NONLINEAR FIRST-PASS METABOLISM OF PROPRANOLOL IN THE RAT

TOKUJI SUZUKI, TOSHIAKI OHKUMA,* AND SADAO ISOZAKI**

Faculty of Pharmaceutical Sciences, University of Chiba,* Yayoi-cho, Chiba, Japan and Research Laboratory, Department of Pharmaceutical Services, University of Tokyo Hospital,** Hongo, Bunkyo-ku, Tokyo, Japan

(Received August 18, 1980)

The mean hepatic extraction ratio \( (\overline{ER})_{ip} \) of propranolol depending on the inflowing blood concentration to the liver was estimated directly by simultaneous measurements of arterial, hepatoportal, and hepatic venous blood concentrations of the drug following intravenous, intraportal, and intraduodenal administration in the rat. It was shown that the inflowing blood concentration to the liver caused considerable variation depending on the route of administration. The mean hepatic extraction ratio of propranolol in the first pass through the liver \( (\overline{ER})_{ip} \), and that of the drug after escaping the hepatic first-pass metabolism \( (\overline{ER})_{ir} \) were assessed by simultaneous administration of intraportal unlabelled propranolol and intravenous \(^{14}\text{C}\)-propranolol over a 50-min period. Consequently, a relation of \( (\overline{ER})_{ip} < (\overline{ER})_{ir} < (\overline{ER})_{ir} \) was observed in higher propranolol doses, if \( (\overline{ER})_{ir} \) refers to the overall mean hepatic extraction ratio following intraportal administration of propranolol. The fraction of orally administered dose reaching the systemic circulation for a drug exhibiting nonlinear hepatic first-pass metabolism was discussed. The unusual AUC-dose relationship of propranolol reported previously in the rat could be explained on the basis of both the nonlinear hepatic first-pass metabolism and the nonlinear hepatic metabolism of drug surviving the hepatic first-pass metabolism.

**Keywords**— propranolol; mean hepatic extraction ratio; nonlinear first-pass effect; route of administration; rat; dose-dependent bioavailability; hepatic venous blood sampling; simultaneous administration of unlabelled and \(^{14}\text{C}\)-labelled compounds

INTRODUCTION

Many drugs are known or suspected of having substantial hepatic first-pass metabolism which accounts for low availability in the systemic circulation following oral administration, despite complete gastrointestinal absorption.\(^{11}\) The hepatic clearance of such drugs is commonly high. For drugs which have linear disposition kinetics, the fraction of dose cleared in the first pass through the liver remains constant, and the bioavailability, i.e., the fraction of the administered dose appearing in the systemic circulation, is independent of dose unless absorption is incomplete.\(^{2}\) As a result, the area under the blood concentration-time curve (AUC) is proportional to the size of oral dose.

On the other hand, for drugs which exhibit nonlinear kinetics for hepatic metabolism, the fraction of dose cleared in the first pass through the liver becomes a function of dose and rate of absorption.\(^{1,2,6}\) Depending on the dose, rate of absorption, volume of distribution, and rate of distribution from plasma to tissues it is possible to achieve significantly higher hepatoportal concentrations after oral administration than are seen after intravenous doses, and to be sufficiently high to saturate metabolic enzyme systems.\(^{13}\) Under such circumstances, dose-dependent first-pass metabolism may occur. Remarkably dose-dependent bioavailability were shown for salicylamide,\(^{3}\) chlormethizole,\(^{4}\) 5-fluorouracil,\(^{5}\) and propranolol in man.\(^{6}\) Mechanisms responsible for dose-dependent bioavailability were presumed to be dose-dependent intestinal and/or hepatic me-
tabolism in the absorptive phase during initial transit of drug from the gastrointestinal lumen fluids to the systemic circulation.

