MEK, and HD&5MEK groups, respectively. In nerve tissue, the prolongation of clearance in the co-administered groups was greater than that in serum, the t/2 from 2 to 8 h being 5.2, 9.6 and 19.9 h for the HD, HD&MEK, and HD&5MEK groups, respectively. From 8 to 16 h the t/2 was 1.0, 3.0 and 16.1 h for the HD, HD&MEK, and HD&5MEK groups, respectively. 3) The average ratios of the 2,5-HD concentration in the serum/2,5-HD concentration in the nerve were not significantly different among the three groups (6.2, 5.4, and 6.3 for the HD, HD&MEK and HD&5MEK groups, respectively).

Key words: 2,5-hexanedione, Methyl ethyl ketone, Co-administration, Clearance, Delay, Serum, Nerve, Urine

Introduction

Epidemiological investigations and animal experiments have shown that hexane and methyl n-butyl ketone (MnBK) cause peripheral polyneuropathy. The neurotoxicity of hexane and MnBK is thought to be induced ultimately by 2,5-hexanedione (2,5-HD), the major metabolite of hexane and MnBK. Several studies have shown that the neurotoxicity of hexane and MnBK was enhanced by co-administration with methyl ethyl ketone (MEK), one mechanism proposed for this enhancement being that MEK could influence the metabolism of hexane and MnBK, especially the metabolism of 2,5-HD. When rats inhaled hexane and MEK, the concentration of 2,5-HD in the serum and urine and the area under the serum concentration versus time curve (AUC) decreased or increased in different studies. When the rats were pretreated with MEK orally and then treated with hexane by inhalation, the concentration of 2,5-HD in the serum, sciatic nerves and testis increased relative to that in sham-treated controls. Further, the concomitant oral administration of 2,5-HD and MEK has been shown to reduce serum 2,5-HD clearance and to increase the concentration and AUC in serum.
Differences between results in the above mentioned studies may be connected with differences in the administration procedure, doses of hexane or 2,5-HD and MEK, the duration of the experiments, or other factors. The purpose of the present study was to clarify whether co-administration of different doses of MEK with 2,5-HD influenced the concentration of 2,5-HD in the serum, sciatic nerve, and urine; and whether the clearance of 2,5-HD from the sciatic nerve and serum was affected was also investigated.

Materials and methods

Animal and treatment: One hundred and eight male Wistar rats (Seiwa Experimental Animal Institute, Japan, average body weight 272±19) were used. 2,5-HD, MEK, and other chemicals used in this experiment were purchased from Wako Chemicals. Purity was more than 99.0%. The rats were divided into three groups: 2,5-HD alone (HD), 2,5-HD plus an equal dose of MEK (HD&MEK), and 2,5-HD plus a dose of MEK 5 times that of 2,5-HD (HD&5MEK). For all three groups, the 2,5-HD 2.6mmol/kg was injected subcutaneously into the rat's back; and 2.6mmol/kg and 13.0mmol/kg MEK were then injected at separate sites into the rats in the HD&MEK and HD&5MEK groups. The rats were killed 0.5, 1, 2, 4, 8, and 16h after injection under anesthesia. The rats to be killed at 16h were maintained in metabolism cages and urine samples were collected until 16h. Blood samples were collected from the abdominal aorta, and 2-ml serum samples were obtained by centrifugation. The sciatic nerves were excised, cut into pieces, and homogenized for 5 minutes with 2ml distilled water. A 2-ml aliquot of dichloromethane containing cyclohexanone (20μl/l), as the internal standard was added to each 2-ml sample (serum, homogenized nerve, and urine). The mixtures were then shaken and centrifuged (3,000 rpm) for 10 minutes. A 2μ1 aliquot of the extract sample was injected into a Shimadzu GC15A gas chromatograph. A Hicap-CBP1 (50 m, i. d. 0.25 mm) fused silica capillary column was used for detection. The column oven temperature was initially 80°C, and was increased to 110°C at the rate of 2°C/min and then to 180°C at 5°C/min. The final temperature of 180°C was maintained for 5 minutes. Both the injection port and detector temperature were 230°C. Helium was used as the carrier gas at 1.7 kg/cm². Hydrogen and air pressure were 0.7 kg/cm² and 0.6 kg/cm², respectively.

