EFFECT OF CURCUMIN ON THEOPHYLLINE PHARMACOKINETICS IN RABBITS

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Theophylline is a widely used bronchodilator with narrow therapeutic index and undergoes extensive metabolism by CYP1A2. Curcumin is a natural polyphenol with various beneficial biological activities and was shown to be a potent inhibitor of CYP1A2. The purpose of this study was to investigate the effect of curcumin on the pharmacokinetics of theophylline in rabbits. The result showed that curcumin only significantly increased the absorption half-life of theophylline from 0.20 ± 0.02 h to 0.36 ± 0.05 h, other pharmacokinetic parameters were not affected. It indicated that coadministration of curcumin delayed the onset of theophylline but would not result in the acute intoxication of theophylline.

Key words: Curcumin, Theophylline, Pharmacokinetics, Interaction.

INTRODUCTION

Theophylline is a widely used bronchodilator and characterized by a narrow therapeutic index with high interindividual variation in plasma concentration[1,2]. Plasma concentrations below 10 μg/ml may be associated with inadequate therapy while those above 20 μg/ml potentially cause a variety of serious side effects in humans[3]. Theophylline is thought to be primarily oxidized by two isozymes CYP1A2 and CYP2E1 of the hepatic microsomal mono-oxygenase system via N-demethylation and 8-hydroxylation pathways, respectively[4-6].

Curcumin is an active constituent of *Curcuma longa* L., *Curcuma aromatica* Salisb. and *Curcuma zedoaria* (Berg.) Rosc., which were used in clinical Chinese medicine as aromatic stomachic, choleretic and for the treatment of menstruation irregularity. In recent decades, pharmacological studies reported that curcumin possessed various

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promising biological activities: hypocholesteremic[7], anti-inflammatory[8,9], anti-platelet[10], antioxidant[11,12], cancer chemopreventive[13-17], anticancer[18-20], antimutagenic[21,22], and anti-HIV[23-25], etc. It was reported by National Cancer Institute of U.S.A. that the preclinical toxicity testing demonstrated the safety of curcumin and currently curcumin is in the stage of clinical trials for AIDS patients. Based on its various beneficial activities and low toxicity, curcumin was currently marketed as a dietary supplement and could be a compound with potential clinical application in the future. On the other hand, curcumin was found to be a potent inhibitor of rat liver P450 1A1/1A2[26], therefore, there is a possibility that curcumin might affect the metabolism of theophylline if curcumin is concomitantly administered. This study attempted to investigate the effect of curcumin on theophylline pharmacokinetics in rabbit.

MATERIALS AND METHODS

Materials

Theophylline, caffeine, curcumin and glycofurol were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. Curcumin was further purified by Silica gel column chromatography. All other chemicals and solvents used were of analytical grade or HPLC quality. Milli-Q plus water (Millipore, Bedford, MA, U.S.A.) was used for all preparations.

Animal treatment

Six male New Zealand white rabbits, weighing 2.1 ~ 2.6 kg, were used throughout this study. Animals were housed in a 12-h light-dark, constant temperature environment prior to study. All rabbits were fasted for 1 day before the experiment. Water was supplied *ad libitum*. In this study, the pharmacokinetic experiments were performed with six rabbits in a randomized crossover design. For treatment with theophylline alone, rabbits were given a vehicle (glycofurol, 2 ml/kg) right before theophylline administration. Theophylline was given as aqueous solution (8 mg/ml) at a dose of 25 mg/kg. For combined treatment, the rabbits were fed with freshly prepared curcumin solution at a dose of 200 mg/kg in glycofurol (100 mg/ml) before theophylline administration (25 mg/kg). One week was allowed for wash-out. Drug administration was carried out via gastric gavage throughout the study.

Pharmacokinetic analysis

Blood sample (1 ml) were withdrawn via right ear vein at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h after administration of theophylline. The blood was centrifuged for 15 min at $9860 \times g$ and the serum samples obtained were stored at $-20°C$ until analysis.

