INFLUENCE OF THE ROUTE OF ADMINISTRATION ON THE MEAN HEPATIC EXTRACTION RATIO OF PROPRANOLOL IN THE RAT

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The mean hepatic extraction ratio (ER) of propranolol was estimated directly by simultaneous measurements of arterial and hepatic venous blood concentrations of the drug following systemic venous and portal venous administration in the rat. The ER was greater than 0.9 in the dose range of 2.5 to 12.5 mg/kg following rapid infusion of propranolol into the femoral vein and was not dependent on infusion rate. On the other hand, the ER following intraportal constant-rate infusion decreased progressively with increasing dose, although the ER at an intraportal dose of 2.5 mg/kg was as high as that found after administration into the femoral vein. In addition, it was found that the ER at an intraportal dose of 12.5 mg/kg of propranolol was significantly influenced by infusion rate. The unusual AUC-dose relationship of propranolol previously reported in the rat could be explained on the basis of the present nonlinear hepatic extraction depending on the route and rate of administration which was clarified in vivo. The nonlinear hepatic extraction was further confirmed by determining the remarkably decreased ER of 14C-propranolol given intravenously after pretreatment or during portal venous administration of unlabelled propranolol.

Keywords—propranolol; mean hepatic extraction ratio; first-pass effect; route of administration; rats; rate-dependent elimination; hepatic venous blood sampling

INTRODUCTION

It has been shown that low availability of the orally administered propranolol results from the fact that all of the absorbed drug in the portal vein must pass through the liver where a substantial part of it can be eliminated before reaching the systemic circulation.1) Of particular interest is the finding that the relationship between the area under the blood concentration curve (AUC) and oral dose was unusually complicated in that the availability of the dose administered is dose-dependent despite complete gastrointestinal absorption.2)

Similar results were obtained in our studies in the rat by hepatic portal venous and systemic venous administration of propranolol.3, 4) No propranolol appeared in the systemic circulation after portal venous infusion at doses less than 2 mg/kg. The mean AUC of propranolol dosed intraportally with 5 mg/kg of propranolol was 42% of that found after intravenous infusion, and the AUC after portal venous infusion increased progressively with increasing infusion rate at a constant dose. In addition, the bioavailability of propranolol assessed by comparison of the AUC’s following hepatic portal venous and systemic venous administration was found to increase with increasing dose, but remained almost constant at a constant infusion rate in the range of about 5 to 12.5 mg/kg. Therefore, the hepatic elimination of the drug during the first passage through the liver was both dose-dependent and rate-dependent in the rat.

Elimination of propranolol occurs almost wholly by metabolic transformation, and therefore it is quite possible that the AUC of propranolol varies with the efficiency of extraction by the liver. Extraction of propranolol by the dog liver was studied by simultaneous measurements in vivo of arterial and hepatic venous concentrations
of the drug during continuous intravenous infusion.\textsuperscript{5} The hepatic extraction ratio was greater than 0.9 at systemic blood levels of 0.1 $\mu$g/ml which would produce maximum blockade of endogenous $\beta$-adrenergic stimulation. Later, George et al. showed that relatively high concentrations of propranolol appeared in the arterial blood of the dog following portal venous administration.\textsuperscript{6} This data was interpreted on the presumption that a larger amount of the drug than predicted from the high hepatic extraction ratio determined after continuous intravenous infusion reached the systemic circulation when administered by the portal venous infusion. These findings suggest that the hepatic extraction ratio of propranolol is highly dependent on the route of administration.

It is the purpose of this study to present the effect of the route of administration of propranolol on its hepatic extraction ratio in the rat, and to elucidate the mechanisms responsible for its nonlinear first-pass effect.