Statistics and kinetic analysis: Data were analyzed by ANOVA (F test), ONEWAY (F test) and regression and correlation (least squares method) with SPSS software (SPSS Inc. 1990). The area under the serum and nerve tissue 2,5-HD concentration versus time curve (AUC) was calculated by the 'trapezoidal rule'. The biological half-life (t1/2) of 2,5-HD was calculated according to the formula t1/2 = 0.693/k.

Results

Concentration of 2,5-HD in serum and nerve: Changes in the 2,5-HD concentration in serum are shown in Fig. 1. From 1 to 16 h after administration, the 2,5-HD concentration in serum increased in the order HD<HD&MEK<HD&5MEK (p<0.001). For the different times, the concentration increased in the order 16h<8h<4h<2h<0.5h<1h in the three groups (p<0.001). The most significant difference among the three groups was found at 16 h, the mean concentration of 2,5-HD at this time being 2.4, 26.0, and 165.5 mg/l for the HD, HD&MEK, and HD&5MEK groups, respectively. As shown in Fig. 1, the 2,5-HD concentration versus time curves were two phase curves (from 1 to 16 h), so two of the t1/2 values for each
phase (1 to 8 h and 8 to 16 h) were estimated. From 1 to 8 h, the half-life for the HD, HD&MEK, and HD&5MEK groups was 6.5, 5.8 and 12.0 h respectively; from 8 to 16 h, the \( t_{1/2} \) for the HD, HD&MEK, and HD&5MEK groups was 1.2, 3.2, and 16.7 h respectively. These results showed that 2,5-HD clearance from serum was prolonged in the 2,5-HD and MEK co-administered groups; moreover, the higher the MEK dose and the longer the experiment period, the greater was the prolongation.

With regard to nerve tissue, the time course of the concentrations of 2,5-HD in the sciatic nerve is shown in Fig. 2. The order of 2,5-HD concentration in the three groups was the same as that of serum, namely, HD<HD&MEK<HD&5MEK (p<0.001). The mean concentration of 2,5-HD at different times decreased in the order 16 h<8 h<4 h<0.5 h<1 h<2 h (p<0.001). The highest concentration in the nerve appeared about 1 h later than the highest concentration in serum. On estimation of 2,5-HD \( t_{1/2} \), the 2,5-HD clearance from the nerve was also found to be delayed in the co-administered groups. The 2,5-HD half-life in the nerve in the HD, HD&MEK, and HD&5MEK groups from 2 to 8 h was 5.2, 9.6 and 19.9 h, respectively, but for the same 2 to 8 h, the half-life of 2,5-HD in serum was 5.8, 5.9, and 10.7 for the HD, HD&MEK and HD&5MEK groups, respectively. These results showed that the prolongation in the nerve tissue was greater than the prolongation in serum.

Concentration of 2,5-HD in urine and its relationship with some parameters: Table 1 shows the urinary 2,5-HD concentration (UC), urinary 2,5-HD amount (UA), the AUC of serum and nerve (SAUC and NAUC), and the UA/AD (AD for administration dose) ratios. The urinary 2,5-HD concentration in the HD&5MEK group was significantly greater than that of the HD and the HD&MEK groups (p<0.05). The values for 2,5-HD concentration in urine, the urinary 2,5-HD amount, and the UA/AD in the HD&5MEK group were about twice those of the 2,5-HD alone group (p=0.0597), but, there was no significant difference between the HD and HD&MEK groups.

Ratio of 2,5-HD concentration in serum/2,5-HD concentration in the sciatic nerve: The ratios for the 2,5-HD concentration in serum divided by the 2,5-HD concentration in the sciatic nerve were determined. The mean ratios for the three groups (0.5 to 8 h) were not significantly different. At 16 h, the sciatic nerve concentration in the HD group was less than the detectable value, so the ratio could not be calculated (Table 2).

