Neurotoxic Convolutions Induced by Theophylline and Its Metabolites in Mice

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To evaluate the risk of neurotoxicity induced by theophylline and its main metabolites, 1-methylxanthine (1-MX), 3-methylxanthine (3-MX), 1,3-dimethyluric acid (1,3-DMUA) and 1-methyluric acid (1-MUA), we compared their convulsive potency to central nervous system (CNS) after intracerebral administration to mice. All compounds studied induced clonic convulsion in a dose-dependent manner, and the ED50 values for convulsion were 490, 546, 1107, 360 and 620 nmol/kg for theophylline, 1-MX, 3-MX, 1,3-DMUA and 1-MUA, respectively. These compounds were also administered intravenously to mice by constant rate infusion until the onset of convulsion. Clonic convulsion was induced by i.v. infusion of theophylline, 1-MX and 3-MX, while convulsion was not observed during 1,3-DMUA or 1-MUA infusion for 60 min. This finding may be due to the poor blood–brain barrier permeability of both 1-MUA and 1,3-DMUA as compared with theophylline, 1-MX and 3-MX. However, it may be also necessary to consider the possibility of 1,3-DMUA-induced neurotoxicity judging from its intrinsic convulsive potency.

Key words theophylline; metabolite; neurotoxicity; mouse

It is well known that an overdose of theophylline induces several neurotoxicities such as nausea, vomiting and convulsions in severe cases.1–3) Theophylline is mainly metabolized in liver, and about 90% of the dose is excreted in urine as metabolites such as 3-methylxanthine (3-MX), 1-methyluric acid (1-MUA) and 1,3-dimethyluric acid (1,3-DMUA).4) An intermediate metabolite, 1-methylxanthine (1-MX) was not detected in plasma or urine, perhaps because it is readily metabolized to 1-MUA by xanthine oxidase. The pharmacological efficacy of these metabolites is relatively weak as compared with the parent compound, therefore reduction of the dose is not necessary in patients with renal failure.5) No toxic effect to the central nervous system (CNS) was observed with a high dose of 3-MX, 1-MUA or 1,3-DMUA in rats.6) The concentration of these compounds in the brain was lower than that in plasma, so that poor permeability of blood–brain barrier might be one reason for the inactivity of theophylline metabolites. If theophylline metabolites have intrinsic neurotoxic potential, the disturbance of blood–brain barrier permeability may possibly cause neurotoxic reactions in a disease state such as renal failure.

A case of neurotoxicity was recently reported in a renal failure patient with sub-therapeutic concentration of theophylline and high concentration of its metabolites, especially 1,3-DMUA, in plasma,7) this suggests the neurotoxic potency of theophylline metabolites. In the present study, theophylline and its main metabolites were intracerebrally administered to mice to evaluate their intrinsic convulsive potency to CNS. An intravenous infusion study was also carried out until the onset of convulsion to evaluate their convulsive efficacy.

MATERIALS AND METHODS

Chemicals and Reagents Theophylline, 1-MX, 3-MX, 1-MUA and 1,3-DMUA were purchased from SIGMA (U.S.A.) and used without further purification. All other chemicals and reagents were purchased from commercial sources and were of analytical grade.

Intracerebral Administration Study Each compound was dissolved in a small amount of 1 m NaOH and pH was adjusted to 7–11 with 1 m HCl, then diluted with water. Behavioral change was not observed by intracerebral administration of drug-free solution within this pH range. The osmotic pressure range between 150–560 mOsm and the concentration of chloride ion between 150–200 meq/l was maintained for each solution, since the convulsive effect was slightly affected by osmotic pressure and chloride concentration.8) Intracerebral administration was performed as described previously.8,9) Briefly, male ddY mice (5 weeks, 20–30 g) were given 10 µl of drug solution in the right lateral ventricle, 3 mm anterior, 2 mm left and 4 mm depth relative to the lambdoid suture; they were then placed in a clear plastic box and observed for 20 min, since in a preliminary study convulsion had not been observed later than 20 min after intracerebral administration. Clonic movement of the limbs lasting more than 3 s was scored as a convulsion.

