The Inhibitory Effects of Cimetidine on Elimination and Distribution of Propranolol in Rats

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We studied the effects of cimetidine on the pharmacokinetics, blood and tissue distribution and plasma protein binding of propranolol in rats.

The plasma disappearance of propranolol after a 10 mg/kg intravenous injection and oral administration were fitted to a two compartment open model. In the cimetidine treated rats, the area under concentration curve after an intravenous injection (AUC_{iv}) was increased by 64% and the plasma total body clearance (Cl_{tot}) and the rate constant at the terminal phase (β) were decreased by 38% and 33% of those of the non-treated rats, respectively. The area under the concentration curve after oral administration (AUC_{po}) was increased by 62% and the plasma oral clearance (Cl_{po}) was decreased by 39% by cimetidine treatment, whereas the bioavailability (F) was not changed. The hepatic blood flow rate (Q_h) and the product of the plasma unbound fraction and the hepatic intrinsic clearance ($f_p \times Cl_{int,h}$) calculated from Cl_{tot} and Cl_{po} were decreased by 30% and 39%, respectively.

The blood-to-plasma concentration ratio (R_b) and the tissue-to-plasma concentration ratio (K_p) of propranolol were not affected by cimetidine treatment, while the binding constant (K_b) in plasma was decreased by 45%. The plasma unbound fractions (f_p) of propranolol were increased by 25–70% in the *in vivo* plasma concentration range (0.1–1.0 $\mu\text{g/ml}$) resulting in the decrease of tissue-to-plasma unbound concentration ratio ($K_{p,u}$) in lung, heart, spleen, brain and muscle. Cimetidine was shown to have the inhibitory effects on elimination and distribution of propranolol in rats.

Keywords — cimetidine; propranolol; drug interaction; elimination; distribution; inhibitory effect

Introduction

Cimetidine, a H_2 -receptor antagonist, is prescribed widely for the therapy of peptic ulcers and is well known to decrease the metabolism of a number of drugs metabolized by the hepatic microsomal oxidation system,^{1–3)} reduce the distribution volume of cationic drugs^{4–6)} and diminish the histamine-induced hepatic blood flow.^{7,8)}

The hepatic drug elimination by metabolism is largely determined by (1) the activity of enzymes, (2) the hepatic blood flow and (3) the degree of drug binding to plasma protein. It is important to study these factors of the drug interaction in the hepatic drug elimination.

Propranolol, a β -adrenergic receptor antagonist, is eliminated from the body only by the hepatic microsomal oxidation system and has a high hepatic clearance.^{10,11)} Then, the primary determinant of the total body clearance is not the activity of metabolizing enzymes, but the

hepatic blood flow rate. We have reported that the distributions of quinidine and lidocaine that obey the pH-partition theory were reduced by cimetidine treatment.^{4,5)} The mechanism of this inhibitory effect was thought to be the increase of the intracellular pH in tissues resulting in the decrease of the pH-partitioning of these drugs.¹²⁾

As the distribution of propranolol was reported to be similar to that of quinidine,¹³⁾ it was expected to be inhibited by cimetidine treatment.

Therefore, we studied the effects of cimetidine on the factors controlling the elimination (the hepatic intrinsic clearance and the hepatic blood flow rate) and the distribution (the plasma protein binding, tissue-to-plasma concentration ratio (K_p) and tissue-to-plasma unbound concentration ratio ($K_{p,u}$)¹⁴⁾ of propranolol in rats.

Materials and Methods

Materials — *dl*-Propranolol hydrochloride

and cimetidine were purchased from Sigma Chemicals Co., Ltd. (St. Louis, MO, U.S.A.). All other reagents were commercial products and of analytical grade.

