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건강한 한국인 자원자에서 theophylline 약동학에 미치는 Cimetidine의 효과

권준택 · 채석 · 손동렬 · 염윤기 · 김형기
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Effect of Cimetidine on Pharmacokinetics of Theophylline in Healthy Korean Volunteers

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Theophylline은 methylxanthine계열 기관지확장제중 가장 강력하며 기관지 천식이나 만성 폐쇄성 호흡기 질환환자에서 사용된다. Theophylline은 치료지수가 5-20 mg/L로 매우 좁은 치료지수를 갖는 약물이다. Cimetidine과 theophylline은 주로 간에 존재하는 cytochrome P450 (CYP) 효소에 의해 대사되며 theophylline은 유전적 다형성이 보고된 바 있는 CYP1A2에 의해 대부분 대사된다. 본 연구는 theophylline의 약동학에 미치는 cimetidine의 영향을 평가하고 *CYP1A2* 유전적 다형성의 영향유무를 검증하였다. 8명의 건강한 비흡연자인 한국인 자원자를 모집하여 공개, 2기 교차시험을 실시하였다. 8명의 자원자에게 1기 시험의 첫날 100 mg의 aminophylline을 경구로 단회 투여하였으며 약물투여 후 36시간까지 채혈하여 혈장을 보관하였다. 공혈장 채취를 위해 채혈한 혈액중 일부를 가지고 유전자형 검색을 실시하였다. 1주일의 휴약기를 거친 후 2기 시험을 진행하였다. 2기 시험의 첫날 같은 용량의 aminophylline과 200 mg의 cimetidine을 같이 경구로 단회 투여하였다. 혈장 중 theophylline농도는 고성능 액체 크로마토그래피를 이용하여 측정하였으며 1기와 2기 시험에서 각종 약동학적 경수를 컴퓨터를 이용하여 계산하였다. 8명의 자원자에서 PCR-RFLP를 이용하여 유전자형 검색을 실시하였다. 8명(남자 4명, 여자 4명)의 자원자에서 약물과 관련한 약물이상반응은 시험기간동안 발생하지 않았다. 약동학적 분석에서 theophylline의 혈장 농도 곡선하 면적(AUC)와 최고혈중농도(C_{max})가 cimetidine과 theophylline을 동시에 투여하였을 때 통계적으로 유의하게 증가하였으며 경구 청소율(CL/F)은 유의하게 감소하였다. 8명의 *CYP1A2* 유전자형 검색에서 돌연변이 유전자형은 발견하지 못하였으며 *CYP1A2*1C* 유전자형 검색에서 모두 (G/G) homozygote였으며 *CYP1A2*1F* 유전자형 검색에서는 5명이 (A/A) homozygote이고 3명이 (A/C) heterozygote였다. 따라서 theophylline대사에 *CYP1A2*유전자형에 따른 대사능의 차이는 관찰할 수 없었다. 이상의 결과를 요약하면 theophylline의 약동학은 cimetidine에 의해 유의한 차이를 보였으며 *CYP1A2*유전자형에 따른 영향은 관찰할 수 없었다. *CYP1A2*유전자형에 따른 생체내 대사능을 관찰하는 실험이 향후 이루어 져야 할 것으로 사료된다.

□ Keywords – cimetidine, theophylline, CYP1A2, drug interaction, pharmacokinetics.