In the preceding papers, the route- and rate-dependent hepatic first-pass metabolism of propranolol were demonstrated in the rat by intravenous and intraportal administration. Furthermore, the mean hepatic extraction ratios for both routes of administration were estimated directly by simultaneous measurements of arterial and hepatic venous blood drug concentrations, and the dose-dependent bioavailability of propranolol could be interpreted on the basis of the observed nonlinear hepatic extraction depending on the route and rate of administration.9)

It is the purpose of this study to present quantitative interpretations on the nonlinear first-pass metabolism of propranolol and the relationship between its bioavailability and the ratio between the AUC’s for intraportal and intravenous administration.

THEORETICAL
Mean Hepatic Extraction Ratio of Drug following Intravenous and Oral Administration

The present analysis relates to drugs which are not necessarily eliminated from the body only by hepatic metabolism, and assumes that gastrointestinal absorption is complete and gut wall metabolism is negligible. If the blood flow across an eliminating organ is assumed constant, then the mean extraction ratio \( \bar{E} \bar{R} \) of a drug by the eliminating organ is defined as:

\[
\bar{E} \bar{R} = \frac{\int_0^\infty C_i \, dt - \int_0^\infty C_o \, dt}{\int_0^\infty C_i \, dt}
\]

(Eq. 1)

where \( C_i \) and \( C_o \) are the concentrations of drug entering and leaving the eliminating organ at the same instant of time.9) The \( \bar{E} \bar{R} \) may be regarded as a ratio of the total amount of drug extracted to the cumulative amount of drug entering the organ by multiplying both the numerator and denominator of Eq. 1 by the blood flow across the organ.

The liver receives blood from the hepatic portal vein and hepatic artery. Thus, the drug concentration entering the liver \( (C_i)_H \) is given by:

\[
(C_i)_H = \frac{V_{HP} C_{HP} + V_{HA} C_{HA}}{V_{HV}}
\]

(Eq. 2a)

where, \( C_{HP} \) and \( C_{HA} \) are the concentrations of drug in the hepatic arterial and hepatoporal blood, and \( V_{HP}, V_{HA}, \) and \( V_{HV} \) are the blood flow in the hepatoporal vein, hepatic artery, and hepatic vein \( (V_{HV} = V_{HP} + V_{HA}) \), respectively. Integrating Eq. 2a between \( t=0 \) and \( t=\infty \) yields:

\[
\int_0^\infty (C_i)_H \, dt = \frac{V_{HP} \int_0^\infty C_{HP} \, dt + V_{HA} \int_0^\infty C_{HA} \, dt}{V_{HV}}
\]

(Eq. 2b)

Let drug be administered via the systemic venous routes, i.e., an intravenous dose. The \( C_{HA} \) in Eq. 2b may be replaced by the concentration in any artery \( (C_{art}) \). In addition, if the hepatoporal venous blood is collected from noneliminating organs and tissues, the AUC evaluated using concentrations in the hepatoporal blood equals that evaluated using concentrations in the arterial blood, i.e., \( \int_0^\infty C_{HP} \, dt = \int_0^\infty C_{HA} \, dt = \int_0^\infty C_{art} \, dt \). Substituting this relation into Eq. 2b gives \( \int_0^\infty (C_i)_H \, dt = \int_0^\infty C_{art} \, dt \). Consequently, the mean hepatic extraction ratio following intravenous administration \( (\bar{E} \bar{R})_iv \) is given by:

\[
(\bar{E} \bar{R})_iv = \frac{\int_0^\infty C_{art} \, dt - \int_0^\infty C_{HV} \, dt}{\int_0^\infty C_{art} \, dt}
\]

(Eq. 3)

Let drug be introduced via the hepatoporal vein, i.e., an oral dose. After passing through the liver, the drug distributes into the rest of the body and then enters liver again. The drug passes the liver repeatedly until it is eliminated completely. This recirculated drug results in a concentration \( (C_{HA})' \) in the hepatic arterial blood and a concentration \( (C_{HP})' \) in the hepatoporal blood. Assuming that the drug is introduced at a concentration \( (C_{HP})' \) in the hepatoporal blood, the total hepatoporal concentration entering the liver is equal to the sum of \( (C_{HP})' \) and \( (C_{HP})' \). Con-
sequently, the cumulative amount of drug entering the liver (\(M_{\text{oral}}\)) is given by:

\[
M_{\text{oral}} = V_{HF} \int_0^\infty C_{\text{HP}}' dt + V_{HF} \int_0^\infty (C_{\text{HP}})' dt + V_{HA} \int_0^\infty C_{\text{HA}}' dt
\]  
(Eq. 4)