Preparation of calibration curve

Theophylline was accurately weighed and dissolved in water to give various concentrations within the ranges of 5.0 ~ 250.0 µg/ml. Twenty µl of theophylline solution was spiked into 180 µl serum to afford serum standards consisting of 0.5, 2.5, 5.0, 10.0 and 25.0 µg/ml. To 100 µl of serum standard, 400 µl of acetonitrile containing 5.0 µg/ml of caffeine as the internal standard was added. The mixture was vortexed for 30 sec and then centrifuged at $9860 \times g$ for 15 min, the clear supernatant was removed and evaporated to dryness by blowing nitrogen. The residue was reconstituted with 100 µl of mobile phase, and 20 µl of this solution was subjected to HPLC analysis. The peak ratios (theophylline to caffeine) of serum standard were determined in duplicates. The calibration curves were drawn after linear regression of the peak-area ratios with concentrations of theophylline.

Instrumentation and HPLC conditions

Theophylline concentrations in serum were determined by high-performance liquid chromatography. The HPLC apparatus included one pump (LC-6AD, Shimadzu, Japan) and a chromatopac (C-R6A, Shimadzu, Japan). The assay employed an ODS-2 column (4.6×250 mm, 5 µm). Caffeine was used as the internal standard. Chromatographic separation was achieved by using a mobile phase consisting of methanol and water (20:80, v/v) with a flow rate of 1.0 ml/ min. An UV detector was set at 270 nm.

Sample preparation

Sample was prepared by adding 400 µl of acetonitrile solution containing 5.0 µg/ ml of internal standard to 100 µl of serum. After vortexed for 30 sec and then centrifuged at 9860 x g for 15 min, the clear supernatant was transferred to another microtube and evaporated to dryness by blowing nitrogen. The residue was reconstituted with 100 µl of mobile phase, and 20 µl of this solution was subjected to HPLC analysis.

Validation of assay method

The precision and accuracy of the assay method was evaluated by the intra-day and inter-day reproducibilities of triplicates of the same serum standards over a period of three days. The recovery of the method was obtained by spiking theophylline into blank serum and water, respectively, to afford concentrations of 25.0, 5.0, 0.5 µg/ml and comparing the detected concentrations between serum and water.

Data analysis

The theophylline serum concentrations obtained from HPLC were fitted to a one-compartment open model equation with the aid of the program WINNONLIN version 1.1 (SCI software, Statistical Consultants, Inc., Lexington, KY). The statistical significance of the differences between the pharmacokinetic parameters of two treatments mentioned above was estimated by paired Student's t-test. A p value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

This assay method developed and validated in our laboratory was simple by using acetonitrile for protein denaturization and different from a previous method using liquid-liquid partition[27]. The HPLC chromatogram of theophylline with internal standard (caffeine) in a serum sample was shown in Fig. 1. The linearity of calibration curve $Y = 0.194 X + 0.046$ (r = 0.999) was found very good within the range $0.5 \sim 25.0$ μg/ml. The intraday and interday precision analysis indicated CVs were below 4.2 % and 4.8 %, respectively, and the relative errors of intraday and interday assays were below 3.3 % and 3.4 %, respectively as shown in Table 1. The recoveries of theophylline from serum were almost quantitative for all concentrations tested as shown in Table 2. The LOQ (limit of quantitation) was 0.5 μg/ml and the LOD (limit of detection) was 0.2 μg/ml.