An interaction is known to occur when the effects of one drug are changed by the presence of another drug, food, drink or by some environmental chemical agent. The outcome may be harmful if the interaction causes an increase or decrease in the efficacy and toxicity of the drug. The more drugs a patient takes the greater the likelihood that an adverse reaction will occur. One hospital study reported that the rate of adverse reaction was 7% in those taking 6-10 drugs but 40% in those taking 16-20 drugs which represents a disproportionate increase.[1)]

Theophylline has been used in the treatment of asthma and chronic obstructive pulmonary disease (COPD) for over 60 years and remains one of the most widely prescribed drugs for the treatment of airway diseases. Among the methylxanthine drugs, theophylline is most effective bronchodilator, and it has been shown to be effective repeatedly on relieving airflow obstruction in acute asthma as well as on reducing the severity of symptoms. Theophylline base is only slightly soluble in water, so it has been administered as several salts containing varying amount of theophylline base. The two

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commonly used theophylline salts are aminophylline, which contains 86% theophylline by weight; and oxtriphylline, which contains 64%. Theophylline has very narrow therapeutic index. Improvement in pulmonary function is correlated with plasma concentration in the range of 5-20 mg/L.

Theophylline is metabolized in human by the hepatic enzyme CYP1A2. CYP1A2 is well known for its role in the metabolic activation of environmental and food-borne carcinogens, including arylamines and heterocyclic amines and thus is a key enzyme in chemical carcinogenesis.[2] CYP1A2 is also responsible for the oxidative metabolism of commonly used drug including imipramine, caffeine, paracetamol, phenacetin, tarcrine, mexiletine and clozapine.[3] In vivo measurement of CYP1A2 activity in several human population has shown wide interindividual variability, and population studies have reported either unimodal[4], bimodal or trimodal distributions of CYP1A2 activity.[5-7] The variation may be due to the enzyme induction or inhibition by other drugs or environmental exposure to a large extent. The wide interindividual variation and possible polymodal distribution of CYP1A2 activity are suggestive of polymorphic control of enzyme activity.

Cimetidine, a histamine H_2-receptor blocking drug, is also widely prescribed in patient with peptic ulcer disease and related gastrointestinal complaints. Considering that cimetidine is over-the-counter drug in many countries, there is possibility of coadministering with theophylline or other drugs. Cimetidine is reported to reduce the clearance of warfarin[8], diazepam[9], theophylline[10,11] and other drugs[12], which are metabolized on the hepatic mixed-function oxidase system.

In the present study, drug interaction between cimetidine and theophylline and each *CYP1A2* genotype was evaluated in non-smoking 8 healthy Korean volunteers.

Materials and Methods

Study designs and subjects

This study was open-label, two-period crossover study consisting of one period of monotherapy and one period of coadministered therapy separated by 1 week washout period. The clinical protocol was reviewed and approved by local ethics committee which was certified by Korean Food and Drug Administration(KFDA). All subjects submitted their written informed consents.

Pretrial screening was performed within 2 weeks from the first study period. Subject underwent a full medical examination including medical history, vital signs and laboratory analyses, concomitant illness/medication history. Women subjects had a pregnancy test in addition. Standard exclusion criteria included various listed conditions, such as renal, cardiac, respiratory, hepatic, metabolic, neurologic, or psychiatric disorder, and pregnant state, or if they had taken any drug within 7 days before the study. Food and beverage containing xanthine were prohibited during the study.

On the morning of day 1 in first period, each subject received a single 100 mg tablet of aminophylline(Daewon Pharmaceuticals, Korea). After 1 week of washout period, same subject received a same 100 mg tablet of aminophylline and 200 mg tablet of cimetidine(Yuhan Pharmaceuticals, Korea). All blood samples were collected from an indwelling venous catheter or by venipuncture. On day 1 of first period, blood samples for determination of CYP1A2 genotype were collected before the administration of study drugs. For measurement of plasma theophylline levels, blood samples were collected immediately before dosing and at 0.2, 0.4, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hours after the dosing. Blood samples were centrifuged for 20 minutes at 1500g. Plasma was collected and stored at -80^{o}C until assay.

In the present study, 8 healthy, non smoking Korean adults were enrolled.