The oral dose is:

\[
(Dose)_{\text{oral}} = V_{HF} \int_0^\infty C_{\text{HP}}' dt
\]  
(Eq. 5)

Since \(\int_0^\infty (C_{\text{HP}})' dt = \int_0^\infty (C_{\text{HA}})' dt = \int_0^\infty C_{\text{art}} dt\), Eq. 4 is simplified as:

\[
M_{\text{oral}} = (Dose)_{\text{oral}} + V_{HF} \int_0^\infty C_{\text{art}} dt
\]  
(Eq. 6)

Therefore, the mean hepatic extraction ratio of drug following oral administration (\(\overline{E}R\))_{oral} is shown as:

\[
(\overline{E}R)_{\text{oral}} = \frac{(Dose)_{\text{oral}} + V_{HF} \int_0^\infty C_{\text{art}} dt - V_{HF} \int_0^\infty C_{\text{HV}} dt}{(Dose)_{\text{oral}} + V_{HF} \int_0^\infty C_{\text{art}} dt}
\]  
(Eq. 7)

By defining (\(\overline{E}R\))_{oral}' and (\(\overline{E}R\))_{oral}'' as the mean hepatic extraction ratio of drug cleared in the first pass through the liver and that of drug cleared during recirculation after escaping hepatic first-pass metabolism, respectively, \((\overline{E}R)_{\text{oral}}\) may be represented also by:

\[
(\overline{E}R)_{\text{oral}} = \frac{(\overline{E}R)_{\text{oral}'} (Dose)_{\text{oral}} + (\overline{E}R)_{\text{oral}''} V_{HF} \int_0^\infty C_{\text{art}} dt}{(Dose)_{\text{oral}} + V_{HF} \int_0^\infty C_{\text{art}} dt}
\]  
(Eq. 8)

Substituting Eq. 7 into Eq. 8 appropriately, the fraction of the dose cleared in the first passage is written by:

\[
(\overline{E}R)_{\text{oral}'} = \frac{(Dose)_{\text{oral}} + [1 - (\overline{E}R)_{\text{oral}''}] V_{HF} \int_0^\infty C_{\text{art}} dt - V_{HF} \int_0^\infty C_{\text{HV}} dt}{(Dose)_{\text{oral}}}
\]  
(Eq. 9)

Fraction of Administered Dose Which reaches the Systemic Circulation for a Drug Exhibiting Nonlinear Metabolic Kinetics

The AUC of a drug intravenously administered is given by dividing an administered dose by the mean systemic clearance of the drug, and this clearance is the sum of the mean hepatic clearance \(V_{HF}(\overline{E}R)_{\text{iv}}\) and the mean clearance of elimination processes (\(CL_{\text{remaining}}\)) except for the hepatic metabolism.\(^{10}\) Then the (AUC)_{iv} may be shown by:

\[
(AUC)_{\text{iv}} = \frac{(Dose)_{\text{iv}}}{V_{HF}(\overline{E}R)_{\text{iv}} + CL_{\text{remaining}}}
\]  
(Eq. 10)

On the other hand, since the fraction of orally administered dose reaching the systemic circulation (\(F\)) is:

\[
F = 1 - (\overline{E}R)_{\text{oral}'}
\]  
(Eq. 11)

the (AUC)_{oral} of the drug may be shown by Eq. 12, assuming that \(CL_{\text{remaining}}\) is not changed depending on the route of administration.