MATERIALS AND METHODS

Materials — Propranolol and $^{14}$C-propranolol were kindly furnished by Sumitomo Chemical Co., Ltd., and were used as hydrochloride and racemic compounds throughout this study without further purification (mp 162—163\textsuperscript{o}). The $^{14}$C-propranolol was labelled at the carbon adjacent to isopropylamino group with a specific activity of 12.9 $\mu$Ci/mg. The labelled compound was synthesized by the method of Yoshitake, et al.\textsuperscript{7} Its radiochemical purity was estimated to be more than 98\% by thin-layer chromatography developed in a solvent system (CHCl\textsubscript{3}: $n$-propanol:NH\textsubscript{4}OH = 12:7:1). Bromsulphalein (BSP) used in this study was a 5\% (w/v) commercial injection (Hepatosulphalein, Daichiseiyaku Co., Ltd.). All other chemicals used in this study were of reagent grade.

Analytical Methods — Blood concentrations of propranolol were determined spectrophotofluorometrically by a minor modification of the method of Shand, et al.\textsuperscript{1} One milliliter of distilled water was added to 0.1 to 0.2 ml of whole blood. The mixture was then alkalized with 1 ml of 1N NaOH, and extracted into 12 ml of $n$-heptane containing 1.5\% isoamylalcohol. After shaking mechanically for 10 min and centrifuging, 10 ml of the heptane layer was extracted into 1 ml of 0.1N HCl, and the fluorescence of the acid layer was determined in a HITACHI Model 204 spectrophotofluorometer (maximum excitation at 295 nm and maximum emission at 340 nm). $^{14}$C-Propranolol were determined by measuring the $^{14}$C-radioactivity of the acid layer mentioned above. Fifteen ml of a scintillator (PPO 12 g, dimethyl-POPOP 0.4 g, anisole 125 ml, toluene 50 ml, dioxane 750 ml) was added to 0.5 ml of the acid layer and the radioactivity of the acid layer was counted in a Beckman DPM 100 liquid scintillation spectrophotometer. The correction for quenching was made by use of a correction curve based on the external standard method. The minimum quantitative concentrations for propranolol and $^{14}$C-propranolol were 40 ng/ml and 10 ng/ml for 0.1 ml whole blood sample, respectively. Plasma BSP concentrations were determined after addition of 0.02 ml of plasma to 0.2 ml of 0.05N NaOH immediately before assay. Optical densities were recorded at 578 nm against blanks using a HITACHI 181 spectrophotometer.

Animal Experiments — Male Wistar rats weighing 270—350 g were used in all experiments, and had free access to food and water till before the experiments. The animals were anesthetized lightly with ether at suitable intervals as needed during surgery. A cannula inserted into the right femoral vein was used for rapid intravenous administration of propranolol and/or BSP and also for transfusion of an equal volume of blood to a withdrawn blood sample in order to avoid circulatory disturbance resulted from blood loss. The blood for transfusion was prepared by adding 0.4 ml of 10\% sodium citrate to 4.6 ml of blood taken from another rat of the same strain. Another cannula was inserted into the left femoral vein when BSP was infused at a constant rate. A portal venous dose of propranolol was given through a cannula inserted into the portal vein via the pyloric vein.\textsuperscript{3} Blood samples (0.1 to
0.2 ml) were withdrawn simultaneously from the femoral artery and hepatic vein. The arterial blood samples were taken through a cannula inserted into the femoral artery, and the hepatic venous blood samples were taken through another cannula introduced to the inferior vena cava by the technique for sampling hepatic venous blood reported previously. The experiments were started at least 90 min after the operations mentioned above.

**Determination of Hepatic Blood Flow** — A decrease in hepatic blood flow was estimated according to the method of Bradley, et al. A loading dose of 25 mg/kg BSP was injected into the femoral vein of the rat and immediately followed by a constant-rate infusion of the dye into the opposite femoral vein at a rate of 0.83 mg/kg/min throughout the experiment. Five blood samples (0.15 ml each) were simultaneously withdrawn from the hepatic vein and femoral artery every 10 min starting at 30 min after the initiation of the BSP injection. A dose of either 2.5 mg/kg or 12.5 mg/kg propranolol was given through the femoral vein or hepatic portal vein 10—20 min after the last sampling. Five more blood samples were withdrawn in the same way as the previous sampling from 15—20 min after propranolol administration. The plasma separated by centrifugation was stored at 4°C until assay. Hematocrits were measured in the first and in the last arterial and hepatic venous blood samples and averaged. The estimated hepatic blood flow (Q) in ml/min was calculated from the following formula:

\[
Q = \frac{R}{(A - H)} \times \frac{100}{100 - \text{Hematocrit}} \quad (\text{Eq. 1})
\]

where \(A\) and \(H\) are the arterial and hepatic venous plasma concentrations of BSP in \(\mu g/ml\), respectively, and \(R\) is the hepatic removal rate of the dye in \(\mu g/\text{min}\). When the BSP level was not kept constant, \(R\) was calculated by Eq. 2.

