Effect of Food on Propranolol Oral Clearance and a Possible Mechanism of This Food Effect

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To better define the mechanism of the increased plasma concentration of propranolol (PL) after meals, the effect of the dietary constitution of a meal on the kinetics of PL and β -naphthoxylactic acid (NLA), a main metabolite, after administration of the drug, was investigated in rats. Additionally, the hepatic uptake of PL and cytochrome P-450 (P-450) content and uridine 5'-diphosphoglucuronyltransferase (UDPGT) activity in liver were measured after glucose intake. As a result, protein (skim milk) intake slightly, but not significantly, increased the area under the plasma concentration–time curve (*AUC*) and bioavailability of PL, with a slight increase (16%) in hepatic blood flow, and enhanced PL metabolism to NLA. Soybean oil and fatty acid intake significantly decreased the bioavailability of PL, while glucose intake dramatically decreased the hepatic uptake of PL and P-450 content at high glucose levels, resulting in a decrease in the plasma PL concentration at the initial time period and in the inhibition of a metabolic conversion to NLA. Thus, a possible mechanism involved in the effect of food on PL bioavailability could have been due largely to the decreased microsomal P-450 content and hepatic uptake of PL after glucose intake, but only partly to the increased hepatic blood flow after protein intake.

Keywords propranolol; food effect mechanism; rat; P-450 inhibition; hepatic uptake inhibition; metabolic inhibition

Since its introduction as a β -adrenoreceptor-blocking drug, propranolol (PL) has become a mainstay in the treatment of many clinical disorders.^{1,2)} Pharmacokinetic studies in healthy adults and patients with various disease states have demonstrated as much as a 10- to 20-fold variation in plasma concentrations of PL between individuals, as well as low bioavailability after oral doses of the drug.^{3–5)} However, when oral doses of PL are given together with food, plasma PL concentrations are increased in comparison to the fasting state, resulting in a 60% increase in the area under the plasma concentration–time curve (*AUC*) (food effect).^{6–8)} Additionally, food decreased the first-pass liver extraction of PL during first-pass transit.⁶⁾

The most likely mechanism for the increased plasma concentration of PL is a food-induced transient increase in hepatic blood flow, resulting in the decreased hepatic extraction of PL during the absorption phase.^{9,10)} However, it has been proposed that the effect of food on PL bioavailability is not principally the result of a change in hepatic blood flow.¹¹⁾ Therefore, there may be another mechanism involved in this food effect, such as the direct inhibition of PL metabolism.

In this study, we specifically investigated the effects of the dietary constituents of meal, such as glucose, protein, fat and fatty acid, on the kinetics of PL; and we studied the effect of glucose and protein meals on the kinetics of α -naphthoxylactic acid (NLA), a main metabolite, after administration of the drug in rats. In addition, to clarify the inhibition of PL metabolism at a high glucose level, the cytochrome P-450 (P-450) content and uridine 5'-diphosphoglucuronyltransferase (UDPGT) activity in liver and the hepatic uptake of PL were measured after glucose intake.

MATERIALS AND METHODS

Materials PL hydrochloride was a generous gift from

Ono Pharmaceutical Industry Co. (Osaka, Japan). NLA and verapamil hydrochloride, an internal standard for HPLC, were purchased from Sigma Chemical Co. (St. Louis, MO). Skim milk (protein 34.8%, fat 0.8%) was obtained from Yukijirushi Milk Co. (Tokyo, Japan). All other chemicals were of analytical or special grade.

Preparation of Meals Glucose and skim milk were dissolved at 20% (w/v) concentration in 1.5% sodium carboxymethyl cellulose (CMC). Soybean oil and a mixture of oleic acid and lauric acid (1:1, mol/mol) were homogenated in 1.5% CMC to attain 20% (w/v) emulsion, respectively, using a Waring blender.