\[
(AUC)_{\text{oral}} = \frac{[1 - (\overline{E}R)_{\text{oral}'}] (Dose)_{\text{oral}}}{V_{HF}(\overline{E}R)_{\text{oral}'} + CL_{\text{remaining}}}
\]  
(Eq. 12)

when an equal dose is administered, the ratio between the AUC's for both routes of administration is given by:

\[
\frac{(AUC)_{\text{oral}}}{(AUC)_{\text{iv}}} = \frac{[1 - (\overline{E}R)_{\text{oral}'}] V_{HF}(\overline{E}R)_{\text{oral}''} + CL_{\text{remaining}}}{V_{HF}(\overline{E}R)_{\text{oral}'} + CL_{\text{remaining}}}
\]  
(Eq. 13)

So that the bioavailability of the drug is:

\[
F = \frac{(AUC)_{\text{oral}} [V_{HF}(\overline{E}R)_{\text{oral}'} + CL_{\text{remaining}}]}{(AUC)_{\text{iv}} [V_{HF}(\overline{E}R)_{\text{iv}} + CL_{\text{remaining}}]}
\]  
(Eq. 14)

Therefore, the fraction of the orally administered dose reaching the systemic circulation is estimated by the AUC ratio for both routes of administration only when the relation (\(\overline{E}R\))_{iv} = (\(\overline{E}R\))_{oral} is valid.\(^{21}\) For a drug exhibiting dose-dependent metabolic kinetics, the fraction of the orally administered dose reaching the systemic circulation estimated from the AUC ratio for oral and intravenous administration is different from the true value, when (\(\overline{E}R\))_{iv} \neq (\(\overline{E}R\))_{oral}. In such a case, the fractional loss of the oral dose as it passes through the liver for the first time becomes
different from the fraction estimated from one minus the AUC ratio.

MATERIALS AND METHODS

**Materials** — Propranolol, ^14^C-propranolol and all other chemicals used in this study were the same as those reported previously.9)

**Analytical Methods** — Propranolol and ^14^C-propranolol were assayed by the fluorometric method of Shand et al.11) and by liquid scintillation spectrometry, respectively. The analytical procedures were described in the previous paper.9) An unlabelled propranolol concentration were calculated by subtracting a labelled propranolol concentration from a total propranolol concentration (unlabelled and labelled) determined fluorometrically when unlabelled and labelled propranolol were administered simultaneously.

**Animal Experiments** — Male Wistar rats weighing 270–350 g were used in all experiments, and free access to food and water until 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 intravenous administration. Another cannula was inserted into the left femoral vein for transfusion of blood equal in volume to the withdrawn blood sample in order to avoid circulatory disturbances resulting from blood loss. The blood for transfusion was prepared by adding 0.4 ml of 10% sodium citrate to 46 ml of blood taken from another rat of the same strain. An intraportal dose of propranolol was given through a cannula inserted into the hepatoportal vein via the pyloric vein.12) An intraduodenal dose of propranolol was given by injection into the duodenal tract over 30 sec after a midline abdominal incision along the linea alba. The abdominal incision was closed by suture following intraduodenal administration.

Propranolol was given intravenously in doses of 2.5 and 12.5 mg/kg at a constant rate over a 50-min period using an infusion pump (Natsume Model KN-1H), and intraduodenally in doses of 12.5, 25, and 50 mg/kg. In another series of experiments, unlabelled propranolol in doses of 2.5, 7.5, and 12.5 mg/kg and ^14^C-propranolol in doses of 1, 3, and 3 mg/kg were simultaneously administered into the hepatoportal vein and femoral vein, respectively. Both unlabelled and labelled propranolol were administered at a constant rate over a 50-min period using an infusion pump.

Blood samples (0.1 to 0.2 ml) were withdrawn simultaneously from the artery, hepatoportal vein (only when propranolol was not administered intraportally), and hepatic vein. The blood samples were taken at –5, 20, 40, 50, 60, 75, 90, 120, and 180 min following intravenous and intraportal administration, and 7 to 8 blood samples were removed appropriately following intraduodenal administration. The arterial blood samples were taken through a cannula inserted into the femoral artery, and the hepatoportal blood samples were taken through a cannula inserted into the hepatoportal vein via the pyloric vein.12) 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.13) The experiments were started at least 90 min after the operations mentioned above.

