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 ease the symptoms. Among the methylxanthine drugs, theophylline is considered to be most effective bronchodilator, and it has been shown to ease the symptoms. Among the methylxanthine drugs, theophylline is considered to be most effective bronchodilator, and it has been shown to ease the symptoms.
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. CYP1A2 is also responsible for the oxidative metabolism of commonly used drug including imipramine, caffeine, paracetamol, phenacetin, tarcrine, mexiletine and clozapine. In vivo measurement of CYP1A2 activity in several human population has shown wide interindividual variability, and population studies have reported either unimodal, bimodal or trimodal distributions of CYP1A2 activity. 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 H2-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, diazepam, theophylline and other drugs, 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 1500 g. Plasma was collected and stored at -80°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×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. The primers were obtained from a DNA synthesizer laboratory with documented protocol. The 5'-flanking region and intron I CYP1A2 polymorphisms were analyzed according to Nakajima et al. and Sachse et al., respectively. Briefly, the intron I sequence was amplified by PCR using 500 ng of genomic DNA using following primers: forward primer P1f, 5'-CAACCCTGCAATCTCAACGCA-3' (located on exon 1) and reverse primer R, 5'-GAAGCTCCTGTGGCCGAGAAGG-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 Apa I (Fermentas Inc., Hanover, MD) The PCR products were separated by electrophoresis 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\textsubscript{max} achieved after a single oral dose of aminophylline 100 mg in the absence of cimetidine was 3.07\pm0.93 \mu g/ml, compared with 5.72\pm1.51 \mu g/ml in the presence of cimetidine (p<0.05). Except T\textsubscript{max}, all the pharmacokinetic parameters of theophylline showed statistically significant difference between monotherapy and polytherapy.

**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.\textsuperscript{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-

![Image](image_url)

**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.**

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

<table>
<thead>
<tr>
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<th>Theophylline only</th>
<th>Theophylline + Cimetidine</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>AUC\textsubscript{t} (\mu g·h/ml)</td>
<td>40.54\pm11.85</td>
<td>73.38\pm22.39</td>
<td>0.012</td>
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<tr>
<td>AUC\textsubscript{inf} (\mu g·h/ml)</td>
<td>48.47\pm15.59</td>
<td>79.78\pm24.30</td>
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<tr>
<td>C\textsubscript{max} (\mu g/ml)</td>
<td>3.07\pm0.93</td>
<td>5.72\pm1.51</td>
<td>0.012</td>
</tr>
<tr>
<td>T\textsubscript{max} (hour)</td>
<td>1.31\pm0.37</td>
<td>2.00\pm1.20</td>
<td>0.140</td>
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<tr>
<td>CL/F (L/h)</td>
<td>1.83\pm0.76</td>
<td>1.08\pm0.36</td>
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<tr>
<td>t\textsubscript{1/2} (hour)</td>
<td>14.59\pm4.36</td>
<td>10.25\pm2.53</td>
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<tr>
<td>Vd/F (L)</td>
<td>38.16\pm17.10</td>
<td>15.71\pm5.35</td>
<td>0.012</td>
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</table>

AUC\textsubscript{t} = area under the concentration-time curves from time zero to time of last quantifiable concentration; AUC\textsubscript{inf} = area under the concentration-time curves from time zero to infinite time; C\textsubscript{max} = maximum plasma concentration; T\textsubscript{max} = time to C\textsubscript{max}; CL/F = oral clearance; t\textsubscript{1/2} = half-life; Vd/F = apparent volume of distribution.
Effect of Cimetidine on Pharmacokinetics of Theophylline in Healthy Korean Volunteers

thyluric acid. Several cytochrome P-450 isoenzymes including CYP1A2 are thought to be involved in these metabolic pathways. 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. 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 AUCt and Cmax 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., 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. 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 AUCt and Cmax 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.

**Acknowledgment**

This study was supported by Soonchunhyang univer-

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<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Genotype distribution (%)</th>
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<th>Genotype distribution (%)</th>
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<tr>
<td>1</td>
<td>2</td>
<td>1 \ 1 \ 2 \ 2</td>
<td></td>
</tr>
<tr>
<td>CYP1A2*1Ca</td>
<td>16 0</td>
<td>(1) 0</td>
<td></td>
</tr>
<tr>
<td>CYP1A2*1Fb</td>
<td>13 3</td>
<td>(0.1875) 0.625</td>
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*Allele 1 is G, allele 2 is A
*Allele 1 is A, allele 2 is C
sity research fund (20040171).

References