Drug Administration Experiment Male Wistar rats (200–280 g) were used in this study. The animals were allowed to acclimate to a standard environment in the animal care room for 3–4 d before the study. An external jugular vein cannula was implanted 24 h prior to drug administration. PL hydrochloride dissolved in saline was administered intravenously through the tubing to rats which had free access to an MF diet (Oriental Yeast Co.) at a 4 mg/kg (free PL equivalent) dose. The animals, divided at random into 3–4 groups, each consisting of 4–8 rats, were fasted for 24 h before the oral (*p.o.*) administration experiment and for 3 h after the oral drug administration. Various meals (3, 6 ml in the experiment for NLA determination) were given orally to rats *via* a stomach tube, and then 30 min after meals, PL hydrochloride (20 mg/kg, PL equivalent) was administered orally. For the comparison, some rats received orally 1.5% CMC alone (3 ml), followed by the administration of PL (20 mg/kg) (CMC rat), and other rats were treated with oral PL alone at the same dose (fasting rat). After the administration of PL, blood samples were collected from the cannulated jugular vein periodically into a heparinized syringe. The plasma was separated immediately by centrifugation and stored frozen until assay.

Preparation of Hepatic 9000g Supernatant The animals were sacrificed by decapitation 1 or 3 h after the

administration of PL. The livers were thoroughly perfused *in situ* with ice-cold 0.9% NaCl solution, and were then homogenized with 4 volumes of 1.15% KCl solution in a Potter homogenizer with a Teflon pestle. The homogenate was centrifuged at $9000 \times g$ for 25 min. The supernatant fraction was used for the experiment.

Enzyme Assays The concentration of P-450 in the $9000 \times g$ supernatant was determined by the method of Omura and Sato.¹²⁾ Aniline hydroxylase activity was assayed at 37°C by the method of Ariyoshi and Takabatake.¹³⁾ UDPGT activity was estimated using *p*-nitrophenol as a substrate according to the method described by Mulder.¹⁴⁾

Analytical Procedures PL in plasma or blood was determined by the method of Katayama *et al.*,¹⁵⁾ as described in a previous paper.¹⁶⁾ NLA was determined by the method of Takahashi *et al.*¹⁷⁾ For determination of PL in liver, the rat liver, thoroughly perfused *in situ* with ice-cold 0.9% NaCl, was excised and homogenized with 5 volumes of 10 mM phosphate buffer (pH 7.4). To the homogenate (1 vol.), acetonitrile (2 vol.) was mixed, followed by centrifugation at 10000 rpm for 10 min. The supernatant fraction was used for the determination (95% the recovery) of PL by the method described above.^{15,16)} Glucose levels in the liver were determined by the method of Sasaki¹⁸⁾ following homogenization with a 10 mM phosphate buffer, pH 7.4.

Analysis of Data Kinetic parameters were calculated by using the least-squares fit program, MULTI.¹⁹⁾ The plasma PL concentration data after oral administration were fitted to the equation²⁰⁾:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} - (A + B)e^{-k_a t} \quad (1)$$

where C_t is the drug concentration at time t , and A , α , B and β are the biexponential equation constants, and k_a is the apparent absorption rate constant.

The area under the plasma concentration–time curve (AUC) was determined by the trapezoidal method to the last observed data point. The residual area beyond the last sampling time was estimated as C'/β , where C' is the last observed concentration. The absolute bioavailability was calculated using the AUC values. The area under the first moment curve ($AUMC$) and the mean residence time (MRT) were calculated by means of the moment analysis:

$$AUMC = \int_0^{\infty} t \cdot C dt \quad (2)$$

$$MRT = AUMC/AUC \quad (3)$$

where k_e is the elimination rate constant.

All plasma concentrations were multiplied by the blood-to-plasma concentration ratio to convert the values to blood concentrations. The systemic clearance (Cl_s), the hepatic extraction ratio (E) and the apparent hepatic blood flow (Q) were calculated by means of the following equations:²¹⁾

$$Cl_s = \text{dose}_{iv}/AUC_{iv} \quad (4)$$

$$E = 1 - F \quad (5)$$

$$Q = Cl_s/E \quad (6)$$

where F is the bioavailability. AUC_{iv} is the AUC after intravenous (i.v.) injection of PL.