**Calculation of the Area under the Blood Concentration-Time Curve (AUC)** — The AUC for propranolol in the arterial and hepatic venous blood was determined. Eq. 15 shows the method used to obtain the total AUC, (i.e., from time zero to infinity).

\[
AUC = AUC_{(0 \rightarrow t)} + AUC_{(t \rightarrow \infty)} \quad (\text{Eq. 15})
\]

where \(t\) represents the time at which the last data point is collected. The AUC \((0 \rightarrow t)\) was obtained by the trapezoidal rule whereas AUC \((t \rightarrow \infty)\) was calculated by:

\[
AUC_{(t \rightarrow \infty)} = 1.44 C_t t_{1/2} \quad (\text{Eq. 16})
\]

where \(C_t\) equals the blood concentration at the last sample time point, and \(t_{1/2}\) is an estimated half-life from data points at the terminal log-linear portion of the blood concentration–time curve. It should be noted that in most cases, the AUC \((t \rightarrow \infty)\) accounted for less than 10% of the total AUC.
RESULTS

Mean Hepatic Extraction Ratio depending on Concentration of Drug Reaching the Liver

The mean hepatoportal, arterial, and hepatic venous blood concentrations of propranolol during constant-rate intravenous and intraportal ad-

![Graphs showing blood concentration-time curves for propranolol in different veins and arteries.](image)

**FIG. 1.** Blood Concentration-Time Curves of Propranolol in Hepatoportal Vein (HP), Artery (art), and Hepatic Vein (HV) after Intravenous, Intraportal, and Intraduodenal Administration.

Propranolol was administered intravenously or intraportally at a constant rate over a 50-min period. Intraduodenal doses were administered over 30 sec into the duodenal tract.

- □: 2.5 mg/kg, ▽: 7.5 mg/kg, ○: 12.5 mg/kg, ◊: 25 mg/kg, Δ: 50 mg/kg.

The data during 60 min after the initiation of infusion were plotted against time and the subsequent data were omitted. Vertical bars show standard errors of the mean from 3 to 4 experiments except 10 experiments of an intraportal administration in a dose of 12.5 mg/kg. Fig. 1B illustrated by using the data of the previous paper. The blood concentration of propranolol in the hepatoportal vein after intraportal administration was calculated as (infusion rate)/$V_{HP} + C_{art}$ (see text).
ministration over a 50 min-period are shown in Figs. 1A and 1B, respectively. Figure 1B was illustrated by using the data of the previous paper. The propranolol concentration in the femoral artery was substituted for that in the hepatic artery. The hepatopetal propranolol concentration following intraportal administration was calculated by dividing an infusion rate of propranolol by the hepatopetal blood flow and adding this concentration of the infused drug to the arterial blood propranolol concentration. A hepatopetal blood flow of 2.1 ml/min/100 g body weight was obtained as three-fourths of the hepatic blood flow of the rat (2.8 ml/min/100 g body weight) estimated in the preceding study. This fraction of hepatic blood flow was approximated by the data determined in the sheep and dog.

Figure 1A shows a close agreement between arterial and hepatopetal blood concentrations during intravenous infusion, demonstrating that the gut did not metabolize the drug as was previously reported by Shand et al. The hepatic venous blood concentrations of propranolol in rats receiving intravenous doses of 2.5 and 12.5 mg/kg were negligibly small, whereas those in rats receiving intraportal doses of 2.5, 7, 5, and 12.5 mg/kg increased greatly with increasing dose (Figs. 1A and 1B). The hepatopetal propranolol concentrations during intraportal infusion were much larger than those during intravenous infusion, and the contribution of drug surviving the hepatic first-pass metabolism to the total hepatopetal blood concentration was minor. The mean hepatopetal, arterial, and hepatic venous blood concentrations of propranolol following intraduodenal administration is shown in Fig. 1C. The time courses of blood propranolol concentration in the three different vascular sites being sampled following intraduodenal administration were found to be similar as those during intraportal administration. The propranolol concentration entering the liver \((C_i)_H\) following intraportal administration equals the propranolol concentration in any artery \((C_{art})\) since \(C_{HP} = C_{HA} = C_{art}\), whereas the \((C_i)_H\) following intraportal administration equals \((\text{Infusion rate})/V_{HV} - C_{art}\) since \(C_{HA} = C_{art}\), and \(C_{HP} = (\text{Infusion rate})/V_{HP} - C_{art}\). The mean \((C_i)_H\) over a 50-min period after the initiation of infusion were calculated to be 0.46 and 2.4 \(\mu g/ml\) for intravenous doses of 2.5 and 12.5 mg/kg, and 1.8, 5.9, and 10.7 \(\mu g/ml\) for intraportal doses of 2.5, 7.5, and 12.5 mg/kg, respectively. From these calculated data the propranolol concentration entering the liver following intraportal administration was found to be much larger than that following intravenous administration of an equal dose.