\[
R = I - (\Delta P \times V_d) \quad (\text{Eq. 2})
\]

Where \(I\) is the rate of infusion in \(\mu g/\text{min}\), \(\Delta P\) is the changing rate of the arterial BSP concentration in \(\mu g/ml/\text{min}\) and \(V_d\) is the volume of distribution of BSP in ml. The volume of distribution was determined in three rats by giving loading and continuously infusing doses of BSP as mentioned above. The changing rate \((\Delta P_3)\) of the arterial BSP concentration was determined using four blood samples withdrawn every 10 min beginning at 30 min after the initiation of the BSP infusion. Further, the changing rate \((\Delta P_3)\) of the arterial BSP concentration was determined in the same way after the infusion rate was doubled. The volume of distribution was estimated from the following equation, assuming that the hepatic removal rate was constant.

\[
V_d = \frac{I}{\Delta P_2 - \Delta P_1} \quad (\text{Eq. 3})
\]

The mean volume of distribution was estimated to be 12.2±0.6(S.E.) ml/100 g body weight.

**RESULTS**

The instantaneous clearance (\(\bar{V}_{CL}\)) of a drug by an eliminating organ was defined as

\[
\bar{V}_{CL} = \bar{V}_B \left( \frac{C_i - C_o}{C_i} \right) \quad (\text{Eq. 4})
\]

where \(C_i\) and \(C_o\) are the concentrations of drug entering and leaving the elimination organ at the same instant of time and \(\bar{V}_B\) is the blood flow across the organ. The term in parentheses is the instantaneous extraction ratio of the drug by the organ. The mean clearance was defined by Eq. 5.

\[
\bar{V}_{CL} = \frac{\int_0^\infty \bar{V}_B (C_i - C_o) \, dt}{\int_0^\infty C_i \, dt} \quad (\text{Eq. 5})
\]

The mean extraction ratio (ER) may be defined by Eq. 6 involving the integrated \(C_i\) and \(C_o\) between \(t=0\) and \(t=\infty\), assuming the blood flow remains constant, and therefore it may be regarded as the ratio of the total extracted
\[ ER = \frac{\int_0^{\infty} C_i \, dt - \int_0^{\infty} C_o \, dt}{\int_0^{\infty} C_i \, dt} \tag{Eq. 6} \]

amount of drug to the cumulative amount of drug entering the organ.

The liver receives blood from the hepatic portal vein and hepatic artery. The mean concentration of propranolol entering the liver is given by

\[ \bar{C}_i = \frac{\bar{V}_{HP} C_{HP} + \bar{V}_{HA} C_{HA}}{\bar{V}_{HV}} \tag{Eq. 7} \]

where \( C_{HP} \) and \( C_{HA} \) are concentrations of propranolol in the hepatic portal vein and hepatic artery, and \( \bar{V}_{HP} \), \( \bar{V}_{HA} \), and \( \bar{V}_{HV} \) are the blood flow in the hepatic portal vein, hepatic artery, and hepatic vein, respectively (\( \bar{V}_{HV} = \bar{V}_{HP} + \bar{V}_{HA} \)). \( C_{HA} \) can be replaced by the concentration of propranolol in any artery (\( C_{art} \)), and the AUC of propranolol determined using the portal venous blood was found to equal that determined using the arterial blood after rapid or 50 min intravenous administration of propranolol.\(^{12} \)

Therefore, the cumulative amounts of drug (\( M_{iv} \) and \( M_{ipv} \)) entering the liver via systemic venous and hepatic portal venous routes are given Eq. 8 and 9.