Pharmacokinetic Study — 1) Intravenous Injection: Four male Sprague Dawley rats, weighing 250–300g were used in each of the non-treated and the cimetidine treated studies. The rats were anesthetized with sodium pentobarbital (i.p., 44 mg/kg body weight) and fixed on a board. The left femoral artery and both femoral veins were cannulated with a polyethylene tubing (PE-50, Clay-Adams, NJ, U.S.A.). The body temperature was kept at 37 °C by a heat lamp. Either the loading dose of cimetidine (25 mg/ml, 20 mg/kg) or saline (0.8 ml/kg) was followed by the infusion (cimetidine; 0.28 mg/kg/min or saline; 0.132 ml/kg/min) by a constant rate infusion pump (JP-WG, Furue Sci., Tokyo, Japan). The infusion was continued until the end of blood sampling. Sixty minutes after the initiation of the infusion, the rats were injected with propranolol (10 mg/ml, 10 mg/kg body weight). Blood samples (0.3 ml) were taken in heparinized polyethylene centrifuge tubes at 1, 3, 5, 7, 10, 15, 20, 30, 60, 90 and 120 min after the propranolol administration. Plasma was separated by centrifugation at $1000 \times g$ for 10 min. A 0.15 ml portion of plasma was used to determine the propranolol concentration.

2) Oral Administration: Five male Sprague Dawley rats, weighing 250–300 g were used in each of the non-treated and in the cimetidine treated studies. The rats were fasted for 15 h prior to the experiment, but water was allowed *ad libitum*. The surgery and the conditions of the rats were the same as described above. Sixty minutes after the initiation of the cimetidine or the saline infusion, the rats were orally administered with a saline solution of propranolol by a stomach tubing (10 mg/ml, 10 mg/kg). Blood samples (0.3 ml) were taken at 1, 3, 5, 10, 20, 30, 60, 90, 120 and 180 min after the propranolol administration. Plasma was separated by centrifugation and the propranolol concentration was determined.

Blood and Tissue Distribution of Propranolol — Four male Sprague Dawley rats were

used in each of the non-treated and the cimetidine treated studies. The rats were operated as described above. Blood samples (1.2 ml) were taken at 30, 60, 90 and 120 min after the propranolol injection. A 0.15 ml portion of blood was removed for the propranolol determination and the remaining blood was centrifuged at $1000 \times g$ for 10 min. A 0.15 ml portion of plasma was used to determine the propranolol concentration and the remaining plasma was used for the plasma protein binding study. Two hours after the propranolol injection, the rats were decapitated and tissues were excised and rinsed with an ice cold saline solution. The tissues were weighed and homogenized in three fold volumes of 0.01 M phosphate buffer that contained 0.15 M KCl (pH 7.0) by using a high speed homogenizer (Phycosoron Homogenizer, Nichion Irica Seisakusho, Tokyo, Japan). The propranolol concentrations in tissue homogenates were determined.

Plasma Protein Binding of Propranolol *in Vivo* — The plasma protein binding of propranolol was determined by an ultrafiltration technique (MPS-1, Amicon Co., Cambridge, MA) in the remaining plasma of the distribution study. A 0.7 ml portion of plasma was placed in an ultrafiltration device equipped with an ultrafiltration membrane (YMT membrane, Amicon Co., Cambridge, MA) and centrifuged at $2000 \times g$ for 15 min at 20 °C.¹⁵⁾ An adsorption of propranolol to the membrane was negligible. The propranolol concentrations in both sides of the membrane were determined.

Analytical Method — The propranolol concentration in samples was determined spectrofluorometrically.¹⁶⁾ To 0.15 ml of plasma, 1.0 ml of water, 1.0 ml 0.1 N NaOH and 5.0 ml of *n*-heptane containing 1.5% iso-amylalcohol were added. After shaking and centrifugation at $1000 \times g$ for 10 min, 4.0 ml of organic layer was transferred to a glass tube that contained 2.0 ml of 0.1 N HCl. After shaking and centrifugation, the intensity of fluorescence was measured in the aqueous phase at 295 nm for excitation and 360 nm for emission (Hitachi Fluorophotometer F-3000, Hitachi Seisakusho, Japan). The same procedures were employed with 0.15 ml of blood, 0.3 ml of ultrafiltrated solution or 0.5 ml

of tissue homogenates. The extraction percentage was 97% and the minimum sensitivity of propranolol in samples was 5 ng/ml, respectively.