Discussion

Ralston et al.\(^{18}\) treated rats orally with 2.2 mmole/kg 2,5-HD alone and with equimolecular MEK once a day for 7 days. They found that the clearance of 2,5-HD in the co-administered groups was delayed, and that the 2,5-HD concentration (from 2 to 9 h) in the co-administered groups was significantly greater than that in the groups receiving 2,5-HD alone. In the same study, it has also been reported that the values for the AUC in co-administered groups were 1.6-1.8 times those for the 2,5-HD alone groups\(^{18}\). In the present experiment, in which rats were injected subcutaneously with 2,5-HD 2.6 mmol/kg alone or with this dose combined with an equal dose or 5 times the dose (13.0 mmol/kg) of MEK, both the value for the 2,5-HD concentration of 2,5-HD and the AUC in the co-administered groups were significantly greater than that in the 2,5-HD alone group (Fig. 1 and Table 1). Further, the higher the dose of MEK,
Table 1. Urinary 2,5-HD concentration and amount (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>UC (mg/L)</th>
<th>UA (mg/16 hour)</th>
<th>SAUC</th>
<th>NAUC</th>
<th>UA/AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>6</td>
<td>133±33.5</td>
<td>1.26±1.14</td>
<td>2060</td>
<td>358</td>
<td>.015±.013</td>
</tr>
<tr>
<td>HD&amp;MEK</td>
<td>6</td>
<td>178±37.9</td>
<td>1.37±0.60</td>
<td>2380</td>
<td>499</td>
<td>.016±.007</td>
</tr>
<tr>
<td>HD&amp;MEK5</td>
<td>5</td>
<td>271±65.1*</td>
<td>2.56±1.04</td>
<td>3380</td>
<td>673</td>
<td>.030±.012</td>
</tr>
</tbody>
</table>

*: UC: urine concentration of 2,5-HD (0-16 hours), UA: total amount of urinary 2,5-HD (0-16 hours), SAUC: serum AUC (mg/16 hours), NAUC: nerve AUC (µg/g/16 hours), AD: administration dose (mg/rat).

*: p<0.05 vs HD group.

Table 2. Ratios of 2,5-HD concentration in serum/2,5-HD concentration in nerve

<table>
<thead>
<tr>
<th></th>
<th>0.5h</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>8h</th>
<th>16h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>6.5±0.59</td>
<td>6.2±1.48</td>
<td>5.7±1.47</td>
<td>6.1±1.59</td>
<td>6.6±2.71</td>
<td>Not detected*</td>
</tr>
<tr>
<td>HD&amp;MEK</td>
<td>5.3±1.05</td>
<td>6.5±1.48</td>
<td>5.8±0.86</td>
<td>4.8±0.94</td>
<td>4.4±0.66</td>
<td>5.2±1.94</td>
</tr>
<tr>
<td>HD&amp;MEK5</td>
<td>7.6±1.59</td>
<td>6.4±0.80</td>
<td>6.2±0.91</td>
<td>5.9±0.63</td>
<td>5.4±1.06</td>
<td>5.5±1.36</td>
</tr>
</tbody>
</table>

*: Concentration in the nerve was below the detectable level.

the stronger was the capture effect of MEK, but when hexane instead of 2,5-HD was co-administered with MEK (co-inhalation or co-administration by gavaging), the 2,5-HD concentrations in the serum were both increased or decreased.

Values for 2,5-HD in serum, especially when the 2,5-HD was co-administered with MEK, have merely been reported. The values for t\textsubscript{1/2} that we approximated from the values given by Ralston et al.\textsuperscript{(18)} were 3.5 and 10.0 for 2,5-HD alone or for the 2,5-HD plus MEK group, respectively. That is, the co-administration of 2,5-HD with MEK prolonged 2,5-HD t\textsubscript{1/2} in the serum, similar to ours in the present series of experiments. Moreover, in the present series of experiments, the difference between the t\textsubscript{1/2} of the HD and HD&MEK groups in the early period (1 to 8 h) was not significant, whereas in the same 1- to 8- h period, when the MEK dose was increased to 5 times the 2,5-HD dose (13.0 mmol/kg), the t\textsubscript{1/2} of the HD&5MEK group was 1.9 fold that of the HD group. In the 8- to 16- h period, the t\textsubscript{1/2} of the HD&MEK and HD&5MEK groups was 2.8 and 13 times, respectively, that of the 2,5-HD alone group. These results suggest that the t\textsubscript{1/2} is prolonged more significantly during the later period and also when 2,5-HD is co-administered with a greater dose of MEK.