Sample preparation

For the determination of the theophylline concentration, the plasma sample (500 μl) was acidified by adding 100 μl of 1N HCl. After the addition of 7-(β-hydroxy propyl) theophylline (50 μg/ml) 50 ml as an internal standard, the mixture was extracted with 5 ml of diethyl ether by shaking for 10 minutes. After centrifugation at 1,000 g for 10 min, the organic fraction

was evaporated with a vacuum evaporator at 40°C. The residue was reconstituted in 200 μl of the mobile phase and then an 50 μl of the sample was injected to HPLC.

Apparatus and chromatographic condition

Chromatography was performed using instruments (Shiseido Nanospace SI-1 HPLC system, Shiseido, Japan) equipped with autosampler, column thermostat, and Shiseido Nanospace SI-1 UV detector. The system was controlled through a HPLC interface module and a personal computer. Data acquisition was performed by dsChrome™ software. Separations were achieved by using a Shiseido Capcell Pak ODS column, 5 μm, 150x 2.0 mm I.D. (Shiseido, Tokyo, Japan). Mobile phase consisted of sodium phosphate buffer (0.02M, pH 3) and acetonitrile (90:10, v/v). Mobile phase was filtered through a Millipore filter (0.45 μm) and was degassed prior to use. Column temperature was maintain at 30°C by a column thermostat and flow-rate was kept at 200 μl/min. Column effluent was quantified at a wavelength of 280 nm. Theophylline was quantified by comparison with the standard curves using the peak area ratios to internal standard.

Pharmacokinetic and statistical analysis

Noncompartmental methods were used to determine pharmacokinetic parameters. Maximum plasma concentration (C_{max}) and corresponding time to C_{max} (T_{max}) were obtained through direct observation on plasma concentration-time curves. Area under the plasma concentration-time curves from time zero to time of the last quantifiable concentrations (AUC_t) were calculated using linear trapezoidal approximation. The elimination rate constant (k_e) was calculated from semi-log regression on the terminal phase of the plasma concentration-time curve. Plasma half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_e$. The apparent clearance was calculated using the formula CL/F = dose/AUC (F is oral bioavailability). The apparent volume of distribution was estimated from the terminal phase of the plasma concentration-time curve, $Vd/f = dose/k_e \times AUC$

The pharmacokinetic parameters C_{max}, $t_{1/2}$, AUC_t, AUC_{inf}, Vd/F, T_{max} and CL/F were compared using nonparametric Wilcoxon matched-pairs signed-rank test (SPSS™). Data are expressed as mean±standard deviation(SD) and statistical significance was set at $p<0.05$.

Genotype of *CYP1A2*

Genomic DNA was isolated and purified from peripheral leukocytes of each subject by conventional methods.[13] The primers were obtained from a DNA synthesizer laboratory with documented protocol.[14] The 5'-flanking region and intron I *CYP1A2* polymorphisms were analyzed according to Nakajima *et al*.[15] and Sachse *et al*.[16], respectively. Briefly, the intron I sequence was amplified by PCR using 500 ng of genomic DNA using following primers: forward primer P1f, 5'-CAACCCTGCCAATCTCAAGCAC-3' (located on exon 1) and reverse primer R, 5'-AGAAGCTCT-GTGGCCGAGAAGG-3' (located on exon 2). PCR was performed with an initial denaturation for 4 minutes at 94°C followed by 35 cycles of 1 minute at 94°C, 30s at 60°C, 1 minute at 72°C, and a terminal extension for 4 minutes at 72°C. PCR products were further digested with A*pa* I (Fermentas Inc., Hanover, MD) The PCR products were sepatated by electroporesis on agarose gel and visualized by ethidium bromide staining under UV light.

Results

Demographic characteristics

Eight healthy, non-related Korean adults aged 20 to 25 years (21.4±2.0) were enrolled. They were all non-smokers. Among eight subjects, four were men and the others were women. Mean weight and height were 58.4 ±14.7 kg and 167.6±12.4 cm, respectively. Throughout the whole study period, there was no adverse event or any clinically relevant change of laboratory test.