Pharmacokinetic Parameters — The observed plasma concentrations (C_p) of propranolol after the intravenous injection were fitted to a two compartment open model by using Eq. 1.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

The pharmacokinetic parameters of propranolol such as the area under the concentration curve after the intravenous injection (AUC_{iv}), the plasma total body clearance (Cl_{tot}) and the volume of distribution at steady state (V_{dss}) were calculated by means of the following equations.

$$AUC_{iv} = A/\alpha + B/\beta \quad (2)$$

$$Cl_{tot} = D_{iv}/AUC_{iv} \quad (3)$$

$$V_{dss} = D_{iv} \times (A\beta^2 + B\alpha^2)/(A\beta + B\alpha)^2 \quad (4)$$

where D_{iv} is the dose of propranolol injected intravenously.

The C_p of propranolol observed after an oral administration were fitted to a two compartment open model with a first order absorption by using Eq. 5.

$$C_p = A'e^{-\alpha t} + B'e^{-\beta t} - (A' + B')e^{-k_{at}} \quad (5)$$

The area under plasma concentration curve (AUC_{po}), the plasma oral clearance (Cl_{po}) and the bioavailability (F) of propranolol after the oral administration were calculated by using the following equations;

$$AUC_{po} = A'/\alpha + B'/\beta - (A' + B')/k_a \quad (6)$$

$$Cl_{po} = D_{po}/AUC_{po} \quad (7)$$

$$F = D_{iv} \cdot AUC_{po}/D_{po} \cdot AUC_{iv} = Cl_{tot}/Cl_{po} \quad (8)$$

where D_{po} is the dose of propranolol administered orally.

Hepatic Blood Flow Rate (Q_h) and Intrinsic

Clearance ($Cl_{int,h}$) — Propranolol is eliminated only from the liver^{10,11)} and is absorbed completely,¹⁷⁾ then Cl_{tot} and F are written as

$$Cl_{tot} = Cl_h = Q_h \cdot R_b \cdot E_h \quad (9)$$

$$F = 1 - E_h \quad (10)$$

where Cl_h is the hepatic clearance, R_b and E_h are the blood-to-plasma concentration ratio and the hepatic extraction ratio, respectively. From Eq. 8, Cl_{tot} is also written as

$$Cl_{tot} = Cl_{po} \times F \quad (11)$$

Substitution for F , according to Eq. 10, into Eq. 11 yields

$$Cl_{tot} = (1 - E_h) \times Cl_{po} \quad (12)$$

From Eq. 9 and 12, Q_h is given by

$$Q_h = Cl_{tot} \times Cl_{po}/(Cl_{po} - Cl_{tot})/R_b \quad (13)$$

The $f_p \times Cl_{int,h}$ is given by

$$f_p \times Cl_{int,h} = Q_h \times Cl_{tot} \times R_b/(Q_h \times R_b - Cl_{tot}) \quad (14)$$

where f_p is the plasma unbound fraction.

Plasma Protein Binding Parameters — The concentrations of unbound (C_u) and bound (C_b) propranolol were fitted to the following equation;

$$C_b = nP \times K_b \times C_u/(1 + K_b \times C_u) \quad (15)$$

where n , P and K_b denote the number of binding site of a protein molecule, the protein concentration and the binding constant, respectively.

Tissue-to-Plasma Concentration Ratio (K_p) of Propranolol — The apparent K_p ($K_{p,app}$) of propranolol is defined as

$$K_{p,app} = C_t/C_p \quad (16)$$

where C_t is the tissue concentration. The K_p value was calculated by the method of Terasaki

*et al.*¹⁸⁾ For the non-eliminating tissues except for lung,

$$K_{p,t} = K_{p,app,t} / (1 + \beta \times V_t \times K_{p,app,t} / Q_t / R_b) \quad (17)$$