The mean hepatic extraction ratio of propranolol was plotted against the mean \((C_i)_H\) reaching the liver over a 50-min period (Fig. 2). The \((C_i)_H\) for intraduodenal administration was calculated as a mean over an interval of 60 min after administration by using Eq. 2a, and its hepatic extraction ratio also was calculated as a mean during 60 min, \((\int_0^{60} (C_i)_H dt - \int_0^{60} C_{HV} dt)/\int_0^{60} (C_i)_H dt\). The correlation coefficient was 0.954 for the regression line of the mean hepatic extraction ratio \((E_R)\) on \((C_i)_H\), \(E_R = 0.977 - 0.04 (C_i)_H\). Figure 2 reveals that the mean hepatic extraction ratio is dependent on the concentration of drug entering the liver.

Assessment of Nonlinear Hepatic First-Pass Metabolism and Dose-Dependent Bioavailability

The previous studies have shown that the apparent bioavailability of propranolol assessed by comparison of the AUC’s following intraportal and intravenous administration increases with increasing dose and infusion rate, and the mean hepatic extraction ratio of propranolol following intraportal administration decreases progressively with increasing infusion rate, that made a large fraction of the administered dose available in the systemic circulation. In addition, the hepatic extraction of labelled propranolol following intravenous infusion was remarkably decreased by simultaneous intraportal infusion of unlabelled propranolol. These data suggest that the present nonlinear hepatic extraction may be responsible for the dose-dependent bioavailability of propranolol and the bioavailability of propranolol.
may not be assessed by a ratio between the AUC's following intraportal and intravenous administration.

Figure 3 shows typical unlabelled and labelled propranolol concentration-time curves determined in hepatoporal, arterial, and hepatic venous blood following intraportal infusion of 12.5 mg/kg unlabelled propranolol and simultaneous intravenous infusion of 3 mg/kg of labelled propranolol into the femoral vein over a 50-min period. The hepatoporal blood concentrations of labelled and unlabelled propranolol were approximated by the labelled propranolol concentration in the femoral arterial blood and the calculated concentration as (Infusion rate)/$V_{HP} + C_{art}(unlabelled)$, respectively, as mentioned in the preceding section. The inflowing concentration of labelled propranolol to the liver was evaluated to be approximately one-fifteenth that of unlabelled propranolol by using Eq. 2a. Therefore, the mean hepatic extraction ratio of unlabelled propranolol following intraportal ad-

![Graph](image-url)

**FIG. 2.** Relationship between Mean Hepatic Extraction Ratio ($ER$) and Mean Inflowing Blood Concentration of Propranolol to Liver ($C_{i}$)$_{H}$ via Various Routes of Administration

Propranolol was administered intravenously in doses of $\Box$: 2.5 mg/kg, $\bigcirc$: 12.5 mg/kg, intraportally in doses of $\blacksquare$: 2.5 mg/kg, $\blacktriangledown$: 7.5 mg/kg, $\bullet$: 12.5 mg/kg at a constant rate over a 50-min period, and intraduodenally in doses of $\oslash$: 12.5 mg/kg, $\blacklozenge$: 25 mg/kg, $\blacktriangle$: 50 mg/kg over 30 sec. Vertical bars show standard errors of 3 to 4 experiments except 10 experiments of intraportal administration in a dose of 12.5 mg/kg. The dotted line shows a regression line. The ($C_{i}$)$_{H}$ and mean hepatic extraction ratio for intraduodenal administration were calculated as a mean during 60 min (see text).

