Effect of Traditional Chinese Herbal Medicines on the Pharmacokinetics of Western Drugs in SD Rats of Different Ages. I. Aminophylline—Tin Chuan Tang and Aminophylline—Hsiao Ching Long Tang

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The effect of the traditional Chinese herbal medicines (Tin Chuan Tang and Hsiao Ching Long Tang) on the serum concentrations and pharmacokinetics of aminophylline was examined in three different ages of SD rats. Each traditional Chinese herbal medicine was orally preadministered to SD rats for one week and then aminophylline was administered intravenously. The serum concentrations and pharmacokinetic parameters of theophylline were estimated by a two-compartment open model. The liver isolated after the last blood sampling was homogenized and the activity of hepatic cytochrome p-450 was determined. Significant difference was found in some pharmacokinetic parameters of theophylline such as K_{10} , $t_{1/2}$, Cl and V_d for three different ages of SD rats without pretreatment with Chinese herbal drugs ($p < 0.05$). However, pretreatment with Tin Chuan Tang or Hsiao Ching Long Tang did not affect the pharmacokinetic parameters of theophylline in three different ages of SD rats ($p > 0.05$). We also found that there was no correlation between age and activity of cytochrome p-450 of SD rats ($p > 0.05$). The decline in some pharmacokinetic parameters of theophylline in the elderly rats perhaps might be attributed to the decrease in hepatic blood flow and liver volume. It is concluded that there was no drug interaction between theophylline and Tin Chuan Tang or Hsiao Ching Long Tang in the different ages of SD rats.

Keywords — western drug; aminophylline; theophylline; Chinese herbal medicine; Tin Chuan Tang; Hsiao Ching Long Tang; pharmacokinetics; drug interaction; cytochrome P-450

Introduction

The traditional Chinese herbal medicines have been developed and used to treat diseases or as an alternative form of health care, with clinical experiences accumulated over some thousand years in China. Although such traditional herbal medicines as compared with western drugs, are not highly appreciated in the modern medical sciences, the fact that many adverse side effects caused recently by the western drugs have renewed interest in it again in clinical application. Recently, the reduced side effects of the traditional herbal medicines have brought wide use for the treatment of chronic diseases of the elderly patients. The decoctions are prepared from crude drugs and water or from the granulated extract prepared by the modern pharmaceutical technology.

If a western drug was comedicated with tradi-

tional Chinese herbal medicine, the pharmacokinetics and pharmacodynamics of western drugs perhaps may alter, especially in the elderly patients due to the alteration of enzymes and drug disposition by aging.¹⁾ The risk of such Chinese-western drug interactions was seldom studied, although some studies have reported that the combined effect of the traditional Chinese herbal medicines with western drugs frequently reduced the dose and side effect of the western drugs.^{2,3)} The therapeutic efficacy and pharmacological effect of the combined use of western drugs and Chinese herbal medicines have been investigated²⁾ but the effects on the pharmacokinetics of western drugs were unknown.

In this preliminary study, aminophylline as a model drug was intravenously administered into SD rats of different ages pretreated with Tin Chuan Tang or Hsiao Ching Long Tang for one week. The effect of these two traditional Chinese

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herbal formulas on the bioavailability and pharmacokinetic parameters of theophylline was examined. The activity of hepatic microsomal cytochrome P-450 of SD rats pretreated with Chinese herbal medicines was determined. The SD rats without pretreatment with Chinese herbal medicines were used as a control group.

Materials and Methods

Animals — Male Sprague-Dawley rats with different ages (young age: 4 ± 1 months, middle age: 10 ± 1 months, old age: 16 ± 1 months), obtained from Animal Center of Yang Ming Medical College, Taipei, R.O.C., were acclimatized in our facility for at least one month before use. They were given free access to water and chow. Before administering the Chinese herbal medicines, blood was withdrawn from the tail to determine glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and creatinine. If the values of GOT and GPT of SD rats were less than 40 and 35 unit/ml, respectively, and the value of creatinine was below 0.8 mg/ml, these SD rats were chosen for further study.

Preparation of Chinese Herbal Medicines

— Tin Chuan Tang and Hsiao Ching Long Tang were used as model Chinese herbal medicines. The herbal materials were extracted with 500 ml water three times. Three decoctions were combined, filtered and concentrated by boiling to appropriate volume (about 1 g plant material per ml extract) and finally stored at -4°C for use. The formulas for both Chinese herbal medi-

cines are listed in Table I.