For lung,

$$K_{p,Lu} = K_{p,app,Lu} / (1 - \beta \times V_A / Q_{Lu}) \quad (18)$$

For the eliminating tissue (liver),

$$K_{p,Li} = K_{p,app,Li} \times (R_b \times Q_h + f_p \times Cl_{int,h}) / (\beta \times V_{Li} \times K_{p,app,Li} + R_b(Q_{Li} - \sum (1 - K_{p,app}/K_p) Q)) \quad (19)$$

$$\begin{aligned} & \sum (1 - K_{p,app}/K_p) Q \\ &= (1 - K_{p,Sp}/K_{p,app,Sp}) Q_{Sp} \\ &+ (1 - K_{p,St}/K_{p,app,St}) Q_{St} \\ &+ (1 - K_{p,In}/K_{p,app,In}) Q_{In} \end{aligned} \quad (20)$$

where Q and V represent the blood flow rate and the volume of tissue and subscript t, Lu, A, Li, Sp, St and In denote tissue, lung, artery, liver, spleen, stomach and intestine, respectively.

In consideration of protein binding, tissue-to-plasma unbound concentration ratio ($K_{p,u}$) can be defined as¹⁴⁾

$$K_{p,u} = K_p / f_p \quad (21)$$

Prediction of $K_{p,u}$ of Propranolol with Cimetidine Treatment ($K_{p,u \text{ pred'}}$) — The $K_{p,u \text{ pred'}}$ is given by

$$K_{p,u \text{ pred'}} = (q'/q) \times K_{p,u \text{ obs}} \quad (22)$$

where $K_{p,u \text{ obs}}$ is observed $K_{p,u}$ without cimetidine treatment. The values of q' and q are the tissue-to-plasma partition coefficient of unbound drug with and without cimetidine treatment, respectively. They are given by Henderson-Hasselbalch's equation.

$$q = (1 + 10^{pK_a - pH_i}) / (1 + 10^{pK_a - pH_e}) \quad (23)$$

where pK_a is the negative logarithm of dissociation constant of propranolol and is 9.3.¹¹⁾ The pH_i and pH_e are the intra- and extra-cellular pH for the case without cimetidine treatment. The q' is calculated in the same way for the case with cimetidine treatment. The pH_i and pH_e with or without cimetidine treatment were cited from our previous report and are listed in Table I.¹²⁾

Statistical Analysis — All means are presented with their standard errors (mean $\pm$

TABLE I. Intracellular pH and Plasma pH with or without Cimetidine Treatment^{a,b)}

Tissue	No Treatment	Cimetidine treatment
Lung	6.89 $\pm$ 0.021	6.95 $\pm$ 0.012
Liver	7.21 $\pm$ 0.029	7.30 $\pm$ 0.010 ^{c)}
Kidney	7.18 $\pm$ 0.022	7.15 $\pm$ 0.018
Heart	7.09 $\pm$ 0.024	7.15 $\pm$ 0.021
Spleen	7.02 $\pm$ 0.024	7.15 $\pm$ 0.021 ^{c)}
Stomach	6.83 $\pm$ 0.027	6.77 $\pm$ 0.175
Intestine	6.93 $\pm$ 0.019	7.07 $\pm$ 0.026 ^{c)}
Brain	6.88 $\pm$ 0.040	7.02 $\pm$ 0.039 ^{c)}
Muscle	7.04 $\pm$ 0.007	7.17 $\pm$ 0.040 ^{c)}
Skin	6.82 $\pm$ 0.029	7.02 $\pm$ 0.032 ^{c)}
Plasma	7.41 $\pm$ 0.020	7.42 $\pm$ 0.070

a) Values are the means $\pm$ S.E. of three experiments.

b) Cited from ref. 12). c) Significantly different from the non-treated group ($p < 0.05$).

Fig. 1. Disappearance of Propranolol from Plasma after a Bolus Intravenous Injection with or without Cimetidine Treatment

Each point and vertical bar represent the means $\pm$ S.E. of four experiments. Lines are simulated using the parameters obtained from curve fitting.