![Graph](image-url)

**FIG. 3.** Typical Blood Concentration-Time Curves of Unlabelled and Labelled Propranolol in Hepatoporal Vein, Artery, and Hepatic Vein following Simultaneous Administration of Intraportal Unlabelled Propranolol and Intravenous $^{14}$C-Propranolol

Unlabelled propranolol and $^{14}$C-propranolol in doses of 12.5 and 3 mg/kg were simultaneously administered over a 50-min period, respectively. $\bullet$: Unlabelled propranolol concentration, $\bigcirc$: Labelled propranolol concentration.

The AUC's of unlabelled propranolol in the arterial and hepatic venous blood were calculated to be 311 and 403 min·μg/ml, and those of labelled propranolol in the arterial and hepatic venous blood were 117 and 31 min·μg/ml, respectively.
TABLE 1. Area under Blood Concentration-Time Curves (AUC) of Unlabelled and Labelled Propranolol in Artery and Hepatic Vein following Simultaneous Administration of Intraportal Unlabelled Propranolol and Intravenous $^{14}$C-Propranolol

<table>
<thead>
<tr>
<th>Unlabelled propranolol dose (mg/kg)</th>
<th>$^{14}$C-Propranolol dose (mg/kg)</th>
<th>AUC of unlabelled propranolol (min $\cdot$ µg/ml)</th>
<th>AUC of $^{14}$C-Propranolol (min $\cdot$ µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>1</td>
<td>66.6±2.2</td>
<td>250±2.9</td>
</tr>
<tr>
<td>7.5</td>
<td>3</td>
<td>108.6±9.7</td>
<td>94.3±1.9</td>
</tr>
<tr>
<td>12.5</td>
<td>3</td>
<td>245.3±27.6</td>
<td>97.7±7.9</td>
</tr>
</tbody>
</table>

Results represent the mean ± standard error of 3 experiments.

![Graph](image_url)  

FIG. 4. Relationship between Various Mean Hepatic Extraction Ratios of intraportally administered Propranolol and Dose

Δ: $(\bar{E}R)_{ipv}$ denoted the mean hepatic extraction ratio of propranolol following intraportal administration.

○: $(\bar{E}R)_{ipv}$ denoted the mean hepatic extraction ratio of propranolol after escaping the hepatic first-pass metabolism.

●: $(\bar{E}R)_{ipv}$ denoted the mean hepatic extraction ratio of propranolol in the first pass through the liver.

Vertical bars show standard errors of the mean from 3 experiments.

ministration $(\bar{E}R)_{ipv}$ seems to be little affected by the labelled propranolol administered simulta-
neously. The subscript of $ipv$ was used to denote intraportal administration, which simulates complete gastrointestinal absorption, so that $(\bar{E}R)_{ipv}$ equals $(\bar{E}R)_{oral}$ shown in THEORETICAL.

The $(\bar{E}R)_{ipv}$ of unlabelled propranolol was calculated to be 0.47 by substituting 311 and 403 min $\cdot$ µg/ml obtained from data of Fig. 3 for the AUCs of unlabelled propranolol in the arterial and hepatic venous blood in Eq. 7. The hepatic extraction ratio of the labelled propranolol intravenously administered was decreased by the existence of unlabelled propranolol in the liver as shown in the preceding paper, and was calculated to be 0.74 by substituting 117 and 311 min $\cdot$ µg/ml obtained from data of Fig. 3 for the AUCs of labelled propranolol in the arterial and hepatic venous blood in Eq. 3. The hepatic extraction ratio of unlabelled propranolol cleared during recirculation after surviving hepatic first-pass metabolism $(\bar{E}R)_{ipv}$ was approximated by that of the labelled propranolol administered simultaneously with the unlabelled propranolol, because the elimination process of labelled propranolol and of unlabelled propranolol surviving hepatic first-pass metabolism is considered to be identical. Consequently, it is possible to calculate the mean hepatic extraction ratio of unlabelled propranolol in the first pass through the liver from Eq. 9 by substituting 0.74 for $(\bar{E}R)_{ipv}$ and 311 and 403 min $\cdot$ µg/ml for the AUCs of arterial and hepatic venous unlabelled propranolol. The calculation revealed that 28% of the propranolol intraportally administered was eliminated in the first pass.
TABLE II.  Comparison of Extent of Bioavailability estimated by Using Two Different Methods

