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Effect of Cimetidine on Theophylline Disposition and Metabolic Pathways

In-Jin Jang, Sun-Hee Lee*, Jae-Gook Shin, Sang-Goo Shin, Chan-Woong Park

*Department of Pharmacology, Seoul National University College of Medicine, Clinical Pharmacology Unit, Seoul National University Hospital and Department of Clinical Pharmacology, National Institute of Safety Research**

ABSTRACT

The effect of cimetidine on theophylline metabolism was examined in dogs. Single dose intravenous theophylline kinetic studies were performed in corss-over manner before and after one week intravenous cimetidine (30 mg/kg/day) treatment.

Cimetidine decreased theophylline clearance by an average of 31% ($p<0.05$) and prolonged theophylline half-life by an average of 29% ($p<0.01$) compared to those in control peirods. However, steady-state volume of distribution and protein binding of theophylline were not changed significantly. Twenty-four hours urinary excretion of 3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, which are the major metabolites of theophylline, were all decreased after cimetidine treatment, whereas the excreted fractions of individual metabolites were unchanged by cimetidine. From the above data, it could be susggested that cimetidine decreases theophylline clearance and prolongs the half-life by non-specific inhibition of the demethylations and 8-hydroxylation pathways.

Key Words: Theophylline, Cimetidine, Drug metabolism, Drug interactions

INTRODUCTION

Theophylline is a frequently prescribed drug as a bronchodilator for asthma and chronic obstructive lung disease patients. Because methylxanthine products can increase acid secretion in stomach, patients may have exacerbration of peptic uler and be given the histamine H_2-receptor antagonist concurrently.

Cimetidine, most frequently used H_2-receptor blocker, is well known for vast of drug interactions with such drugs as warfarin (Hetzel *et al.*, 1979), some of benzodiazepines (Desmond *et al.*, 1980; Klotzu *et al.*, 1980) and antipyrine (Puurunen *et al.*, 1980). Cimetidine is reported to inhibit hepatic metabolism of these drugs by competitively and reversibly blocking part of cytochrome P_{450} monooxygenase system (Puurunen *et al.*, 1980; Robert *et al.*, 1982; Grygiel *et al.*, 1984). During early 1980's, many of theophylline toxicity with concurrent administration of cimetidine have been reported (Weinberger *et al.*, 1981; Baumann *et al.*, 1982; Andrew *et al.*, 1984) and a number of investigators reported that cimtidine decreased theophylline clearance in healthy volunteers and patients (Breen *et al.*, 1982; Vestal *et al.*, 1983; Grygiel *et al.*, 1984; Powell *et al.*, 1984; Roberts *et al.*, 1984). However, few studies investigated the effects of cimetidine on the individual pathways of theophylline metabolism. While one investigator reported that cimetidine decreased 1-and 3-demethylation but did not inhibit 8-hydroxylation of theophylline in liver (Grygiel *et al.*, 1984), Vestal *et al.* (1987) reported that only 1-demethylation was significantly decreased. By contrast, De Angelis *et al.* (1983) found no change in the urinary excretion patterns of theophylline metabolites in human.

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For these reasons, we conducted a pharmacokinetic study of the cimetidine-theophylline interaction under the cross-over design to re-evaluate the influence of cimetidine on the differential pathways of theophylline metabolism.

METHODS AND METERIALS

Study animals and theophylline kinetic study

Six male adult dogs weighing 10 to 13kg were used as experimental animals. Each dog was anesthesized by intravenous pentobarbital with loading dose of 20 mg/kg and followed by constant infusion of 2 mg/kg/hr dose during 6 hours of blood sampling period. Two intravenous lines and one femoral arterial line were placed with angiocatheter and the blood pressure was continuously monitored through femoral arterial line during the study period. A 16F Foley catheter was placed in urinary bladder for urine collection. After initial hydration with normal saline 0.5L to 1L for more than one hour, normal saline was infused at a rate of 2.4 ml/minute with peristaltic pump (Cole-Parmer) to maintain constant urine output and to compensate for insensible water loss and sampled blood loss.

Theophylline 7 mg/kg was diluted with normal saline 20-25ml and infused for 10 minutes with Sage infusion pump. Serial 7ml blood samples were taken through arterial line at the times of 0, 5, 7, 10, 11, 12, 13, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300 and 360 minutes after starting the infusion. Additional 10ml blood samples was collected at the time of 90 min for protein binding assay. Urine samples were collected at 3, 6, 12 and 24 hours after the start of drug administration, and voided urine volume at each interval was measured. Blood samples were anticoagulated with EDTA and centrifuged for 10 minutes at 3,000 rpm, then the plasma was taken to be frozen at $-20°C$. Urine samples were also stored at $-20°C$ until drug analysis.