○, no treatment; ●, cimetidine treatment

TABLE II. Pharmacokinetic Parameters of Propranolol after Intravenous Injection in Rats with or without Cimetidine Treatment^{a,b)}

	No treatment	Cimetidine treatment ^{c)}
<i>A</i> (μg/ml)	6.43 ± 3.02	5.64 ± 1.23
<i>α</i> (min ⁻¹)	0.482 ± 0.116	0.446 ± 0.079
<i>B</i> (μg/ml)	1.40 ± 0.102	1.53 ± 0.144
<i>β</i> (10 ⁻² min ⁻¹)	1.16 ± 0.066	0.78 ± 0.018 ^{d)}
<i>AUC</i> _{iv} (μg·min/ml)	133.9 ± 10.5	219.3 ± 22.3 ^{d)}
<i>Cl</i> _{tot} (ml/min/kg)	76.2 ± 6.38	47.1 ± 4.97 ^{d)}
<i>V</i> _{dss} (l/kg)	5.98 ± 0.384	5.87 ± 0.436

a) Values are the means ± S.E. of four experiments. b) Propranolol was intravenously injected (10 mg/kg). c) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection. d) Significantly different from the non-treated group ($p < 0.05$).

S.E.). The Student's *t*-test was utilized to estimate a significant difference between the non-treated group and the cimetidine-treated group, with $p = 0.05$ as the minimal level of significance.

Results

Effects of Cimetidine Treatment on Pharmacokinetics of Propranolol after Intravenous Injection and Oral Administration

The disappearance of propranolol in plasma after the intravenous injection with or without cimetidine treatment are shown in Fig. 1. The plasma concentrations of propranolol at the elimination phase in the cimetidine treated rats were significantly higher than those in the non-treated rats. The plasma concentration curves in both groups were fitted to a two compartment open model. The pharmacokinetic parameters are listed in Table II. The value of *AUC*_{iv} was increased by 64% by cimetidine treatment; *Cl*_{tot} and the rate constant at the terminal phase (*β*) were decreased by 38 and 33%, respectively ($p < 0.05$). However, *V*_{dss} was not altered.

The plasma concentration profiles of propranolol after the 10 mg/kg oral administration with or without cimetidine treatment are shown in Fig. 2. The disappearance of propranolol was fitted to a two compartment open model with first order absorption. The pharmacokinetic parameters are listed in Table III. The values of *AUC*_{po} increased by 62% and the *Cl*_{po} decreased by 39% with cimetidine treatment, but *k*_a and *F*

did not change.

Effects of Cimetidine Treatment on Blood Distribution and Plasma Protein Binding of Propranolol

Table IV demonstrates *R*_b of propranolol with or without cimetidine treatment. The values of *R*_b were constant during the elimination phase both in the non-treated and cimetidine treated rats.

The binding parameters of propranolol in rat

Fig. 2. Disappearance of Propranolol from Plasma after Oral Administration with or without Cimetidine Treatment

Each point and vertical bar represent the means ± S.E. of five experiments, respectively. Lines are simulated using the parameters obtained from curve fitting.

○, no treatment; ●, cimetidine treatment

TABLE III. Pharmacokinetic Parameters of Propranolol after Oral Administration in Rats with or without Cimetidine Treatment^{a,b)}

	No treatment	Cimetidine treatment ^{c)}
α (min ⁻¹)	0.295 ± 0.092	0.379 ± 0.136
β (10 ⁻² min ⁻¹)	0.984 ± 0.276	0.457 ± 0.096 ^{d)}
k_a (min ⁻¹)	0.793 ± 0.173	0.498 ± 0.099 ^{d)}
AUC_{po} (μg · min/ml)	30.6 ± 2.47	49.6 ± 4.77 ^{d)}
Cl_{po} (ml/min/kg)	335.7 ± 26.6	205.3 ± 18.0 ^{d)}
F	0.228 ± 0.018	0.226 ± 0.022

a) Values are the means ± S.E. of five experiments. b) Propranolol was orally administered (10 mg/kg). c) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection. d) Significantly different from the non-treated group ($p < 0.05$).

