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ORIGINAL RESEARCH

Enhanced theophylline metabolism in patients with bronchial asthma at age 4 and under

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ABSTRACT: The plasma levels of theophylline (TP) and its metabolites were measured in patients with bronchial asthma who were treated with a slow-release preparation of TP. The ratios of the plasma levels of these metabolites to TP levels in the group aged 1–4 years were larger than those in the group aged 5 years and older, suggesting enhanced activity of drug-metabolizing enzymes during infancy. Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Round-the-clock (RTC) therapy with a slow-release preparation of theophylline (TP) is widely used in clinical practice as a therapy in which the plasma level is maintained within the therapeutic range for prophylaxis of asthma attacks. Approximately 85–90% of the administered dose of TP is metabolized by the hepatic microsomal enzyme cytochrome P450. One major step in the metabolic pathway is hydroxylation at position 8 of TP to generate 1,3-dimethyluric acid (DMU); other steps are *N*-demethylation to form 1-methylxanthine and 3-methylxanthine (3MX). 1-Methylxanthine is rapidly converted to 1-methyluric acid (1MU) by xanthine oxidase (Tserng *et al.*, 1981). The rate of metabolism of TP varies considerably from one individual to another; therefore, effective, safe TP therapy requires optimization by measuring plasma levels of TP and these metabolites, especially in patients with abnormal TP metabolism (Kizu *et al.*, 1999).

We previously measured the plasma levels of TP and its metabolites by HPLC in patients aged 6–80 years old receiving RTC therapy. We found a strong correlation between the plasma levels of DMU and TP, and a consistent ratio of DMU to TP (DMU:TP) of 0.054 ± 0.011. We found no correlation between the plasma levels of other metabolites and TP (Kizu *et al.*, 1999). In this study we examined TP metabolism in plasma samples from patients of a wide range of ages receiving RTC therapy, including infants.

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Abbreviations used: DMU, 1,3-dimethyluric acid 1mu 1-methyluric acid; RTC, round-the-clock; TP, theophylline.

EXPERIMENTAL

Eighty-four plasma samples were collected from 46 out-patients and in-patients with bronchial asthma (27 males and 19 females, aged 1–55 years) who were being treated with TP. Informed consent was given in all cases. When different doses were administered in the same patient, the samples were regarded as different. When doses were the same, the mean value was used.

The HPLC method used to measure plasma TP and metabolites was essentially the same as that reported previously (Kizu *et al.*, 1999). Briefly, 300 μL of acetonitrile, containing 0.3 μg of *β*-hydroxyethyltheophylline as an internal standard, was added to 100 μL of plasma sample. After vigorous mixing, the sample was centrifuged at 5000***g*** for 5 min at room temperature. Three hundred microliters of the supernatant were transferred into a new tube and dried in a heat-block at 40°C. The residue was dissolved in 30 μL of mobile phase [20 mM sodium acetate buffer (pH 4.8), acetonitrile, and methanol (900:35:65, v/v)], and 5 μL of the solution was injected onto the HPLC column (TSK ODS-80TM reversed-phase column, 250 × 4.6 mm i.d., Tosoh Co., Tokyo, Japan) operated at room temperature. The flow rate was 1.0 mL/min. The eluate was monitored at 275 nm.

RESULTS

The mean doses of TP administered were 18.5 mg/kg/day

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Table 1. Correlation coefficients (*r*) of the plasma DMU, 1MU, and 3MX levels with TP levels

Metabolite	Patient group		
	1–4 years ($n = 25$)	5–12 years ($n = 21$)	13–55 years ($n = 18$)
DMU	0.856[b]	0.851[b]	0.857[b]
1MU	0.719[b]	0.231	0.258
3MX	0.742[b]	0.496[a]	0.649[a]

[a] $p < 0.05$; [b] $p < 0.01$ (Fisher's *Z*-transformation).

for patients aged 1–4 years ($n = 25$, average body weight 15.1 kg), 15.5 mg/kg/day for those aged 5–12 years ($n = 21$, average 26.0 kg), and 12.3 mg/kg/day for those aged 13–55 years ($n = 18$, average 52.0 kg). The mean plasma TP levels were 9.18, 11.2 and 12.0 μg/mL, respectively. The plasma TP levels were lower in younger patients in spite of the administration of higher doses. The plasma levels of TP and its metabolites were not significantly different between both sexes in any group, and therefore the results were pooled for both sexes.

The plasma DMU level was strongly correlated with the TP level in all three groups shown in Table 1. In contrast, the plasma 1 MU level and 3 MX (Fig. 1A) levels were strongly correlated with the TP level only in the 1–4-year-old group (Table 1). The DMU:TP ratio of the 13–55-year-old group was 0.055 ± 0.012, identical to that reported previously (Kizu *et al.*, 1999), but those of the 5–12 and 13–55-year-old groups were slightly higher (0.058 ± 0.013 and 0.063 ± 0.013, respectively). The 1MU:TP and 3MX:TP ratios of the 1–4-year-old group were significantly higher than those of the other groups ($p < 0.001$ or 0.05; Fig. 1B).