Pharmacokinetic characteristics

No interference was observed in human plasma and limit of quantification for theophylline was determined to be 0.1 μg/ml (S/N ratio : 10). The intra- and inter-

day coefficient of variation were less than 8.1% and 19.0%, respectively.

Mean plasma theophylline concentration time profiles with or without cimetidine (200 mg) tablet coadministration are presented in the Fig. 1. There was a significant overall increase of theophylline concentration after cimetidine coadministration. A summary of theophylline's pharmacokinetic characteristics in the presence or absence of cimetidine pretreatment is presented in the Table 1. The mean theophylline C_{max} achieved after a single oral dose of aminophylline 100 mg in the absence of cimetidine was 3.07±0.93 μg/ml, compared with 5.72±1.51 μg/ml in the presence of cimetidine ($p<$ 0.05). Except T_{max}, all the pharmacokinetic parameters of theophylline showed statistically significant difference between monotherapy and polytherapy.

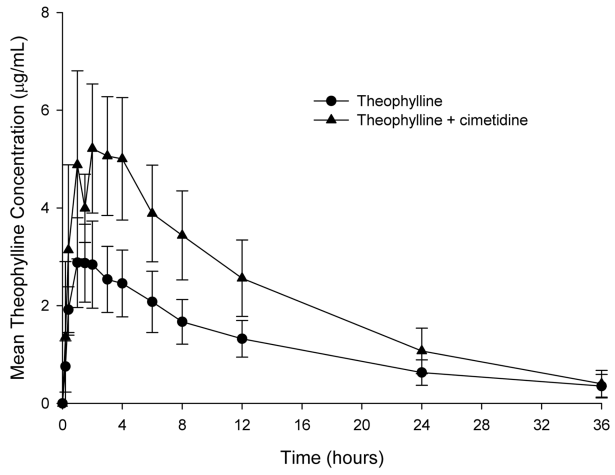

Fig. 1. Plasma theophylline concentration versus time (mean±SD) in 8 healthy volunteers administered theophylline 100 mg in the absence and presence of cimetidine 200 mg coadministration.

CYP1A2 genotype

Genomic DNA from 8 subjects were analyzed according to the *CYP1A2* single nucleotide polymorphism(SNP) located in the 5'-flanking region and intron I. Genotype frequencies for the 5'-flanking region polymorphism found in the various ethnic populations were published. The genotypic frequencies of the wild-type(G/G) in *CYP1A2*1C* was 100%, and the wild-type(A/A) in *CYP1A2*1F* and heterozygote(A/C) were 62.5% and 37.3%, respectively. The summary of allele and genotypic frequency was shown in Table 2. The mutant of *CYP1A2* was not identified in 8 subjects.

Discussion

Among the many CYP families, the metabolism of xenobiotics in humans is mainly catalyzed by isoforms belonging to the CYP1, CYP2, and CYP3 families.[17] Theophylline is known to be metabolized by N-demethylation to form 3-methylxanthine and 1-methylxanthine with subsequent conversion to 1-methyluric acid by xanthine oxidase, and by 8-hydroxylation to 1, 3-deme-

Table 1. Mean (±SD) pharmacokinetic variables for theophylline in the absence and presence of cimetidine in 8 healthy subjects.

	Theophylline only	Theophylline + Cimetidine	*p*-value
AUC_t (μg·h/ml)	40.54±11.85	73.38±22.39	0.012
AUC_{inf} (μg·h/ml)	48.47±15.59	79.78±24.30	0.012
C_{max} (μg/ml)	3.07±0.93	5.72±1.51	0.012
T_{max} (hour)	1.31±0.37	2.00±1.20	0.140
CL/F (L/h)	1.83±0.76	1.08±0.36	0.012
$t_{1/2}$ (hour)	14.59±4.36	10.25±2.53	0.012
Vd/F (L)	38.16±17.10	15.71±5.35	0.012

AUC_t = area under the concentration-time curves from time zero to time of last quantifiable concentration; AUC_{inf} = area under the concentration-time curves from time zero to infinite time; C_{max} = maximum plasma concentration; T_{max} = time to C_{max}; CL/F = oral clearance; $t_{1/2}$ = half-life; Vd/F = apparent volume of distribution.