TABLE IV. R_b of Propranolol in Rats with or without Cimetidine Treatment^{a,b)}

Time (min) ^{c)}	No treatment	Cimetidine treatment ^{d)}
30	1.19 ± 0.038	1.10 ± 0.054
60	1.38 ± 0.218	1.01 ± 0.055
90	0.94 ± 0.151	0.90 ± 0.122
120	1.12 ± 0.126	1.12 ± 0.068
Mean	1.16 ± 0.091	1.03 ± 0.050

a) Values are the means ± S.E. of four experiments. b) Propranolol was intravenously injected (10 mg/kg). c) Time after propranolol intravenous injection. d) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection.

TABLE V. Binding Parameters of Propranolol in Rat Plasma with or without Cimetidine Treatment^{a,b)}

	No treatment	Cimetidine treatment ^{c)}
nP (μM)	4.21 ± 0.084	4.72 ± 0.206
K_b (μM ⁻¹)	12.6 ± 1.19	6.82 ± 0.157 ^{d)}

a) Values are the means ± S.E. of four experiments. b) Propranolol was intravenously injected (10 mg/kg). c) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection. d) Significantly different from the non-treated group ($p < 0.05$).

TABLE VI. Q_h and $f_p \times Cl_{int,h}$ of Propranolol in Rats with or without Cimetidine Treatment^{a)}

	No treatment	Cimetidine treatment ^{d)}
Q_h (ml/min/kg) ^{b)}	85.0 ± 3.26	59.3 ± 2.51 ^{e)}
$f_p \times Cl_{int,h}$ (ml/min/kg) ^{c)}	335.5 ± 26.6	205.8 ± 18.1 ^{e)}

a) Values are the means ± S.E. of five experiments. b) Calculated by Eq. 13. c) Calculated by Eq. 14. d) A loading dose (20 mg/kg) was followed by an infusion (0.28 mg/kg/min) 60 min before propranolol administration. e) Significantly different from the non-treated group ($p < 0.05$).

plasma with or without cimetidine treatment are listed in Table V. The concentration of binding site of proteins (nP) was not changed by cimetidine, while the binding constant (K_b) was decreased to 55% of that of the non-treated group, significantly ($p < 0.05$).

Effects of Cimetidine Treatment on Q_h and $f_p \times Cl_{int,h}$ of Propranolol

Table VI summarizes Q_h and $f_p \times Cl_{int,h}$ of propranolol with or without cimetidine treatment. The values of Q_h and $f_p \times Cl_{int,h}$ significantly decreased by 30 and 39%, respectively.

Effects of Cimetidine Treatment on Tissue Distribution of Propranolol

The tissue volumes and tissue blood flow rates used to calculate K_p are listed in Table VII.

Table VIII lists K_p of propranolol with or without cimetidine treatment. The K_p values of stomach and muscle increased by 120 and 28% of those of the non-treated group ($p < 0.05$), respectively; although K_p of other tissues remained unchanged.

Table IX shows $K_{p,u}$ of propranolol with or without cimetidine treatment. The values of f_p used to calculate $K_{p,u}$ were 0.032 ± 0.004 and

TABLE VII. Tissue Volume and Tissue Blood Flow Rate of 290 g Rats^{a)}

Tissue	Volume (ml)	Blood flow rate (ml/min)
Lung	1.2	46.2 ^{b)}
Liver	11.7	24.7
		17.2 ^{c)}
Kidney	2.1	11.4 ^{b)}
Heart	1.0	3.0 ^{b)}
Spleen	0.9	0.5 ^{b)}
Stomach	1.6	1.6 ^{b)}
Intestine	13.5 ^{b)}	13.2 ^{b)}
Brain	1.4	2.0 ^{b)}
Muscle	127.2 ^{b)}	6.3 ^{b)}
Skin	50.5 ^{b)}	2.5 ^{b)}
Blood	23.5 ^{b)}	—
Artery	7.8 ^{b)}	—
Vein	15.7 ^{b)}	—

a) Values are the means of eight rats. b) Calculated from the body weight according to the equation of Biscoff's.¹⁹⁾

c) The cimetidine treated group.