\[ M_{iv} = \bar{V}_{HV} \int_0^{\infty} C_{art} \, dt \tag{Eq. 8} \]
\[ M_{ipv} = (Dose)_{ipv} + \bar{V}_{HV} \int_0^{\infty} C_{art} \, dt \tag{Eq. 9} \]

The mean hepatic extraction ratios (\( \bar{ER}_{iv}^{iv} \) and \( \bar{ER}_{iv}^{ipv} \)) of propranolol for the both routes were determined by Eq. 10 and 11:

\[ \bar{ER}_{iv}^{iv} = \frac{\int_0^{\infty} C_{art} \, dt - \int_0^{\infty} C_{HV} \, dt}{\int_0^{\infty} C_{art} \, dt} \tag{Eq. 10} \]
\[ \bar{ER}_{iv}^{ipv} = \frac{(Dose)_{ipv} + Q \int_0^{\infty} C_{art} \, dt - \int_0^{\infty} C_{HV} \, dt)}{(Dose)_{ipv} + Q \int_0^{\infty} C_{art} \, dt} \tag{Eq. 11} \]

where \( Q \) is the estimated hepatic blood flow, and \( \int_0^{\infty} C_{HV} \, dt \) and \( \int_0^{\infty} C_{art} \, dt \) are AUC’s calculated

FIG. 1. Effect of Propranolol on Hepatic Blood Flow

- ○: Intravenous administration
- △: Intraperitoneal administration

Propranolol was injected over 30 sec, and the hepatic blood flow was determined during 50 min from 15 to 20 min after administration of propranolol. Results are expressed as percent decrease of the initial value before administration of propranolol. Vertical bars show standard errors of the mean from 4 experiments.

FIG. 2. Relationship between Mean Hepatic Extraction Ratio and Dose, and Relationship between the Area under the Blood Concentration – Time Curve (AUC) and Dose after Intravenous Administration of Propranolol over 30 sec

- ○: Relationship between mean hepatic extraction ratio and dose
- △: Relationship between AUC and dose

Vertical bars show standard errors of the mean from 3 to 4 experiments.
using propranolol concentrations in the hepatic venous blood and in any arterial blood, respectively.

The hepatic blood flow was determined before and after administration of propranolol. Fig. 1 illustrates that the estimated hepatic blood flow after propranolol administration was reduced to approximately 70% of its initial value before administration in the range of 2.5 to 12.5 mg/kg, irrespectively of the route of administration. A similar result was previously shown after oral administration of dl-propranolol in six unanesthetized monkeys. A mean hepatic initial flow of 4.12±0.33 (S.E.) ml/min/100g body weight obtained with 15 rats in this study was in a very close agreement with a mean hepatic blood flow of 4.06±0.22 (S.E.) ml/min/100g body weight obtained in our previous study. Therefore, an estimated hepatic blood flow of 2.8 ml/min/100g body weight, 70% of a mean flow rate for these 45 rats, was used in the calculation of the mean extraction ratio (Eq.11) upon hepatic portal administration.

Both the AUC and mean hepatic extraction ratio of propranolol following its administration into the femoral vein are shown in Fig. 2. The AUC of propranolol given into the femoral vein was directly proportional to dose as was previously reported. The mean hepatic extraction ratio of propranolol calculated by Eq. 10 was high in all rats, averaging 0.99 and 0.93 in rats receiving

<table>
<thead>
<tr>
<th>Infusion time (min)</th>
<th>AUC (min·µg/ml)</th>
<th>Mean hepatic extraction ratio</th>
</tr>
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<tbody>
<tr>
<td>0.5</td>
<td>269.1±27.3</td>
<td>0.93±0.01</td>
</tr>
<tr>
<td>50.0</td>
<td>272.4±5.9</td>
<td>0.89±0.02</td>
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A dose of 12.5 mg/kg propranolol was administered at two different constant-rates. Results represent the mean ± standard error of 3 to 4 experiments.
intravenous doses of 5 and 12.5 mg/kg, respectively. The AUC and mean hepatic extraction ratio in rats dosed with 50 min constant-rate infusion of 12.5 mg/kg propranolol into the femoral vein were 101 and 96% of those found after 30 sec administration of an equal dose, respectively (Table I). There was no significant difference between the AUC's and also between the mean hepatic extraction ratios in rats receiving an equal dose at different infusion rates, despite the wide variation in infusion rate. Therefore, the hepatic extraction following systemic venous administration of propranolol is considered to be almost complete and also independent of its infusion rate.