After completing control study, the dog were kept in same diet condition for sufficient washout period. Intravenous cimetidine of 30 mg/kg/day were administered in the morning for one week. Single dose pharmacokinetic study of theophylline was repeated to analyse the cimetidine-theophylline interaction.

Theophylline and metabolite assay

Plasma theophylline levels were determined by fluorescence polarization immunoassay (Abbott Laboratories, TDx) developed by Jolley *et al.* (1981). In the range of 2.5 to 20 μg/ml, the coefficient of variation of the assay was 2.5%. Urinary theophylline metabolite concentrations were measured by a modification of the one described by Muir *et al.* (1980). The reverse phase, ion-pair high performance liquid chromatography (HPLC) method consisting of a Waters pump, a variable wavelength ultraviolet detector (Waters; Model 450), U6K 20μl loop injector and μ-Bondapak ODS column (ID 3.9 × 300mm, Waters) was used. UV detector was set at 280nm, 0.04 AUF. Chromatography was performed under isocratic condition at the constant flow rate of 1.8 ml/minute. The mobile phase was composed of 95% 0.005M tetrabutyl ammonium sulfate/0.01M sodium acetate (50/50) plus 5% methanol with pH adjusted to 4.4.

For the chromatographic analysis, a 0.5ml aliquot of urine was mixed with 0.1ml of internal standard solution (β-hydroxyethyl theophylline, 1 mg/ml), 0.5ml of 0.1 mol/L tetrabutyl ammonium sulfate, and solid amonium sulfate to saturation. The mixture was mixed by a vortex for 30 seconds and then pH was adjusted to 6 to 6.5 with 0.1 mol/L sodium carbonate-bicarbonate buffer (pH 11). The mixture was then extracted with 10ml of methylene chloride-ethylacetate-isopropranol (45:45:10). The organic phase was evaporated to dryness under vacuum at 37°C. The residue was reconstituted in 500μl of mobile phase, and then a 50μl aliquot was injected into the injector.

Figure 1. represents the typical chromatogram of the urine sample.

Protein binding

Theophylline protein binding was determined by equilibrium dialysis method (Shin *et al.*, 1988). We used cellulose dialysis membrane with 4.8μm pore diameter (Bethsda Research Lab.) after equilibrating in 0.9% NaCl − 0.05 mol/L phosphate buffer (pH 7.2). Plasma from 90 minute sample after drug administration was used for measuring protein binding after pH correction to 7.4 with 0.3 mol/L KH_2PO_4 solution. Acrylic plastic dialysis chambers with phosphate buffer and plasma sample were incubated at 37°C for 6 hours with gentle shaking. Percent of protein binding was caculated from the equation: (theophylline concentration in buffer/theophylline concentration in plasma) × 100.

Pharmacokinetic and statistical analyses

Theophylline plasma concentration and urine excretion data were analyzed by nonlinear least-square