0.054 ± 0.005 for the non-treated and the cimetidine treated rats, respectively at 120 min after the intravenous injection when C_p were 0.501 ± 0.07 and 0.774 ± 0.08 µg/ml. The $K_{p,u}$ values of lung, heart, spleen, brain and muscle were significantly decreased by 45, 27, 39, 44

TABLE VIII. K_p of Propranolol with or without Cimetidine Treatment^{a,b)}

Tissue	No treatment	Cimetidine treatment ^{c)}
Lung	100.1 ± 4.58	93.4 ± 3.23
Liver	5.45 ± 0.94	7.13 ± 0.52
Kidney	16.9 ± 2.81	21.0 ± 2.04
Heart	8.42 ± 0.30	10.2 ± 0.58
Spleen	17.1 ± 0.54	17.4 ± 1.08
Stomach	5.99 ± 0.97	13.2 ± 1.26 ^{d)}
Intestine	10.7 ± 1.56	11.2 ± 2.62
Brain	28.8 ± 1.54	26.7 ± 1.37
Muscle	2.31 ± 0.11	2.96 ± 0.08 ^{d)}
Skin	2.25 ± 0.17	2.96 ± 0.44

a) Values are the means ± S.E. of four experiments.

b) Propranolol was intravenously injected (10 mg/kg).

c) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection. d) Significantly different from the non-treated group ($p < 0.05$).

TABLE IX. $K_{p,u}$ of Propranolol with or without Cimetidine Treatment^{a-c)}

Tissue	No treatment	Cimetidine treatment ^{d)}
Lung	3128.1 ± 123.6	1729.6 ± 27.2 ^{e)}
Liver	170.3 ± 29.5	132.0 ± 12.1
Kidney	513.7 ± 85.3	386.0 ± 37.5
Heart	255.9 ± 9.11	187.5 ± 10.7 ^{e)}
Spleen	519.8 ± 16.3	319.9 ± 12.0 ^{e)}
Stomach	182.1 ± 29.5	242.6 ± 23.0
Intestine	325.2 ± 47.4	205.9 ± 48.0
Brain	875.4 ± 46.7	490.8 ± 25.2 ^{e)}
Muscle	70.2 ± 3.37	54.4 ± 0.57 ^{e)}
Skin	68.4 ± 5.07	54.4 ± 9.74

a) Values are the means ± S.E. of four experiments.

b) Calculated by Eq. 21. c) Propranolol was intravenously injected (10 mg/kg). d) A loading dose (20 mg/kg) was followed by an infusion of cimetidine (0.28 mg/kg/min) 60 min before propranolol injection. e) Significantly different from the non-treated group ($p < 0.05$).

and 23% by cimetidine treatment, respectively.

The relationship between the observed and predicted $K_{p,u}$ with cimetidine treatment ($K_{p,u \text{ obs'}}$ and $K_{p,u \text{ pred'}}$) is shown in Fig. 3. The plot of $K_{p,u \text{ obs'}}$ and $K_{p,u \text{ pred'}}$ yields a straight line with a correlation coefficient of 0.980 ($Y = 1.28 X - 22.7$).

Fig. 3. Relationship between $K_{p,u \text{ obs'}}$ and $K_{p,u \text{ pred'}}$ of Propranolol

The abbreviations, Lu, Li, Ki, He, Sp, St, In, Br, Mu and Sk denote lung, liver, kidney, heart, spleen, stomach, intestine, brain, muscle and skin, respectively. a) Calculated by Eq. 22.