The means of all data are presented with their standard deviation (mean $\pm$ S.D.). Statistical analysis was performed using the unpaired Student's *t*-test and the one-way analysis of variance (ANOVA) with Scheffé's multiple comparison procedure, and the significance level adopted was $p < 0.05$.

RESULTS

Plasma Concentration of PL after Single i.v. Administration The plasma concentration after a single i.v. administration of PL hydrochloride (4 mg/kg) showed biexponential kinetics, similarly to our previous paper.¹⁶⁾ The AUC value was 1672.0 ± 73.2 h·ng/ml.

Effect of Various Meals on the Absorption and Disposition of PL PL was orally administered both to rats given 1.5% CMC and to those fasted. The plasma PL concentrations of these rats are shown in Fig. 1. In both groups, the disappearance of PL in plasma was apparently fitted to biexponential kinetics, although the CMC rats had slightly slower elimination profiles at the later time periods. There were no significant differences in k_a , $AUC_{0-\infty}$ or bioavailability between the two groups, as shown in Table I.

PL was administered orally to rats given 20% soybean oil emulsion or 20% lauric acid–oleic acid emulsion. The plasma PL concentrations of the rats are depicted in Fig. 2. In the group given soybean oil emulsion, the peak plasma concentration (C_{max}) appeared slightly later than that after a fatty acid emulsion, suggesting the reduced absorption rate and/or reduced release rate of PL from the oil. Both types of fat containing meals gave significantly less AUC and bioavailability compared to the data of the fasted rats ($p < 0.05$).

The plasma PL concentrations of the two groups which were given 20% glucose and 20% protein (skim milk) meals, and the pharmacokinetic parameters calculated,

Fig. 1. Plasma Concentration of PL after a Single *p.o.* Administration under a Fasting State and Together with CMC (3 ml)

—○—, fasting; —●—, CMC. Each point represents the mean $\pm$ S.D. ($n = 5-6$). Dose: 20 mg/kg as propranolol.

TABLE I. Pharmacokinetic Parameters of PL Following Single *p.o.* Administration under Fasting State and Together with a Meal

Parameter	Fasting	CMC	Soybean oil	Fatty acid	Glucose	Protein
k_a (h^{-1})	3.16 ± 1.22	2.59 ± 1.51	4.88 ± 2.37	3.13 ± 2.06	—	2.63 ± 0.36
β or k_e (h^{-1})	0.484 ± 0.200	0.276 ± 0.176	0.493 ± 0.142	0.275 ± 0.161	0.284 ± 0.170^a	0.365 ± 0.161
$AUC_{0-\infty}$ (ng·h/ml)	2066 ± 343	1981 ± 890	721 ± 327^b	1236 ± 504	2499 ± 780	2763 ± 708
MRT (h)	2.38 ± 0.88	5.51 ± 2.93	2.57 ± 0.66	5.08 ± 0.99	—	4.35 ± 3.03
Bioavailability (%)	24.7 ± 4.1	23.7 ± 10.6	$8.6 \pm 4.9^{c,d}$	14.8 ± 6.1^e	29.9 ± 8.5	33.0 ± 11.9

Each value represents the mean $\pm$ S.D. ($n=4-7$). Dose: 20 mg/kg as PL. $AUC_{i.v.}$ (4 mg/kg): 1672.0 ± 73.2 (ng·h/ml). *a*) k_e , calculated from the slope of elimination phase. *b*) $p < 0.05$ compared to fasting and protein. *c*) $p < 0.005$ compared to fasting. *d*) $p < 0.05$ compared to glucose. *e*) $p < 0.05$ compared to fasting and glucose.

Fig. 2. Plasma Concentration of PL after a Single *p.o.* Administration with Soybean Oil and Fatty Acid (3 ml)

—○—, soybean oil; —●—, fatty acid. Each point represents the mean $\pm$ S.D. ($n=4-5$). Dose: 20 mg/kg as propranolol.