On the other hand, the relationship between the AUC and dose was not linear in rats infused into the portal vein with 2.5 to 12.5 mg/kg propranolol (Fig. 3). Extremely small propranolol concentration was found in the hepatic venous blood at a dose of 2.5 mg/kg. A mean hepatic extraction ratio of 0.97 at a dose of 2.5 mg/kg decreased progressively with increasing dose and fell to 0.46 at a dose of 12.5 mg/kg. The effect of infusion rate on the AUC and mean hepatic extraction ratio was examined at three different infusion rates upon portal venous dosing of 12.5 mg/kg propranolol. The AUC and mean hepatic extraction ratio plotted against infusion time are shown in Fig. 4. The mean hepatic extraction ratio increased with decreasing infusion rate, resulting in the decrease of AUC. From the data described above, it was shown that the mean hepatic extraction ratio is dependent on the rate of administration into the portal vein as well as the route of administration.

![Blood concentration vs Time](attachment:image.png)

**Fig. 5. Arterial and Hepatic Venous Blood concentration—Time Curves of $^{14}$C-Propranolol and Total Propranolol**

- ○: Arterial blood concentration of $^{14}$C-propranolol
- ▲: Hepatic venous blood concentration of $^{14}$C-propranolol
- ○: Total arterial blood concentration of unlabelled propranolol and $^{14}$C-propranolol

(a) Five mg/kg of propranolol was administered into the hepatic portal vein over 30 sec, and after an interval of 30 sec and an equal dose of $^{14}$C-propranolol was administered into the femoral vein over 30 sec. Vertical bars show standard errors of the mean from 3 experiments.

(b) Seven mg/kg $^{14}$C-propranolol alone was administered over 30 sec into the femoral vein in a rat as a control experiment.
To ascertain the nonlinear hepatic extraction of propranolol, the mean hepatic extraction ratio of $^{14}$C-propranolol given intravenously into the femoral vein was determined using rats pretreated with unlabelled propranolol. Five mg/kg of propranolol was administered into the hepatic portal vein over 30 sec, and after an interval of 30 sec an equal dose of $^{14}$C-propranolol was administered into the femoral vein over 30 sec. Another rat was given 7 mg/kg $^{14}$C-propranolol into the femoral vein as a control experiment so that the arterial propranolol concentration was almost equal to the total arterial propranolol concentration obtained in the pretreatment study. The time courses of the arterial and hepatic venous concentrations of propranolol determined by radiochemical and/or spectrophotofluorometrical assay are illustrated in Fig. 5a and 5b. $^{14}$C-Propranolol was hardly detected in the hepatic venous blood of the rat without the pretreatment, and, therefore, the mean hepatic extraction ratio was almost a unity. On the other hand, $^{14}$C-propranolol given into the femoral vein appeared much more in the hepatic venous blood after loading with unlabelled propranolol, and the mean hepatic extraction ratio was calculated to be 0.68±0.03 (mean ± S.E., $n = 3$).

Three mg/kg $^{14}$C-propranolol and 12.5 mg/kg unlabelled propranolol were simultaneously administered over 50 min period into the femoral and portal vein in rats, respectively, to confirm further the nonlinear hepatic extraction of propranolol. As a control experiment, 4 mg/kg $^{14}$C-propranolol alone was administered into the femoral vein of another rat. The time courses of the arterial and hepatic venous concentrations of propranolol were investigated by radiochemical and spectrophotofluorometrical assay.