The commercially available curcumin contained only 70% of curcumin, therefore, silica gel column chromatographic separation was carried out for the purification. Curcumin was eluted with $CHCl_3$. Curcumin was insoluble in water and glycofurol was found to be the best vehicle for dissolving high dose of curcumin. Because of the instability of curcumin, the solution was prepared freshly right before administration.
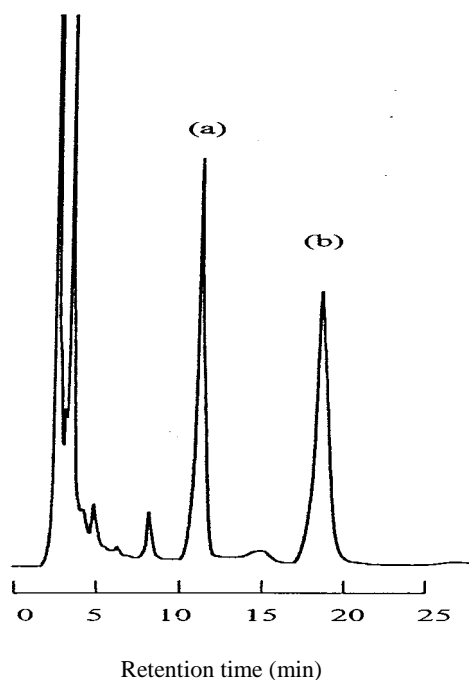

Fig. 1. HPLC chromatogram of theophylline (a) in serum sample. Caffeine (b) was the internal standard.

Table 1. Intraday and interday analytical precision and accuracy of theophylline

Conc. (μg/ml)	Intraday		Interday	
	Precision mean ± S.D. (C.V.%)	Accuracy (%)	Precision mean ± S.D. (C.V.%)	Accuracy (%)
25.0	25.2 ± 0.2 (0.9)	1.0	25.3 ± 0.4 (1.5)	1.1
10.0	10.1 ± 0.1 (1.3)	1.4	10.1 ± 0.1 (0.8)	0.7
5.0	5.0 ± 0.1 (1.1)	0.8	5.0 ± 0.1 (1.0)	0.7

| 2.5 | $2.5 \pm 0.0_3$ | (1.1) | 0.4 | $2.5 \pm 0.0_4$ | (1.7) | −0.3 |
| 0.5 | $0.5 \pm 0.0_2$ | (4.2) | −3.3 | $0.5 \pm 0.0_2$ | (4.8) | −3.4 |

n=3

Table 2. Recovery (%) of theophylline from serum

Conc. spiked (μg/ml)	Conc. detected in serum Mean ± S.D.	Conc. detected in water Mean ± S.D.	Recovery (%)
25.0	25.4 ± 0.2	25.6 ± 0.2	99.4
5.0	$5.1 \pm 0.0_4$	5.1 ± 0.2	99.1
0.5	$0.5 \pm 0.0_1$	$0.4 \pm 0.0_2$	103.5

n = 3
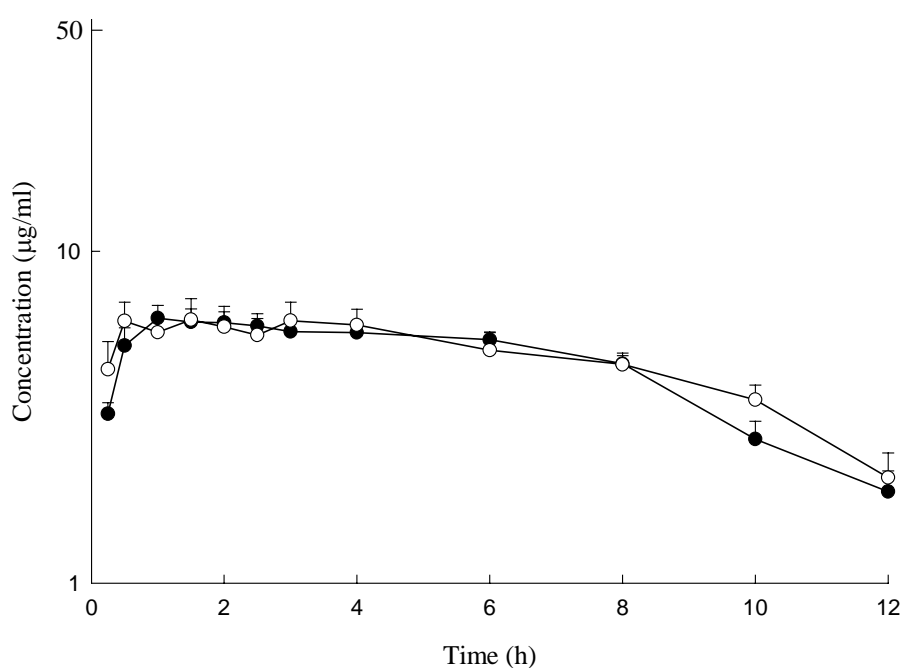
Fig. 2. Mean serum concentrations of theophylline in six rabbits after oral administration of theophylline alone (○) or coadministration with curcumin (●)