Administration of Chinese Herbal Medicines and Aminophylline — The SD rats within the normal range of biofunctions were used. Each extracted herbal medicine was preadministered 1 ml/100 g orally to SD rats for one week. The dosage of each extracted decoction corresponded to approximately 10 times the normal human dosage.

SD rats with or without pretreatment with Chinese herbal medicines were catheterized from the left carotid artery to the aortal junction.⁴⁻⁶⁾ After a 1-d recovery period, each rat was given intravenously 10 mg/kg of aminophylline injection. Serial blood samples were taken through the cannula before administration and at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 h after zero time. The blood samples were centrifuged and the serum portion was pipetted and stored at -20°C until the time of analysis. The serum theophylline concentration was assayed by using a fluorescence polarization immunoassay (FPIA, Abbott Lab., Illinois), as described in our previous studies.⁷⁻⁹⁾

Bioavailability and Pharmacokinetic Analysis — Individual serum theophylline concentrations in each rat after i.v. administration of aminophylline was first fitted to Estrip program to obtain the initial estimate, and then the PCNONLIN program was used to determine the pharmacokinetic parameters of theophylline according to a two-compartment open model.^{10,11)}

Preparation of Liver Microsomes — After the last blood sampling, the rats were sacrificed

TABLE I. Formulas of Tin Chuan Tang and Hsiao Ching Long Tang

Tin Chuan Tang	Hsiao Ching Long Tang
Ginkgo Semen (2 units)	
Perillae Fructus (7.0 g)	
Ephedrae Herba (1.8 g)	Ephedrae Herba (1.8 g)
Armeniacae Semen (1.8 g)	
Pinelliae Tuber (1.8 g)	Cinnamoni Ramulus (1.8 g)
Scutellariae Radix (1.8 g)	
Farfarae Flos (7.0 g)	Asari Herba Cum Radice (3.5 g)
Glycyrrhizae Radix (1.8 g)	
Mori Radix Cortex (7.0 g)	Glycyrrhizae Radix (1.8 g)
Fresh Curcumae Longae Rhizoma (2 pieces)	Curcumae Longae Rhizoma (7.0 g)
	Typhonium Divaricatum (1.8 g)
	Schizandrae Fructus (7.0 g)

Fig. 1. Mean Serum Theophylline Concentrations after i.v. Administration of Aminophylline in the SD Rats of Different Ages

Key: Δ , young-aged rats; $\circ$, middle-aged rats; $\square$, old-aged rats. Mean $\pm$ S.D., $n = 6$. a) $p < 0.05$.

and the livers immediately removed and stored at -80°C until use. The amount of microsomal protein was measured.¹²⁾ The activity of cytochrome P-450 of SD rats was determined from the reduced CO difference spectrum with a Beckman DU-40 spectrophotometer.¹³⁾

Data were expressed as mean $\pm$ standard deviation (S.D.). One-way analysis of variance (ANOVA) tests were applied to analyze the effect of different Chinese herbal medicines on the pharmacokinetic parameters of theophylline. Statistical significance was defined as $p < 0.05$.

Results and Discussion

In order to investigate the drug interaction between Chinese herbal medicine and western drugs, we previously used the SD rat as an animal model to avoid the dangerous effects on patients.

The mean serum theophylline concentration–time profiles after i.v. administration of aminophylline in the SD rats of different ages without pretreatment with Chinese herbal medicines are shown in Fig. 1. A rather inter-animal variation in serum theophylline concentrations was also observed. It is obvious that the serum theophylline concentrations of old-aged SD rats at time 0.25, 0.5, 4, 6 and 8 h, were significantly higher than that of the middle-aged and young SD rats ($p < 0.05$). However, there was significant difference between the middle-aged and young SD rats ($p > 0.05$). The pharmacokinetic parameters of theophylline in rats of three different ages without pretreatment with Chinese herbal medicines are illustrated in Table II. Table II shows the elimination rate constant (K_{10}) and clearance (Cl) of theophylline were significantly decreased in the old-aged SD rats group, and its half-life ($t_{1/2}$) and volume of distribution (V_d) were also significantly longer and higher than that of the middle-aged and young-aged SD rats ($p < 0.05$). However, the other pharmacokinetic parameters such as $C_{\max}$, AUC , K_{12} , K_{21} , MRT and VRT of theophylline for three different ages