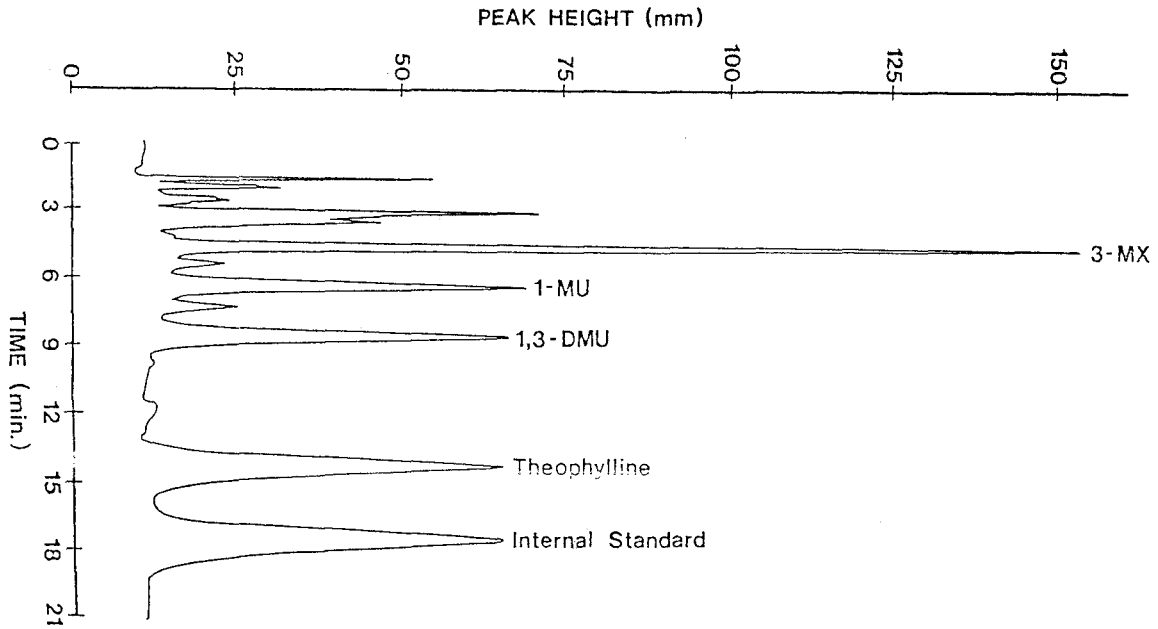

Fig. 1. Chromatogram obtained after injection of extracted urine sample containing 3-MX, 1,3-DMU, 1-MU, theophylline and internal standard, hydroxyethyl theophylline.

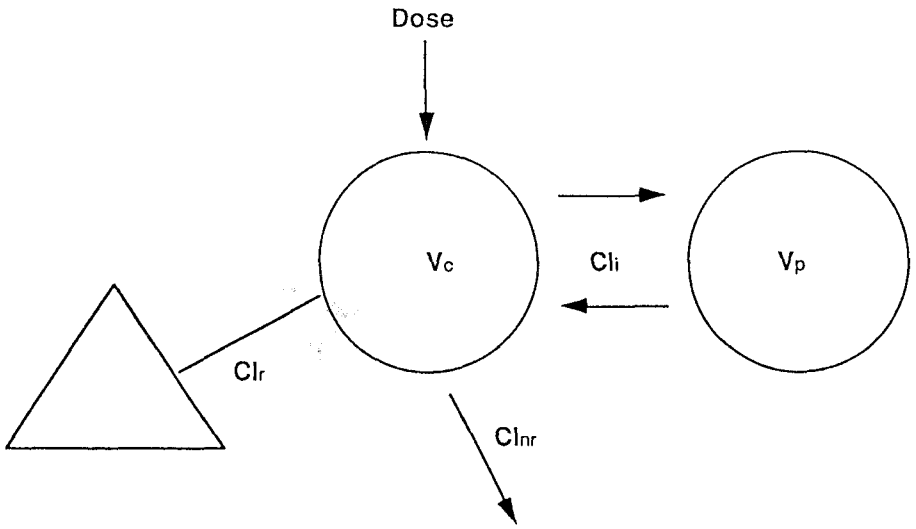

Fig. 2. Two compartment model for kinetics of theophylline distribution and elimination.

regression with NONLIN program developed by Metzler (1974). The kinetics of theophylline distribution and elimination were modeled with a two-compartmental model as Fig. 2. Renal clearances were calculated by dividing 12 hours urinary excreted amount of theophylline by area under the plasma theophylline concentration versus time curve during initial 6 hours ($Clr = \Delta E/AUC$). Urinary recovery of the administered theophylline was calculated on the basis of the excreted molar amount of theophylline and its metabolites. The unbound clearance was estimated by multiplying fraction un-

bound to the corresponding clearance value (Levy, 1980).

Two -tailed paired t-test was used to compare the various kinetic parameters between before and after cimetidine treatment. A value of $p<0.05$ was considered statistically significant.