The relationship between convulsive incidence (E) and intracerebral dose (D) was fitted to the equation $E = D^γ/(ED_{50} + D^γ)$ to estimate $ED_{50}$ and slope factor, γ.

Intravenous Administration Study Intravenous administration of theophylline and its metabolites was performed as described previously.8,9) Each drug solution was infused into the tail vein of male ddY mice at the rate of 24.1 µl/min until the onset of convulsion, at which time infusion was stopped and the mouse was decapitated to collect blood and brain. Blood was immediately centrifuged to obtain plasma. Infusion was also stopped at 60 min if no convulsion was observed. Plasma and brain samples were stored at −20°C until analysis.

Determination of Drugs in Plasma and Brain Concentration of each drug in plasma and brain was determined as described by Ramzan and Levy6) with slight modification. Theophylline, 1-MX and 3-MX were determined by the same procedure, while 1,3-DMUA and 1-MUA were subjected to another procedure. If the con-
centration of administered drug was much higher than the possible metabolites of administered drug, they were determined separately.

For the determination of theophylline, 1-MX and 3-MX in plasma, 25 μl of plasma sample was added to 10 μl of thebromine solution as internal standard, 50 μl of 0.1 M phosphate buffer (pH 5.0), 2.5 ml of chloroform–ethyllactate–2-propanol (45:45:10) mixture, shaken for 10 min and centrifuged at 1600 g for 10 min. Two milliliters of organic phase was evaporated to dryness by nitrogen stream, then dissolved in 100 μl of mobile phase and the 50 μl aliquot was subjected to HPLC.

To determine theophylline, 1-MX and 3-MX in the brain, the brain sample was homogenized with 4 volume of 0.1 M phosphate buffer, 100 μl of internal standard solution added and extracted with 10 ml of chloroform–ethyllactate–2-propanol (45:45:10) mixture. One milliliter aliquot of organic phase was evaporated by nitrogen stream. The residue was dissolved in 100 μl of mobile phase, then centrifuged at 10000 g for 2 min and 20 μl of the supernatant was subjected to HPLC. The HPLC apparatus consisted of an LC-6A pump (Shimadzu, Japan) and an SPD-6A spectrophotometer set at 280 nm. The column was a 250 mm × 4 mm stainless steel tube packed with Nucleosil 5C18 and maintained at 30 °C. The mobile phase was methanol–10 mm sodium acetate buffer (pH 6.5) (1:9) containing 5 mM tetrabutylammonium hydroxysulphate, and pumped at the rate of 1.5 ml/min.

To determine 1,3-DMAU and 1-MUA in plasma, 25 μl of plasma sample was added to 25 μl of β-hydroxypropyltheophylline solution as the internal standard and 50 μl of 6 M HCl, and extracted by 3 ml of chloroform–2-propanol (85:15). Two milliliters of organic phase was evaporated by nitrogen stream, and the residue was dissolved by mobile phase and 50 μl aliquot was subjected to HPLC.

For the determination of 1,3-DMAU and 1-MUA in the brain, the brain sample was homogenized with 4 volumes of 1N HCl, added with 25 μl of the internal standard solution and extracted with 10 ml of chloroform–2-propanol (85:15). One milliliter of organic phase was evaporated by nitrogen stream, and the residue was dissolved by 100 μl of mobile phase, then centrifuged at 10000 g for 2 min and 20 μl of the supernatant was subjected to HPLC. The HPLC apparatus was as described above. The mobile phase was methanol–10 mm sodium acetate buffer (pH 6.5) (16:84) containing 5 mM tetrabutylammonium hydroxysulphate and pumped at the rate of 1.5 ml/min.

The detection limits of all compounds in plasma and the brain were 10 μM and 10 nmol/g with the coefficient of variation less than 10%, respectively.