Fig. 3. Plasma Concentration of PL after a Single *p.o.* Administration with Glucose and Protein (3 ml)

—○—, glucose; —●—, protein. Each point represents the mean $\pm$ S.D. ($n=6-7$). Dose: 20 mg/kg as propranolol.

are shown in Fig. 3 and Table I, respectively. The rats given a skim milk meal showed the highest C_{max} value (1914 ± 365 ng/ml) among the groups tested, resulting in a higher AUC and greater bioavailability. On the other hand, in the rats given a 20% glucose meal, low plasma concentration during the early time periods and a secondary increase in plasma PL decay curve about 2 h post-dosing were observed, although after this time, the decay of plasma PL was approximately parallel to that after skim milk. Thus, a slightly higher bioavailability (29.9%), compared to that of the fasted rats, was obtained in the rats given glucose, as shown in Table I. These results suggest a transient decrease in the hepatic clearance of PL, or a delayed absorption of PL at a high glucose concentration.

Hepatic Blood Flow after the Intake of Various Meals Since PL is totally absorbed after oral administration²²⁾ and completely eliminated by hepatic metabolism,²³⁾ the apparent hepatic blood flow (Q) was calculated from the Cl_s and E by Eq. 6. The Q values (ml/min·kg) calculated were 53.0 ± 3.3 , 52.3 ± 6.7 , 56.9 ± 18.8 and 59.5 ± 18.1 for Fasting, CMC, glucose and protein groups, respectively. Protein (skim milk) feeding caused a slight (12%) increase in the apparent hepatic blood flow, but it was not significant compared to that of the fasted and CMC rats. This result suggests that a main factor influencing the effect of food is not the transient increase in hepatic blood flow, although the protein meal may

partially contribute to the food effect. This was consistent with the data shown by Modi *et al.*¹¹⁾ that the effect of food on PL bioavailability is apparently not principally the result of a change in Q , but must be due to other mechanisms such as a decrease in apparent intrinsic metabolic clearance.

Hepatic Uptake of PL and NLA Level after Glucose and Protein Intake To clarify whether or not the hepatic uptake of PL is inhibited at high glucose concentrations, the hepatic uptake of PL and NLA level was determined after glucose and protein intake. The amount of PL and NLA in the liver at 1 h after dosing of the drug (at 1.5 h after 20% glucose) was 0.67 ± 0.07 and 0.29 ± 0.05 μ g/g wet liver, respectively, for rats given glucose, 12.56 ± 1.29 and 2.09 ± 2.56 μ g/g wet liver, respectively, for rats given protein, and 12.86 ± 5.86 and 1.66 ± 0.72 μ g/g wet liver, respectively, for rats given CMC ($p < 0.01$). These clearly demonstrate that the hepatic uptake of PL was significantly decreased in the presence of glucose at high levels, but the uptake was not affected by the protein meal. The glucose levels in liver at 1.5 h after meals were 1.21 ± 0.25 mg/g wet liver for CMC group and 6.70 ± 0.92 mg/g wet liver for glucose group ($p < 0.01$).

Effect of Protein and Glucose Meals on NLA Disposition NLA, like PL glucuronide, is shown to be a main metabolite in plasma after the oral administration of PL.¹⁷⁾ To further clarify the mechanism involved in the inhibitory effect of dietary constituents on the hepatic

Fig. 4. Plasma Concentration of NLA after a Single *p.o.* Administration of PL with Meal (6 ml)

A: —○—, CMC; —●—, protein. B: —▲—, glucose type 1; —△—, glucose type 2. Each point represents the mean $\pm$ S.D. ($n=5-8$). Dose: 20 mg/kg as propranolol.