Discussion

The pharmacokinetic parameter of propranolol representing the overall elimination from the body (Cl_{tot}) after the 10 mg/kg intravenous injection was decreased by cimetidine treatment. Since propranolol is eliminated from the body only by its metabolism in the liver,^{10,11)} the decrease of Cl_{tot} is thought to be a result of decrease of hepatic clearance. The values of E_h were about 0.8, so the hepatic clearance was blood flow limited. If the reciprocals of $f_p \times Cl_{int,h}$ and Q_h are interpreted as resistance of the hepatic clearance, the partial resistance of $1/(f_p \times Cl_{int,h})$ contributes only 20% to the total resistance, while the contribution of the hepatic blood flow ($1/Q_h$) amounts to 80%. Therefore, the decrease of Q_h by cimetidine treatment decreased Cl_{tot} of propranolol. Several papers have shown the decrease of the tissue blood flow in the presence of cimetidine. Garg *et al.* obtained the results that cimetidine and ranitidine decreased Q_h in humans by 19 and 22%, respectively,²⁰⁾ and cimetidine was reported to decrease the mesenteric,⁷⁾ the gastric⁸⁾ and the hepatic⁹⁾ arterial blood flow by an inhibition on the H_2 -receptor.

The value of $f_p \times Cl_{int,h}$ was decreased by 39%. Because f_p was observed to decrease by 69%, $Cl_{int,h}$ was calculated to decrease by 64% with cimetidine treatment. This shows that cimetidine inhibits the hepatic elimination of propranolol and agrees with the reports that cimetidine inhibited the oxidative metabolism increasing the concentration of concomitant drugs.¹⁻³⁾ However this inhibition contributed only a little to the decrease of Cl_{tot} .

Cimetidine decreased the binding constant (K_b) of propranolol to plasma protein to 55%. Since the binding capacity (nP) of propranolol was not affected by the cimetidine treatment, the mechanism of the decreasing plasma protein binding is thought to be one of competitive inhibition. It was reported that cimetidine inhibited the plasma protein binding resulting in the increases of the volume of distribution of trimazosin²¹⁾ and bupivacaine.²²⁾ Propranolol and these drugs are cationic and may have the same binding sites such as α_1 -acid glycoprotein

and β -lipoprotein in plasma. However we obtained conflicting results in that cimetidine did not inhibit the plasma protein binding of quinidine⁴⁾ and lidocaine.⁵⁾ The binding inhibition of cimetidine in plasma seems not to be common to cationic drugs.

The decrease in the plasma protein binding could increase the tissue-to-plasma concentration ratio (K_p), since K_p of propranolol is the product of the ratio of unbound fraction of plasma to that of tissue (f_p/f_t) and the tissue-to-plasma pH-partition coefficient (q). However K_p of propranolol was not changed by cimetidine treatment (Table VIII). Because K_p is the apparent one, $K_{p,u}$ that represents the distribution of unbound propranolol was calculated by Eq. 21. The $K_{p,u}$ values of propranolol in lung, heart, spleen, brain and muscle decreased by 45, 27, 39, 44 and 23%, respectively by cimetidine treatment ($p < 0.05$).

We reported that cimetidine increased the pH of the intracellular space and so decreased the pH difference between the intra- and extracellular spaces, and that this change caused the decreases in V_{dss} and K_p of quinidine.¹²⁾ Assuming that the distribution of propranolol obeys the pH-partition theory, $K_{p,u}$ can be predicted by using the reported pH values with cimetidine treatment according to Eq. 22 and 23. The observed and predicted $K_{p,u}$ with cimetidine treatment ($K_{p,u,obs'}$ and $K_{p,u,pred'}$, respectively) have a good linear relationship with a slope of 1.28 and a correlation coefficient of 0.980. This shows that the decrease of the tissue distribution resulted from the increased intracellular pH by cimetidine treatment.

In spite of the increased f_p and the constant pH_i in lung and heart, K_p of these tissues did not change. This discrepancy may result from the nonlinear tissue binding of propranolol and the inhibition to it by cimetidine.

In conclusion, cimetidine decreased Cl_{tot} , β , Cl_{po} , the hepatic intrinsic clearance and the plasma protein binding of propranolol, and the hepatic blood flow rate. The tissue distribution of unbound propranolol was decreased by the effect of cimetidine to increase the intracellular pH.

Therefore cimetidine may have some effects

on the behavior of drugs in the body administered concurrently by the mechanisms indicated above.

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