It has been reported that MEK administered orally prior to hexane inhalation increases the 2,5-HD concentration in the sciatic nerve\textsuperscript{(17)}. In the present series of experiments, the 2,5-HD concentration in the sciatic nerve was significantly increased in the co-administered groups. Moreover, we also found that the clearance of 2,5-HD from the nerve was delayed in the co-administered groups. It should be noted that in the 2- to 8- h period, the 2,5-HD t\textsubscript{1/2} in the nerve tissue in the HD& MEK and HD&5MEK groups was 1.9 and 3.9 times that in the HD group (9.6/5.2, 19.9/5.2), whereas in the serum these values were only 1.0 (5.9/5.8) for the HD&MEK/HD groups and 1.9 (10.0/5.8) for the HD&5MEK/HD groups. These findings suggest that MEK delayed the clearance of 2,5-HD in the nerve tissue to a greater extent than that in the serum.

A good correlation (r=0.86, p<0.001) between the concentration in the sciatic nerve and serum was found in this series of experiments. This correlation was not changed in the groups receiving co-administration with MEK. Furthermore, the ratio of the 2,5-HD concentration in the serum/2,5-HD concentration in the nerve did not differ significantly among the three groups. Thus it is possible to estimate the 2,5-HD concentration in nerve from the serum concentration, but reported values for the ratio of the 2,5-HD concentration in the serum/2,5-HD concentrations in the nerve vary in different studies. We estimated that this ratio was about 1.0 in both their hexane and hexane plus MEK pretreatment groups from the reported results of Robertson et al.\textsuperscript{(17)} From the data published by Ralston\textsuperscript{(8)} et al. and Suwita\textsuperscript{(20)} et al., we calculated that the ratios for serum/nerve radioactivity were 0.8 to 1.7 for the 2,5-HD alone group and 0.8 to 2.3 for the co-administered group. As the radioactivity in the Ralston\textsuperscript{(8)} et al. and Suwita et al. studies could reflect that of compounds other than 2,5-
HD, it is difficult to compare our results with the data estimated from these two studies. Furthermore, the administration procedure plays a role.

Suwita et al.\textsuperscript{20)} reported that only 1.2% of the 2,5-HD administered to hens on the skin (50 mg/kg) was eliminated in urine within 24 h. In the present study, we found that 1.5%, 1.6%, and 3.1% of the 2,5-HD administered was eliminated within 16 h in the HD, HD&MEK and HD&5MEK groups, respectively (p=0.059). The amount of 2,5-HD eliminated in the urine of the HD&5MEK group was two times that of the 2,5-HD alone group (Table 1). These results showed that 2,5-HD elimination in urine was increased when the agent was co-administered with 5 times the dose of MEK. Whereas, when it was inhaled with hexane and MEK, 2,5-HD elimination was decreased\textsuperscript{13-15)}.

The urinary concentration correlated well with the serum concentration (r=0.81, p<0.01) and nerve concentration (r=0.80, p<0.01) in the present experiment, indicating that the increase in the urine 2,5-HD concentration in the HD&5MEK group paralleled the increase in serum 2,5-HD concentration in that group.