Table 2. Genotype distribution of the subjects

	Allele frequency		Genotype distribution (%)		
	1	2	1\1	1\2	2\2
CYP1A2*1C[a]	16	0	8	0	0
	(1)	(0)	(1)	(0)	(0)
CYP1A2*1F[b]	13	3	5	3	0
	(0.8125)	(0.1875)	(0.625)	(0.375)	(0)

[a]Allele 1 is G, allele 2 is A
[b]Allele 1 is A, allele 2 is C

thyluric acid.[18,19] Several cytochrome P-450 isoenzymes including CYP1A2 are thought to be involved in these metabolic pathways.[20] In the present study, genetic polymorphisms of CYP1A2 were further analyzed after pharmacokinetic study to evaluate the possible influence of genetic factor as a variability in metabolism. As shown in results, *CYP1A2* is subdivided to *CYP1A2*1C* and *CYP1A2*1F*. These two genotypes were recently found and *CYP1A2*1C* mutant decrease metabolic capacity and *CYP1A2*1F* mutant has high inducibility of metabolic capacity. Mutant frequencies of *CYP1A2*1C* in 116 Japanese subjects and *CYP1A2*1F* in 236 Caucasian subjects were 5.2% and 10.0%, respectively.[15,16] The present study was not focused on *CYP1A2* genotypic frequency but on the drug interaction. With a small number of subjects enrolled in this study, we could not find the meaningful genotypic influence in Korean population for *CYP1A2* polymorphism. To elucidate the influence of genetic polymorphism of *CYP1A2* on disposition of theophylline in a Korean population, further evaluation regarding not only pharmacogenetic study, but well-designed drug interaction study in accordance with various genotype of *CYP1A2* is mandatory.

In the present study, results showed that cimetidine obviously inhibit the theophylline disposition. The cimetidine pretreatment significantly increased mean AUC_t and C_{max} of theophylline. Cimetidine significantly decrease the oral clearance and shorten the elimination half-life of theophylline. These findings, however, contrast with those of Ohashi et al.,[21] who found that cimetidine prolonged elimination half-life in nine healthy male subjects. The discrepancy between their findings and ours may result partly from subject selection and dosage of theophylline and cimetidine. They administered 400 mg of cimetidine and 200 mg of theophylline.

Nix *et al.*[22] reported that interaction between theophylline and cimetidine is dependant on cimetidine dose. They conducted a study with multiple dose of cimetidine and showed that dose-related examinations of this interaction. But in the present study, single oral dose of cimetidine significantly inhibited the theophylline metabolism. Considering that cimetidine is over-the-counter drug in many countries, there are significant potential to evoke an adverse drug reaction of theophylline in asthma or COPD patients.

Summary

The purpose of the present study was to investigate the effect of cimetidine on theophylline pharmacokinetics in Korean healthy normal subjects.

Eight subjects were enrolled and open label, two period cross-over study was conducted without significant drug related adverse reactions. Cimetidine seemed that significantly inhibited the metabolism of theophylline, oral clearance decreased significantly when cimetidine was coadministered. Coadministered cimetidine increased AUC_t and C_{max} of theophylline.

All subjects were genotyped using PCR-RFLP methods to evaluate the differences in metabolic capacity in accordance with *CYP1A2* genotypes, but no mutant genotype was found. This suggests that metabolic capacities were not significantly affected by *CYP1A2* genotypes among subjects.

In conclusion, disposition of theophylline was significantly affected by coadministered cimetidine. Further evaluation with well-designed drug interaction study in accordance with various genotype of *CYP1A2* is needed.

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