RESULT

Plasma theophylline concentration time data were obtained from 6 dogs and one of them is presented in Fig. 3. As shown in the figure, plasma theophylline

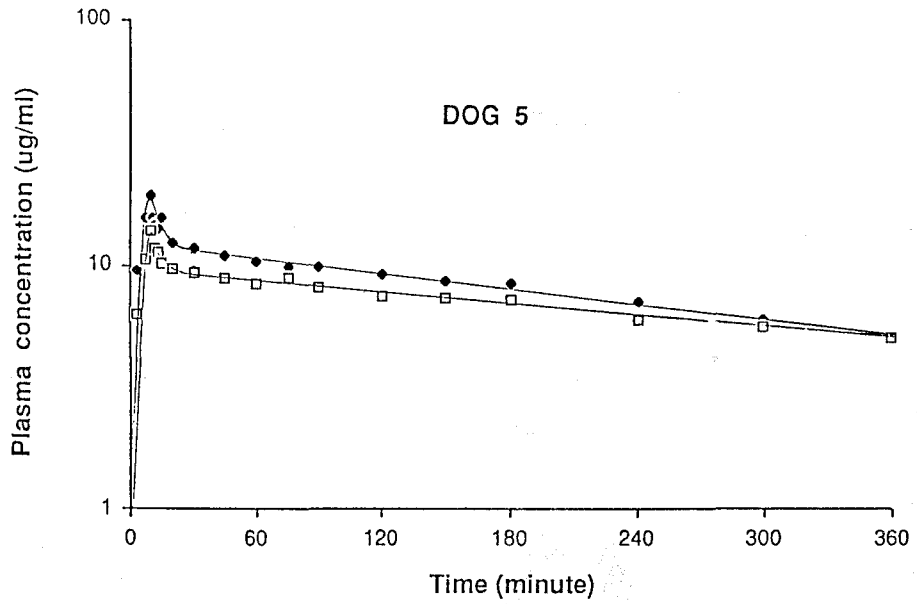

Fig. 3. Plasma concentration-time curves of theophylline after I.V. infusion of theophylline over 10 minutes. (♦) control period, (□) cimetidine treatment for 1 week. Theophylline dose; 8.3mg/kg for control, 7.0mg/kg for cimetidine treatment.

Table 1. Effect of cimetidine on theophylline pharmacokinetic parameters in dog after a single intravenous dose of theophylline

Dog No.	V_c (L/kg)		V_p (L/kg)		V_{dss} (L/kg)		$t_{1/2\beta}$ (min)	
	Control	+Cimet	Control	+Cimet	Control	+Cimet	Control	+Cimet
1	0.197	0.216	0.649	0.608	0.846	0.824	260.7	288.3
2	0.198	0.198	0.513	0.512	0.711	0.710	166.5	234.6
3	0.269	0.203	0.462	0.646	0.731	0.849	280.0	373.3
4	0.286	0.304	0.699	0.686	0.984	0.990	314.7	522.8
5	0.210	0.301	0.442	0.414	0.652	0.741	278.5	363.3
6	0.184	0.239	0.503	0.451	0.687	0.690	323.2	316.4
Mean	0.224	0.224	0.545	0.553	0.768	0.796	270.6	349.8
S.D.	0.043	0.048	0.105	0.110	0.124	0.115	56.2	98.8
Stat. Sig.	N.S		N.S		N.S		$0.01<p<0.05$*	

V_c: Volume of central compartment
V_p: Volume of peripheral compartment
V_{dss}: Volume of steady-state
$t_{1/2\beta}$: Elimination half-life
*: Two-tailed paired T test

Table 2. Effect of cimetidine on theophylline clearance in dog after a single intravenous dose of theophylline

Dog No.	Cl (ml/min)		Protein binding (%)		Club (ml/min)		Clr (ml/min)		Clnr (ml/min)	
	Control	+Cimet	Control	+Cimet	Control	+Cimet	Control	+Cimet	Control	+Cimet
1	24.04	19.32	3.30	2.90	23.25	18.76	13.78	11.07	10.26	8.25
2	33.67	20.85	5.89	6.36	31.69	19.52	12.56	11.90	21.11	8.95
3	25.77	18.48	8.13	8.09	23.67	16.98	8.20	5.56	17.57	12.92
4	29.83	13.72	4.56	2.71	28.47	13.35	11.22	7.39	18.61	6.33
5	20.23	15.11	4.13	3.50	19.40	14.59	11.09	8.27	9.14	6.84
6	17.08	16.11	7.65	6.24	15.77	15.11	9.52	11.40	7.56	4.71
Mean	25.10	17.27	5.61	4.97	23.71	16.39	11.06	9.27	14.04	8.00
S.D.	6.09	2.72	1.96	2.23	5.80	2.44	2.01	2.57	5.72	2.83
Stat. Sig.	$0.005<p<0.01$		N.S		$0.005<p<0.01$		N.S		$0.01<p<0.05$	

Cl: Total clearance (Renal and non-renal clearance)
Club: Unbound clearance
Clr: Renal clearance
Clnr: Non-renal clearance