TABLE II. Pharmacokinetic Parameters of NLA Following Single *p.o.* Administration of PL Together with a Meal

Parameter	CMC	Glucose		Protein
		Type 1	Type 2	
AUC (ng·h/ml)	247 ± 68	163 ± 39^a	187 ± 58	383 ± 78^b
C_{max} (ng/ml)	177 ± 85	132 ± 62	136 ± 66	387 ± 93^c
AUC_{meal}/AUC_{CMC}	1	0.66	0.76	1.55

Each value represents the mean $\pm$ S.D. ($n=5-8$). Types 1 and 2 are rats with a secondary increase in the plasma NLA decay curve and with a very low plasma concentration at the initial time stage, respectively. ^a $p < 0.05$ compared to CMC. ^b $p < 0.01$ compared to glucose. ^c $p < 0.001$ compared to glucose and CMC.

TABLE III. Hepatic 9000 *g*-Supernatant Drug Metabolizing Enzyme Activities 1 or 3 h after Single *p.o.* Administration of PL with CMC and Glucose

Activity	Time (h)	CMC	Glucose
UDPGT (%)	3	100 ± 8.2	98.3 ± 8.5
Cytochrome P-450	1	0.222 ± 0.032	0.175 ± 0.012
(nmol/mg protein)	3	0.220 ± 0.021	0.155 ± 0.008^a

Each value represents the mean $\pm$ S.D. ($n=4-8$). Dose: 20 mg/kg as propranolol. ^a $p < 0.01$ compared to CMC.

metabolism of PL, NLA concentrations in plasma were measured after oral meals (6 ml each) and PL dosing. The results are shown in Fig. 4. Although the plasma decay curve of NLA after skim milk was relatively similar to that after CMC, the plasma concentrations during the initial time periods were much higher than those after CMC, resulting in higher AUC and C_{max} values than the CMC group. The profiles after 20% glucose were abnormal, and there were two types of the plasma concentration profile, each of which had negligible or low concentrations at the initial time stage and the C_{max} at 2–3 h after dosing, with a secondary peak at 3 h post-dosing, as shown in Fig. 4. The kinetic parameters calculated are shown in Table II. The AUC values for the glucose group were significantly decreased compared to those of the CMC group ($p < 0.05$).

Effect of Glucose Meal on the Metabolizing Enzyme Activities A possible mechanism of the increased bioavailability may be the direct inhibition of PL metab-

olism in liver. Thus, both the content of a microsomal oxygenase, P-450, and the UDPGT activity were determined after glucose intake. The P-450 content was significantly decreased (a 19 and 29% decrease at 1 and 3 h, respectively) by glucose intake compared to that after CMC, as shown in Table III. However, UDPGT activity was not altered by glucose intake. This indicated that a high uptake of glucose would decrease the hepatic P-450 content, but the mechanism is not yet clear.

On the other hand, the metabolizing enzyme activities of some drugs, including aniline hydroxylase and UDPGT activities, were determined *in vitro* in the presence of glucose (2% in the incubation medium). No decrease in these activities was observed in the presence of glucose [aniline hydroxylase activity, 0.524 ± 0.093 nmol/min/mg protein (control) and 0.522 ± 0.085 nmol/min/mg protein (glucose); UDPGT activity, 100% (control) and $108.6 \pm 32.2\%$ (glucose)]. This suggests that glucose existing in the liver did not directly inhibit PL metabolism.

DISCUSSION

This study in rats has demonstrated that protein (skim milk) intake slightly increased the AUC and bioavailability of PL and PL metabolism to NLA, while glucose intake significantly decreased the hepatic uptake of PL and P-450 content at high glucose levels, resulting in the inhibition of the metabolic conversion of PL to NLA.

In man the oral systemic bioavailability of PL is significantly enhanced when it is coadministered with food.⁷⁾ This has been shown to result in a mean increase of 60% in the AUC for PL.^{6,24)} However, in rat, the increase in bioavailability when PL was coadministered with food (skim milk) was much less compared to the increase in man. This may be attributed to the species differences, probably due to the fact that rats metabolize drugs at a faster rate than man,²⁵⁾ and to the significant response of the effect of food in man. The food effect has been ascribed to a transient increase (34%) in hepatic blood flow.^{9,10)} Consequently, it is unlikely that the increase in the hepatic blood flow is the principal cause of the food effect. Therefore, a study designed to elucidate other mechanisms, such as a decrease in metabolic

clearance, was carried out in rats.