TABLE II. Pharmacokinetic Parameters of Aminophylline after i.v. Administration in SD Rats of Three Different Ages

Parameters	Animal ages (months)			ANOVA test
	Young (4)	Middle (10)	Old (16)	
$C_{\max}$ ($\mu\text{g/ml}$)	$105.340 \pm 26.004^a)$	92.170 ± 11.199	93.550 ± 11.453	NS ^{b)}
AUC_{0-8} ($\mu\text{g}\cdot\text{h/ml}$)	513.500 ± 122.802	411.150 ± 86.996	486.120 ± 21.088	NS
AUC_{0-24} ($\mu\text{g}\cdot\text{h/ml}$)	1842.140 ± 350.164	2282.697 ± 640.467	6055.490 ± 4716.478	NS
K_{10} (h^{-1})	0.060 ± 0.029	0.040 ± 0.016	0.030 ± 0.016	$p < 0.05^c)$
$t_{1/2}(K_{10})$ (h)	13.270 ± 4.676	16.601 ± 3.677	30.680 ± 16.065	$p < 0.05$
K_{12} (h^{-1})	2.410 ± 0.864	2.920 ± 1.347	1.410 ± 1.015	NS
K_{21} (h^{-1})	6.330 ± 2.145	5.840 ± 1.546	3.980 ± 1.026	NS
V_d (l/kg)	0.190 ± 0.063	0.280 ± 0.041	0.270 ± 0.060	$p < 0.05$
Cl (l·h/kg)	0.0113 ± 0.0037	0.0105 ± 0.0044	0.0057 ± 0.0033	$p < 0.05$
MRT (h)	2.780 ± 0.890	3.080 ± 0.829	3.550 ± 0.106	NS
VRT (h^2)	5.490 ± 0.286	4.220 ± 1.923	4.610 ± 1.245	NS

a) Mean $\pm$ S.D. ($n = 6$). b) NS: no significant difference ($p > 0.05$). c) $p < 0.05$: significant difference.

Fig. 2. Mean Serum Theophylline Concentrations after i.v. Administration of Aminophylline in Young SD Rats Pretreated with or without Traditional Chinese Herbal Medicines

Key: Δ , without treatment; $\blacktriangle$, pretreatment with Tin Chuan Tang; $\bullet$, pretreatment with Hsiao Ching Long Tang. Mean $\pm$ S.D., $n = 6$.

of SD rats were similar.

The mean serum theophylline concentration-time curves after preadministration of Ting Chuan Tang or Hsiao Ching Long Tang to young SD rats for one week are shown in Fig. 2. The statistical analysis did not show a significant difference for young SD rats with or without pretreatment with Chinese herbal medicines. The similar results were also indicated for middle-aged and old-aged SD rats. Moreover, the mean values of the estimated bioavailability and pharmacokinetic parameters obtained for young SD rats are listed in Table III. This table gave the similar bioavailability and pharmacokinetic values, suggesting that there was no statistically significant difference among these treatments ($p > 0.05$). The same results were also observed in middle-aged and old-aged SD rats whether pretreatment with Chinese herbal medicines or not. This indicates that preadministration of Tin Chuan Tang or Hsiao Ching Long Tang did not affect the pharmacokinetics of theophylline for three

TABLE III. Pharmacokinetic Parameters of Aminophylline after i.v. Administration in Young SD Rats Pretreated with or without Traditional Chinese Herbal Medicines

Parameters	No treatment	Tin Chuan Tang	Hsiao Ching Long Tang	ANOVA test
$C_{\max}$ ($\mu\text{g/ml}$)	105.340 ± 26.004^a	101.850 ± 24.792	88.850 ± 9.987	NS ^b
AUC_{0-8} ($\mu\text{g}\cdot\text{h/ml}$)	513.500 ± 122.802	555.690 ± 59.508	476.680 ± 236.129	NS
AUC_{0-24} ($\mu\text{g}\cdot\text{h/ml}$)	1842.140 ± 350.164	2789.940 ± 1878.701	2918.520 ± 1315.107	NS
K_{10} (h^{-1})	0.060 ± 0.029	0.050 ± 0.030	0.040 ± 0.016	NS
$t_{1/2}(K_{10})$ (h)	13.270 ± 4.676	18.990 ± 11.146	23.420 ± 11.340	NS
K_{12} (h^{-1})	2.410 ± 0.864	2.030 ± 0.880	1.870 ± 1.064	NS
K_{21} (h^{-1})	6.330 ± 2.145	5.330 ± 1.528	4.750 ± 1.841	NS
V_d (l/kg)	0.190 ± 0.063	0.170 ± 0.041	0.200 ± 0.043	NS
Cl (l·h/kg)	0.0113 ± 0.0037	0.0104 ± 0.0051	0.0101 ± 0.0032	NS
MRT (h)	2.780 ± 0.890	3.660 ± 0.308	2.560 ± 1.329	NS
VRT (h^2)	5.490 ± 0.286	5.670 ± 0.136	5.350 ± 0.794	NS