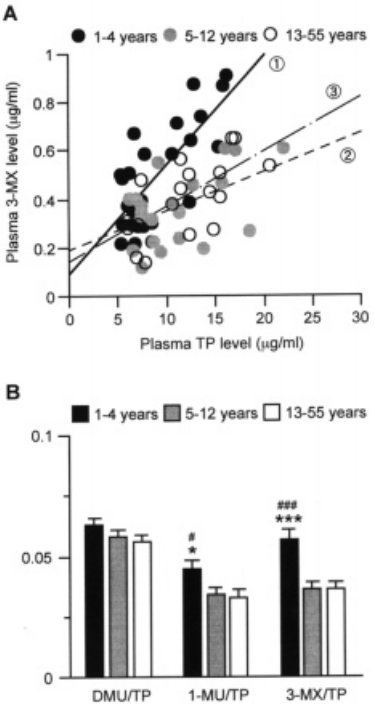

Figure 1. Enhanced metabolism of TP in patients at ages 1–4 years. (A) Correlation between plasma TP and 3MX levels in the three groups given slow-release preparations of TP (①, 1–4 years, $y = 0.045x + 0.092$, $r = 0.742$; ②, 5–12 years, $y = 0.016x + 0.192$, $r = 0.496$; ③, 13–55 years, $y = 0.023x + 0.144$, $r = 0.649$). (B) Comparison of the DMU:TP, 1MU:TP, and 3MX:TP ratios in the three groups. $*p < 0.05$, $***p < 0.001$ as compared with 5–12 years; $\#p < 0.05$, $\#\#\#p < 0.001$ as compared with 13–55 years (Bonferroni test)

DISCUSSION

The plasma TP level attained by RTC therapy in bronchial asthma has to be 10–20 μg/mL (Hendeles *et al.*, 1978). According to the dosing guidelines (Masaki *et al.*, 1981), the average dose is 18 mg/kg/day for patients weighing 21 kg or less, 15 mg/kg/day for patients weighing 21–40 kg, and 600 mg/day for patients weighing more than 40 kg. Although we administered the above doses to the 1–4-year-old children, the plasma levels of most did not increase to the required level.

Cytochrome P450 enzyme subtypes CYP1A2, 2E1 and 3A4 are involved in metabolizing TP to DMU; CYP1A2, 1A1, and 2D6 are involved in metabolizing it to 1MU; and CYP1A2 is specifically involved in metabolizing it to 3MX (Huy Riem *et al.*,1995). There are saturable steps in all metabolic pathways the formation rate of DMU is thought to be the largest in both K_m and V_{max} (Tang-Liu *et al.*, 1982). This may be the reason why DMU was strongly correlated with the plasma TP level in every group in this study.

The DMU:TP ratio was not significantly different in younger patients. In the groups aged 5 or older, the plasma 1MU and 3MX levels were not correlated with the plasma TP levels, suggesting that these metabolic pathways might have been saturated. In contrast, in the 1–4-year-old group, the plasma 1MU and 3MX levels were correlated with the plasma TP levels, and the 1MU:TP and 3MX:TP ratios were significantly higher than in the other groups. These results indicate that at the age of 1–4 years, the steps metabolizing TP to 1MU and 3MX are not saturated, and thus the total metabolism of TP is enhanced. This is why a higher dose is required in infants. The specific increase in the metabolism of TP to 1MU and 3MX at this age suggests that increased activity of CYP1A2 or the expression of some unknown enzymes might be involved.

REFERENCES

Hendeles, L., Weinberger, M. and Johnson, G. 1978. *Clin. Pharmacokinet.* **3**:294.
Huy Riem, H.A., Jinzhen, C., Andreas, U.F. and Ferenc, F. 1995 *Br. J. Clin. Pharmac.* **39**:321.
Kizu, J., Watanabe, S., Yasuno, N., Arakawa, Y., Uzu, S., Kanda, S., Komoda, F., Iwata, T., Hayakawa, H., Hayakawa, T. and Imai, K. 1999. *Biomed. Chromatogr.* **13**:15.
Masaki, T., Nagakura, T. and Iikura, Y. 1981. *Allergy* **30**:190.
Tang-Liu, D.D., Williams, R.L. and Riegelman, S. 1982. *J. Pharmac. Exp. Ther.* **224**:180.
Tserng, K.Y., King, K.C. and Takieddine, F.N. 1981. *Clin. Pharmac. Ther.* **29**:594.