RESULTS AND DISCUSSION

The intracerebral dose–response (occurrence of convulsion) curves of each drug for convulsion is shown in Fig. 1. All compounds used in the present study induced clonic convolution in a dose dependent manner after intracerebral administration. The values of ED<sub>50</sub> and the slope factor for each drug are listed in Table 1. Convulsive potency of 1,3-DMAU and 1-MUA were no less than that of theophylline, while that of 3-MX was smaller than that of theophylline.

Theophylline, 1-MX and 3-MX induced convolution by intravenous infusion, while no convulsive response was observed during intravenous infusion of 1,3-DMAU or 1-MUA for 60 min. The concentration of these drugs in plasma at 60 min was much higher, while in the brain their concentration were much lower than those of other drugs which induce convolution during intravenous infusion (Table 2). The poor permeability of 1,3-DMAU and 1-MUA was quite plausible considering their poor lipophilicity. The ratio of brain concentration/plasma concentration was about 1%, which may be due to drug in the blood remaining in the brain.

These results suggested that 1,3-DMAU and 1-MUA did not manifest toxicity to CNS because of its poor permeability to the blood–brain barrier. Nevertheless their convulsive potency was large enough to induce convolution if directly administered into brain. The possibility of neurotoxicity induced by 1,3-DMAU should therefore be considered because of its intrinsic neurotoxic potency, since the blood–brain permeability may change in disease states such as severe renal failure. The change of protein binding may be one cause of the change of blood–brain barrier permeability, but this may not be true with theophylline or its metabolites because their unbound fractions ranged from 0.3–0.7 in rats.

Among the compounds studied in the present work, 1-MX was usually not detected in plasma or urine of a human administered theophylline. In the animal study, 1-MX and its possible metabolite, 1-MUA, were not
Table 2. Onset Time, Threshold Dose and Concentration for Clonic Convulsion Induced by Intravenous Infusion of Theophylline or its Metabolites in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Theophylline (n = 3)</th>
<th>1-MX (n = 3)</th>
<th>3-MX (n = 3)</th>
<th>1,3-DMUA (n = 3)</th>
<th>1-MUA (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>25.6 ± 1.2</td>
<td>20.3 ± 1.2</td>
<td>23.7 ± 2.2</td>
<td>22.0 ± 0.9</td>
<td>20.3 ± 1.2</td>
</tr>
<tr>
<td>Infusion rate (µmol/min)</td>
<td>2.41</td>
<td>4.82</td>
<td>7.23</td>
<td>7.23</td>
<td>4.82</td>
</tr>
<tr>
<td>Onset time (min)</td>
<td>19.0 ± 1.2</td>
<td>30.4 ± 2.0*</td>
<td>24.5 ± 2.4</td>
<td>60†</td>
<td>60†</td>
</tr>
<tr>
<td>Total dose (mmol/kg)</td>
<td>1.79 ± 0.11</td>
<td>7.21 ± 0.19**</td>
<td>7.48 ± 0.67**</td>
<td>19.74 ± 0.67†</td>
<td>14.25 ± 0.79†</td>
</tr>
<tr>
<td>C₀ (mm)</td>
<td>2.36 ± 0.56</td>
<td>3.91 ± 0.17</td>
<td>5.21 ± 0.63*</td>
<td>68.13 ± 6.72†</td>
<td>21.63 ± 2.98†</td>
</tr>
<tr>
<td>C₀ (µmol/g)</td>
<td>0.74 ± 0.15</td>
<td>0.57 ± 0.03</td>
<td>0.81 ± 0.18</td>
<td>0.56 ± 0.17†</td>
<td>0.28 ± 0.27†</td>
</tr>
<tr>
<td>C₀/C₀ (ml/g)</td>
<td>0.32 ± 0.06</td>
<td>0.15 ± 0.01</td>
<td>0.16 ± 0.05</td>
<td>0.008 ± 0.002†</td>
<td>0.012 ± 0.012†</td>
</tr>
</tbody>
</table>

C₀: Plasma concentration at the end of infusion. C₀: Brain concentration at the end of infusion. * Convulsion was not observed. † Significantly different from theophylline (p<0.05, Dunnett multiple comparison test). ** Significantly different from theophylline (p<0.01, Dunnett multiple comparison test).