Table 3. Effect of cimetidine on renal excretion of theophylline and each metabolite during 24 hours in 4 dogs after single intravenous dose of theophylline

Dog No.	3-MX (molar % excreted)		1-MU (molar % excreted)		1,3-DMU (molar % excreted)		Theohylline (molar % excreted)		molar % of dose recovered	
	Control	+Cimet	Control	+Cimet	Control	+Cimet	Control	+Cimet	Control	+Cimet
1	13.26	12.32	2.36	2.05	13.57	8.90	43.02	45.89	72.21	69.16
2	18.50	16.63	3.23	2.0	19.09	10.21	33.44	44.10	74.26	72.94
5	28.46	11.85	2.52	1.25	20.68	14.83	49.08	48.48	100.70	76.40
6	20.84	13.76	5.29	0.20	12.32	17.81	44.38	42.86	82.83	74.63
Mean	20.27	13.64	3.35	1.38	16.42	12.94	42.48	45.33	82.5	73.28
S.D.	6.32	2.15	1.35	0.86	4.09	4.13	6.56	2.44	12.98	3.09
Stat. Sig.	N.S ($p<0.2$)		N.S ($p<0.2$)		N.S ($p<0.35$)		N.S ($p<0.4$)			

3-MX: 3-Methylxanthine
1-MU: 1-Methyluric acid
1,3-DMU: 1,3-Dimethyluric aicd

level showed rapid declining distribution phase and then slow elimination phase which was best explained by two compartmental model.

Pharmacokinetic parameters before and after cimetidine treatment are summarized in Table 1 and Table 2. Volume of distribution of central compartment (control period: 0.22 ± 0.043 L/kg, cimetidine treated period: 0.22 ± 0.110 L/kg) and steady-state volume of distribution (0.768 ± 0.124 L/kg vs. 0.796 ± 0.005 L/kg) were unchanged by cimetidine treatment. However, elimination half-life was significantly prolonged from 270.6 ± 56.2 minutes to 349.8 ± 98.8 minutes. Total body clearance (25.10 ± 6.09 vs. 17.27 ± 2.72 ml/min) and non-renal clearance (14.04 ± 5.72 vs. 8.00 ± 2.83 ml/min) were decreased significantly. Changes of protein binding (5.61 ± 1.96 vs. 4.97 ± 2.23%) and renal clearance (11.06 ± 2.01 vs. 9.27 ± 2.57 ml/min) were not significant. When the pharmacokinetic changes by cimetidine were taken into consideration, the decreased metabolic clearance might be the cause of the decreased total body clearance and prolonged half-life of theophylline after cimetidine treatment.

Urinary excretion of theophylline and metabolites

from 4 dogs with complete urine collections for 24 hours are presented in molar percent at Table 3. As shown in the table, average 81.8 molar percent of administered dose was recovered in control and 73.3 percent in cimetidine treated experiment during 24 hours. Although urinary excretion of three metabolites showed evidently decreasing trends, the changes did not reach statistical significance. Moreover, the excreted fractions of each metabolites were almost identical after cimetidine treatment. Among the three metaoblites, N-demethylation products of theophylline, 3-methyl xanthine and 1-methyluric acid, were consistently less excreted after cimetidine treatment in all 4 dogs.

DISCUSSION

Pharmacokinetic features of theophylline were similar to previous reports in dogs (Kuze *et al.*, 1988; Steven *et al.*, 1987; Nosaka *et al.*, 1986). Compared with non-smoking healthy human (Shin *et al.*, 1988), dogs had quite different pharmacokinetic parameters. They had larger volume of distribution (0.73 L/kg vs. 0.46 L/kg), shorter half-life (270.6 minutes vs. 492 minutes), greater total body clearance (2.5 ml/min/kg vs. 0.96 ml/min/kg) and less protein binding (5.6% vs. 37.6%). These differences are thought to be due to proportionally bigger liver mass in dogs because liver of various species has similar metabolic capacity per unit tissue mass (Boxenbaum 1986). And minimal protein binding of dog may be the one cause of larger volume of distribution and it also contributes to the greater total body clearance of theophylline because only free theophylline can be filtered through renal glomerulus or undergone metabolism in the liver. From the our experimental resutls, one more striking difference is the proportion of the metabolites in urine between man and dog. In human, about 20% of administered theophylline dose is excreted as a 1-methyluric acid (Tang-Liu DDS *et al.*, 1982), but only 4% in dog. Although Kuze *et al.* (1988) did not mention about 1-methyluric acid, their data suggest that 1-methyluric acid is trivial metabolite compared to 3-methylxanthine and 1,3-dimethyluric acid in dog. This difference in metabolite excretion might be due to different hepatic enzyme system that demethylates theophylline or difference in the activity of xanthine oxidase that further metabolize 1-methylxanthine to 1-methyluric acid. In our study, unchanged theophylline excretion was much larger (42.5%) than other dog study (18.2%) or human study (13.3%). This difference can be partly explained by the fact that in our study we maintained constant urine flow of about 1 ml/min during experimental period, because urinary excretion of theophylline is urine flow dependent. Also, the difference can be explained by the minimal protein binding in dog compared to human because free theophylline is easily filtered through glomerulus.