Our data that showed the *AUC* value for PL after glucose intake to be approximately similar to that after CMC intake and under a fasting state, in spite of the low plasma concentration during the early time periods after glucose intake, suggest that the delayed gastrointestinal absorption of PL was partly involved in the low plasma concentration at the initial time stage after dosing. This result was similar to the report that a low fat (high-carbohydrate) meal slows the absorption rate but does not alter the extent of nifedipine absorption.²⁶⁾ The PL plasma concentration at the initial time stage, however, did not reflect the extremely low hepatic uptake of PL observed 1 h after dosing. This indicates that the transfer of PL into the hepatic cells was dramatically inhibited at high glucose levels, although the mechanism was not indicated by our experiments. However, there is a possibility that the intensive active transport of glucose into the cells or the glucose–insulin balance²⁷⁾ may affect the decreased uptake of PL. Low bioavailability after the intake of either soybean oil or fatty acid may be due to the reduced gastric emptying and decrease in absorption rate based on the slow release rate of PL from the emulsions.

Modi *et al.*¹¹⁾ suggest that the effect of food on PL bioavailability may be due to a decrease in apparent intrinsic metabolic clearance or to alterations in the plasma protein binding of a drug in hepatic sinusoidal blood, presuming there is no change in hepatic blood flow. In this study, glucose intake significantly, but probably transiently, inhibited the metabolism of PL to NLA, although another important metabolic pathway, ring oxidation to 4-hydroxypropanolol, was not determined, due to the low plasma level of the metabolite. However, there is a possibility that the ring oxidation may also be reduced by glucose intake, as well as side chain oxidation to NLA. It has been demonstrated from this study that the inhibition of PL metabolism was largely, but not completely, due to a decrease in hepatic P-450 content and to a transient decrease in the hepatic uptake of PL. The mechanism underlying this glucose-dependent decrease in P-450 content is still obscure. However, this decrease may be due to more diverse effects on intermediary metabolism and hormone balance, not a direct effect on the enzymes. High glucose intake has also been shown to inhibit barbiturate metabolism, and to decrease hepatic P-450 content and biphenyl-4-hydroxylase activity.²⁸⁾

Liedholm and Melander²⁴⁾ suggest that food does not interfere with presystemic hydroxylation or dealkylation of PL, and that concomitant food intake may selectively inhibit presystemic conjugation of PL. Our study clearly demonstrated the inhibition of oxidation, with no inhibitory effect on the glucuronidation. PL is metabolized through three distinct pathways, including oxidation and conjugation.²⁹⁾ NLA is an important metabolite of PL formed by primary dealkylation mediated by P-450 enzymes and secondary oxidation. The decreased hepatic uptake of PL at high glucose levels, however, may partly explain the inhibition of PL conjugation, based on their report of delayed hepatic uptake.²⁴⁾

McLean *et al.*⁶⁾ investigated the effect of food intake

on the plasma concentrations of PL in man, and revealed that protein–lipid and carbohydrate feeding increased the peak concentration and *AUC*. In their case, the drug elimination from plasma was much slower after the carbohydrate feeding than that after protein–lipid feeding. Our result that glucose intake significantly reduced P-450 content reasonably explains the slow elimination of PL after carbohydrate feeding in man, based on the metabolic inhibition.

A possibility that the effect of food may be ascribed to the synergetic effect of glucose and other nutritional elements intaken, or to an alteration in the protein binding of drug, in addition to the results we obtained, remains to be determined.

In conclusion, a possible mechanism for the effect of food on PL bioavailability was due largely to the decreased microsomal oxygenase, P-450, based on the inhibition of biosynthesis, and to the dramatically decreased hepatic uptake of PL after glucose intake, resulting in a decrease in PL metabolism.

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