^a) Mean $\pm$ S.D. ($n = 6$). ^b) NS: no significant difference ($p > 0.05$).

TABLE IV. Effect of Age on the Activity of Hepatic Microsomal Cytochrome P-450 of SD Rats

Age	Activity of cytochrome P-450 (nmol/mg protein)			ANOVA test
	No treatment	Tin Chuan Tang	Hsiao Ching Long Tang	
Young	0.5062 ± 0.0777^a	0.4851 ± 0.2451	0.4950 ± 0.1082	NS ^b
Middle	0.5431 ± 0.1011	0.4461 ± 0.1463	0.4120 ± 0.1699	NS
Old	0.4914 ± 0.1296	0.5288 ± 0.2337	0.5061 ± 0.1342	NS

^a) Mean $\pm$ S.D. ($n = 6$). ^b) NS: no significant difference ($p > 0.05$).

different ages of SD rats.

The effect of age on the activity of hepatic microsomal cytochrome P-450 of rats used is shown in Table IV. No correlation was observed between age and activity of cytochrome P-450, although some reports have shown the decline in hepatic mixed-function oxidase enzyme systems with increasing age in rodents.^{14,15)}

Theophylline is extensively metabolized *via* biotransformation by the hepatic microsomal cytochrome P-450 mediated enzyme system,¹⁶⁾ its clearance and disposition mainly depend on liver drug-metabolizing enzyme activity. There are two major metabolic pathways (N-demethylation and C-8 oxidation) involved in microsomal metabolism of theophylline. These pathways can be enhanced or inhibited by co-medication. The western drugs such as cimetidine, enoxacin, erythromycin or oral contraceptives can inhibit theophylline N-demethylation, resulting in an increase in serum theophylline concentration; whereas carbamazepin can stimulate the oxidation and/or N-methylation, leading to a decrease of elimination half-life of theophylline.^{17–21)} Although age-related differences in theophylline metabolism have been reported to be decreased, increased or unchanged,^{22–24)} it is difficult to evaluate the impact of age on theophylline kinetics since the inclusion of many confounding variables such as sexes, disease states, smoking habit and co-medication.

The old-aged SD rats without pretreatment with Chinese herbal medicines increased their serum half-life of theophylline. This seems to be an age-related difference although there was no age-associated difference in V_d , Cl and $t_{1/2}$ of theophylline for normal elderly subjects as has been reported.²⁵⁾ After one week pre-administration of Tin Chuan Tang or Hsiao Ching Long Tang to each different age of SD rats, there was no significant difference in serum theophylline concentration and pharmacokinetic parameters for each group. This implies that these Chinese herbal medicines did not cause any alteration to body metabolism after one week pretreatment. This result was confirmed by the activity of hepatic cytochrome P-450, as tabulated in Table IV. It indicates that there was no

age-related or Chinese herbal medicine-related difference in the activity of hepatic cytochrome P-450 of SD rats. These results are consistent with those of Rawlins *et al.*²⁶⁾ in which no age-related decline was found in either the cytochrome P-450 content or its specific activity. Thus the statistical difference of some theophylline pharmacokinetic parameters (Table II) between the different ages of SD rats, may be attributed to the reduced hepatic blood flow and liver volume. It has been suggested that a reduction in hepatic blood flow plays a more important role in the decline in metabolism without any change occurring in oxidative enzyme activities with aging.^{27–30)} In this study, the decline in hepatic blood flow in the elderly SD rats, coupled with a reduction in liver volume, may have decreased the elimination rate constant and clearance of theophylline in the elderly rats.

In conclusion, pretreatment with Tin Chuan Tang or Hsiao Ching Long Tang to SD rats of different ages did not affect the pharmacokinetic parameters of theophylline or aminophylline and no correlation seemed to be observed between age and the activity of hepatic microsomal enzyme system.

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