Table 3. Plasma Concentration of Theophylline and its Possible Metabolites at the Onset of Clonic Convulsion

<table>
<thead>
<tr>
<th>Theophylline infusion (n = 3)</th>
<th>1-MX</th>
<th>3-MX</th>
<th>1,3-DMUA</th>
<th>1-MUA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀ (mm)</td>
<td>2.36 ± 0.56</td>
<td>N.D.</td>
<td>0.039 ± 0.022</td>
<td>0.043 ± 0.025</td>
</tr>
<tr>
<td>C₀ (µmol/g)</td>
<td>0.74 ± 0.15</td>
<td>N.D.</td>
<td>0.024 ± 0.008</td>
<td>N.D.</td>
</tr>
<tr>
<td>1-MX infusion (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₀ (mm)</td>
<td>N.D.</td>
<td>3.91 ± 0.17</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>C₀ (µmol/g)</td>
<td>N.D.</td>
<td>0.57 ± 0.03</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>3-MX infusion (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₀ (mm)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>5.21 ± 0.63</td>
<td>N.D.</td>
</tr>
<tr>
<td>C₀ (µmol/g)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.82 ± 0.18</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not detected.

detected in plasma after intravenous administration of theophylline (Table 3). 1-MUA concentration in plasma after intravenous administration of 1-MX was rather high. Therefore, 1-MX dose not usually contribute to the neurotoxic response after theophylline administration since its production from theophylline is small and elimination into 1-MUA is rapid, although its convulsive potency is similar. If xanthine oxidase which metabolizes 1-MX to 1-MUA is inhibited, 1-MX may be accumulated in the body, penetrate the brain and induce neurotoxicity.

3-MX is detected in plasma and the brain after intravenous administration of theophylline and distributed into the brain to a similar extent as 1-MX (Table 2). However, the convulsive potency of 3-MX was less than half that of theophylline (Table 1), therefore the brain concentration of 3-MX to induce convulsion should be twice or more that of theophylline. However, there were no significant difference between the threshold brain concentration of theophylline and 3-MX. The drug concentration in brain does not always reflect the concentration in an effective site in CNS, which may be one of the reasons that the difference between 3-MX and theophylline in the neurotoxic potency could not be evaluated by determination of threshold brain concentration. CSF concentration may be preferable for the quantitative evaluation of neurotoxic potency of several drugs. Since 3-MX is excreted in urine in human, its accumulation due to renal insufficiency may induce neurotoxicity even if the theophylline level is in the sub-therapeutic range.

In the neurotoxicity reported by Leakey et al., CNS adverse reactions were observed with a sub-therapeutic level of theophylline, suggesting that neurotoxicity was caused, at least in part, by theophylline metabolites. Since the concentration of 1-MX was about 1/10 theophylline in this patient, 1-MX did not act to induce CNS adverse reactions. The concentration of 3-MX in plasma was in a similar range to that of theophylline and some portion of it may be transferred to the brain, thus 3-MX may be involved in the neurotoxicity observed in the anuric patient. However, the neurotoxic potency of 3-MX would not be strong enough alone to induce convulsion. The concentration of 1,3-DMUA in serum was 10 times higher than that of theophylline in this patient. A very small amount of 1,3-DMUA may usually be transferred into the brain and may not change the CNS activities. The CNS function of this patient, however, could have been abnormal due to not only anuria but to hepatic encephalopathy. Disturbance of the blood-brain barrier may have resulted in the increase of 1,3-DMUA concentration in CNS and induced neurotoxicity.

In conclusion, all major metabolites of theophylline were demonstrated to have convulsive potency. Both 1-MUA and 1,3-DMUA are predominantly produced by theophylline metabolism and their CNS convulsive potencies are rather strong. Their pharmacological inactivity is probably due to their poor permeability to the blood-brain barrier, and the possibility of neurotoxicity induced by these compounds when the blood-brain barrier is broken down by a disease such as severe renal failure must be considered.

REFERENCES