**FIG. 6.** Arterial and Hepatic Venous Blood Concentration - Time Curves of $^{14}$C-Propranolol

- ● : Arterial blood concentration of $^{14}$C-propranolol
- ▲ : Hepatic venous blood concentration of $^{14}$C-propranolol

(a) Three mg/kg $^{14}$C-propranolol and 12.5 mg/kg unlabelled propranolol were simultaneously administered over 50 min into the femoral and portal vein, respectively. Vertical bars show standard errors of the mean from 3 experiments.

(b) Four mg/kg $^{14}$C-propranolol alone was administered over 50 min into the femoral vein in a rat as a control experiment.
$^{14}$C-propranolol are illustrated in Fig. 6a and 6b. The $^{14}$C-radioactivity in the hepatic vein was almost below the limit of assay after intravenous administration of $^{14}$C-propranolol alone, and the mean hepatic extraction ratio was almost a unity. On the other hand, the mean hepatic extraction ratio was reduced to 0.73±0.03 (mean ± S.E., n = 3) upon the simultaneous portal venous infusion of unlabelled propranolol.

DISCUSSION

Propranolol was virtually completely absorbed after administration in man, rats, and dogs.\textsuperscript{14–16} The drug was eliminated almost entirely by metabolic transformation in the liver in man and rats, and only 1 to 4% of a radiolabelled dose was recovered as unchanged drug in the urine and feces.\textsuperscript{14–16} The relationship between the AUC of propranolol and dose was not linear, and did not extrapolate through the origin following oral administration or portal venous administration simulating complete oral absorption, while the AUC following intravenous administration was proportional to dose.\textsuperscript{1,2,4} The unusual disposition of propranolol depending on the route of administration has been presumed to result from its concentration-dependent hepatic clearance by in vitro perfusion of the rat liver.\textsuperscript{17,18}

The present in vivo experiments have confirmed quantitatively that the hepatic extraction of propranolol following portal venous administration was influenced by the rate of administration. The mean hepatic extraction ratio was 0.9 to 1.0 at intravenous doses of less than 12.5 mg/kg of propranolol, and was assumed to be essentially constant throughout the drug elimination phase. The mean hepatic extraction ratio at an intraportal dose of 2.5 mg/kg remained high, and only a trace of propranolol was detected in the hepatic venous blood. However, the mean hepatic extraction ratio of propranolol decreased progressively with increasing intraportal dose, that made larger portion of the drug available in the systemic circulation. The unusual AUC and dose relationship previously reported in the rat\textsuperscript{3,4} can be explained on the basis of the present nonlinear hepatic extraction depending on the route and rate of administration which was clarified in vivo.

The nonlinear hepatic extraction of labelled propranolol given intravenously was confirmed by the pretreatment and simultaneous administration of unlabelled propranolol into the hepatic portal vein. It is shown in Fig. 5 and 6, as would be expected, that the mean hepatic extraction ratio of labelled propranolol was decreased remarkably by the existence of unlabelled propranolol in the liver. This is probably because the drug concentration in the liver was sufficiently high to saturate metabolic enzyme systems.

The bioavailability of propranolol may not be assessed by comparison of the AUC’s following oral and intravenous administration, since the mean hepatic extraction ratio is quite different depending on the route of administration and the fraction metabolized during the first passage through the liver is dependent on the dose administered. For instance, the AUC’s following intravenous and portal venous administration were not significantly different at a dose of 12.5 mg/kg propranolol (Fig. 2 and 3), and it does not seem to show a first-pass effect. The bioavailability may be calculated to be almost 100%. However, propranolol was subject to a considerable first-pass effect, since the mean hepatic extraction ratio was more than 0.4. The presystemic hepatic extraction following portal venous administration resulted in less propranolol reaching the systemic circulation than following intravenous administration, but the hepatic clearance of the drug after reaching the systemic circulation was decreased following portal venous administration. The change in hepatic clearance counterbalanced the change in fraction of the drug reaching the systemic circulation, resulting in almost equal AUC’s after both administration. Therefore, the relative AUC following oral and intravenous administration of propranolol is not equal to the fraction of the administered drug reaching the systemic circulation. Quantitative in vivo studies on the nonlinear first-pass metabolism of propranolol will be presented in a subsequent paper.
REFERENCES