The extent of change in the pharmacokientic parameters after cimetidine treatment was similar to that in humna with average prolongation of half-life by 29.2% and average decrease of clearance by 31.2%, but no change in the volume of distribution and protein binding (Breen *et al.*, 1982; Vestal *et al.*, 1983; Powell *et al.*, 1984; Grygiel *et al.*, 1984). Although total body clearance and non-renal clearance were significantly decreased by cimetidine, renal clearance was not changed. This observation is consistant with the fact that cimetidine inhibits theophylline clearance by inhibiting hepatic cytochrome P_{450} system (Robert *et al.*, 1982; Puurunen *et al.*, 1980).

From urinary metabolite excretion data, we found that changes of three metabolite excretion were not statistically significant, but decreases of 3-methylxanthine and 1-methyluric acid were consistent in four dogs. Such statistical insignificance seems to be due to the small sample size of our experiment. The decreasing trends of demethylated metabolites are somewhat consistent with the report of Grygiel *et al.* (1984), who found that 1- and 3-demethylation were much more inhibited by cimetidine than 8-hydroxylation and those trends were more profound in smoker, where 1- and 3-demethylation more preferentially induced. Therefore, it was suggested that two demethylations are under common regulatory control and are seperate from 8-hydroxylation and that demethylation and 8-hydroxylation are carried out by different forms of cytochrome P_{450}. However, the large difference of 3-methylxanthine and 1-methyluric acid proportion between human and dog suggests that the two demethylation process can also be explained by the different enzyme system, although we cannot exclude the possibility of different activity of xanthine oxidase which converts 1-methylxanthine to 1-methyluric acid. Vestal *et al.* (1987) also found that cimetidine effect on the clearance of 1,3-diemethyluric acid was significantly less than that on the clearance of 3-methyluric acid.

Although further studies should be done for definite elucidation of cimetidine effect on differential metabolic pathway, it might be said from our experimental data that the three main metabolic pathways of theophylline are under seperate regula-

tory control which are inhibited by cimetidine.

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=국문초록=

Cimetidine의 Theophylline 약동학 및 대사과정에 미치는 효과에 관한 연구

서울대학교 의과대학 약리학교실, 서울대학교 병원 임상약리학 Unit 및
국립안전성 연구원 약효약리과*

장인진 · 이선희* · 신재국 · 신상구 · 박찬웅

Cimetidine이 theophylline의 약동학적 특성과 대사과정에 미치는 효과를 검토코자 6마리의 개를 대상으로 일주일간 정맥내 cimetidine(30mg/kg/day)투여 전후에 단일 용량의 정맥투여에 따른 theophylline의 약동학적 parameter 및 뇨중 theophylline 대사물 배설의 변화를 교차 실험을 통하여 관찰하였다.

대조실험에 비해 cimetidine투여후 theophylline의 청소율은 평균 31%($P<0.05$)감소하였고 혈장반감기는 29%($P<0.01$)연장되었다. 그러나 steady-state의 분포용적 및 혈장 단백 결합의 변화는 관찰할 수 없었다. Theophylline의 주 대사물인 3-methylxanthine, 1-methyluric acid 및 1, 3-dimethyluric acid의 24시간 뇨중 배설량은 cimetidine투여후 모두 감소 하였으나 통계적으로 유의한 변화는 아니었으며 개별대사물의 배설 분획은 변화가 없었다.

이상의 결과로 부터 cimetidine이 theophylline의 demethylation과 8-hydroxylation대사과정 모두를 비선택적으로 억제함으로써 청소율을 감소시키고 반감기를 증가시킬 것으로 추정되었다.