In the present series of experiments, it should be noticed that the time change course of the 2,5-HD concentration in the serum and nerve appeared as a non-linear curve on the semi-logarithm graph (Figs. 1 and 2). That is the t\textsubscript{1/2} was prolonged when the 2,5-HD concentration in serum or nerve was higher than a certain level. This model is thought to predominantly reflect the limitation of liver metabolic capacity\textsuperscript{13,36,27)} in relation to the amount of agents that have being metabolized in liver. According to this explanation, it could be hypothesized that if the total dose of 2,5-HD and MEK is too great to be metabolized in the liver (even if the 2,5-HD amount is relatively small, the 2,5-HD concentration in the serum and nerve could be increased). In the present series of experiments, the clearance of 2,5-HD was prolonged to a greater extent in the nerve than that in serum), and this fact was thought to be related to the neurotoxicity enhancement of 2,5-HD caused by MEK, but the mechanism whereby MEK prolongs 2,5-HD t\textsubscript{1/2} in the nerve should be further investigated.

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References

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A STUDY ON INDICES OF BODY FAT DISTRIBUTION FOR SCREENING FOR OBESITY
Jung Su Lee, Kazuo Aoki, Kiyoshi Kawakubo, and Atsuki Gungi…………………………………………………9-18

冠動脈疾患危険因子（CRF）の高い肥満者を判定する
ために、体脂肪分布指数と CRF の関連について分析を
行った。事務系の仕事に従事する成人男性 938 人を対象
とし、周囲長、皮脂厚を測定して体脂肪分布を表すいくつか
の指数を作成した。その結果、以下の各点が明らかとな
った。①肥満指数 (BMI)、②体脂肪分布指数の HDL-コ
レステロール (TC)、LDL-コレステロールと強い正の相関を
示した。③体脂肪分布指数の最も高い相関があり、中性脂
肪 (TG) と正の、HDL-コレステロールと負の強い相関を
示した。④ WSR は高価、TC、TG 異常者の判別におい
て、敏感度、特異度ともに最も優れていた。⑤ WSR 高
値群（0.49 以上）と低値群（0.49 未満）間ですべての
CRF 有意差が認められ、高値群の異常者は低値群の約 2 倍であっ
た。上述の結果から、冠動脈疾患予防のための肥満判定の
ために、WSR 測定の有用性が示された。
(J Occup Health 1995; 37: 9-18)
(2.6 mmol/kg) 単独投与群と 2, 5-HD (2.6 mmol/kg) + MEK (2.6 mmol/kg) 混合投与群と 2, 5-HD (2.6 mmol/kg) + 5 倍の MEK (13.0 mmol/kg) 混合投与群を作り、0.5, 1, 2, 4, 8, 16 時間後に血清中、坐骨神経中、尿中（16 時間後のみ）2, 5-HD 濃度をガスクロマトグラフィーを用いて測定した。2, 5-HD の血清中濃度及び神経中濃度は、単独投与時に比べて混合投与群では全体的に高く、またクリアランスも速く、この傾向は MEK の濃度が高いほど明らかだった。また、2, 5-HD の血清中濃度は、単独投与群では片対数グラフで 2 相性を示し、それぞれの生理学的半減期は投与後 1 より 8 時間では 6.5 時間であり、8 より 16 時間では 1.2 時間であった。一方 5 倍の MEK 混合投与群の半減期は、8 より 16 時間では 16.6 時間であり、これは 2, 5-HD 単独投与群の 8～16 時間における半減期の 13 倍と非常に速くなっていた。次に神経組織におけるクリアランスについて、投与後 2～8 時間におけるクリアランスは、血清中のクリアランスより速くなる傾向があり、生物学的半減期は、単独投与群では 5.2 時間であり、2, 5-HD と当モルの MEK 混合投与群では 9.6 時間、5 倍の MEK 混合投与群では 19.9 時間であった。2, 5-HD の血清中濃度と神経組織中濃度の比は、4.8 から 6.6 と 2, 5-HD の単独投与、あるいは混合投与にかかわらずほぼ同じ程度であり、神経中濃度は血清中濃度をよく反映していた。投与から 16 時間までの尿中排泄量は、投与量の約 15% から 3.1% であり、2, 5-HD 単独投与群より混合投与群の方が多く、この傾向は MEK の血中濃度が高いほど著明であった。また、血清中濃度及び神経中濃度と尿中濃度の相関係数は、ともに約 0.8 とよい相関がみられた。

(J Occup Health 1995; 37: 19-24)