Table 3. Pharmacokinetic parameters of theophylline in six rabbits after giving theophylline alone and coadministration with curcumin

Parameters	Theophylline alone	Curcumin + Theophylline
Volume/F (l)	8.99 ± 1.30	7.60 ± 0.95
K_{01} (h^{-1})	3.77 ± 0.66	2.23 ± 0.46
K_{10} (h^{-1})	0.08 ± 0.02	0.11 ± 0.02
AUC[a] (μg/ml• h)	103.94 ± 13.47	85.08 ± 8.57
K_{01}-HL[b] (h)	0.20 ± 0.02	0.36 ± 0.05*
K_{10}-HL[c] (h)	10.75 ± 1.71	8.00 ± 1.68
T_{max} (h)	1.14 ± 0.10	1.58 ± 0.16
C_{max} (μg/ml)	6.81 ± 1.09	6.96 ± 0.64

[a] Area under plasma concentration – time curve to time infinity.

[b] The half – life of drug in the absorption phase.

[c] The half – life of drug in the elimination phase.

Results are given as mean $\pm$ S.E. (n = 6)

[*] $p < 0.05$

Pharmacokinetic characteristics obtained for theophylline following oral administration of 25 mg/kg to six rabbits in the present study correspond to those found by other investigators[28].   The peak serum theophylline concentration (C_{max}) of 6.81 ± 1.09 µg/ml occurred at 1.14 ± 0.10 h after oral administration of theophylline alone.

The influence of concomitant administration of curcumin on theophylline disposition was demonstrated by the serum concentration profiles as shown in Fig. 2 and the pharmacokinetic parameters were shown in Table 3.   For both treatments, theophylline was absorbed rapidly, and the mean concentration - time profiles were very close between two treatments. Statistical analysis indicated only the difference of absorption half lives between two treatments was significant. Curcumin increased the absorption half life of theophylline from 0.20 ± 0.02 h to 0.36 ± 0.05 h. It indicated that curcumin would delay the onset of theophylline. Other pharmacokinetics parameters were not significantly affected by coadministration of curcumin. Although CYP1A2 is the main isozymes thought to catalyze theophylline metabolism and curcumin demonstrated potent inhibition on rat liver CYP1A1/1A2, our present study indicated the effects of concomitant administration of curcumin on theophylline systemic clearance were generally negligible. This could be due to the poor absorption of curcumin from the gut[29].   In spite of the fact that numerous studies reported the *in vitro* or *in vivo* bioactivities of curcumin, until recently no information about its pharmacokinetics or bioavailability was available in the literature. Our *in vivo* study indicated that no curcumin was detected in the rabbit serum after oral administration of curcumin (200 mg/kg).

In conclusion, this *in vivo* study failed to show a significant increase of theophylline exposure after coadministration of curcumin. It indicated that coadministration of curcumin would not resulted in the acute intoxication of theophylline.

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薑黃素於兔體中對茶鹼動態學之影響

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茶鹼(Theophylline)為一臨床常用之氣管擴張劑，其治療指數狹窄，主要由 CYP1A2 代謝。薑黃素(Curcumin)為一具有多種優越活性的天然多酚化合物，對 CYP1A2 具強力的抑制作用。本研究探討薑黃素在兔體內對茶鹼動態學之影響。結果顯示薑黃素僅增加其吸收半生期，而對其他的藥物動力學參數沒有顯著之影響。因此併服薑黃素會延緩茶鹼生效，但應不會導致茶鹼急性中毒。

關鍵詞：薑黃素，茶鹼，動態學，交互作用。

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