<table>
<thead>
<tr>
<th>Propranolol dose (mg/kg)</th>
<th>Extent of bioavailability</th>
<th>(F^*)</th>
<th>((AUC)^{<strong>}_{yv}/(AUC)^{</strong>*}_{yv})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td></td>
<td>0.10±001</td>
<td>0.13</td>
</tr>
<tr>
<td>7.5</td>
<td></td>
<td>0.45±004</td>
<td>0.68</td>
</tr>
<tr>
<td>12.5</td>
<td></td>
<td>0.66±004</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*: Calculated as \([1-(\overline{E}R)^{yv}_p]\).  
**: The AUC of unlabelled propranolol in arterial blood after intraportal administration of propranolol at a constant rate over a 50-min period.  
***: The AUC in arterial blood after intravenous administration of propranolol over 30 sec. The data was taken from the previous study.  

Results represent the mean ± standard error of 3 experiments.

through the liver and the remaining 72% reached the systemic circulation. The observed data indicated that magnitudes of \((\overline{E}R)^{yv}_p\), \((\overline{E}R)^{yv}_v\), and \((\overline{E}R)^{yv}_p\) decreased in that order.

The mean AUC's in three rats for intraportal infusion of 2.5, 7.5, and 12.5 mg/kg unlabelled propranolol and simultaneous intravenous infusion of 1 or 3 mg/kg labelled propranolol into the femoral vein are shown in Table I. Various mean hepatic extraction ratios were calculated by using a set of AUC's determined in each rat. Fig. 4 illustrates various mean hepatic extraction ratios of propranolol following intraportal administration over a 50-min period against dose. It can be seen that a relation of \((\overline{E}R)^{yv}_p < (\overline{E}R)^{yv}_v < (\overline{E}R)^{yv}_p\) was observed also in a dose of 7.5 mg/kg, but all the mean hepatic extraction ratios were almost equal in a dose of 2.5 mg/kg. The absolute amount of propranolol reaching the systemic circulation calculated as one minus \((\overline{E}R)^{yv}_p\) are shown in the second column of Table II. The calculated values were smaller than the ratios between the AUC's for both routes shown in the third column.

DISCUSSION

Propranolol was virtually completely absorbed after oral administration in man, rat, and dog, and only 1 to 4% of a radiolabelled dose was recovered as unchanged drug in the urine and feces.\(^{16-18}\) Shand et al.\(^{19}\) showed that the mean systemic clearance of propranolol in rats was high (2.5 ml/g liver/min) and actually greater than the estimated liver blood flow rate in the rat of 1.2 ml/g liver/min, and suggested a significant degree of extrahepatic elimination of propranolol in the rat. The contribution of hepatic clearance in man, monkey, dog and rat was compared.\(^{20}\) Only the rat showed significant extrahepatic metabolism, the estimated hepatic clearance being approximately 36% of the mean systemic clearance. If a drug is eliminated exclusively by the liver, the product of the hepatic blood flow and the difference between the AUC's in the numerator of Eq. 3 for intravenous administration must equal an administered dose, and the numerator of Eq. 7 for oral administration also must equal an administered dose. However, the calculated values in rats were not equal the administered dose in this study, and the extrahepatic elimination was estimated to be approximately 40% using the data following intravenous administration.

The relationship between the AUC and dose following oral or intraportal administration was curvilinear and a threshold dose showing a sudden increase in AUC was observed, although the AUC of propranolol given into the systemic peripheral vein was directly proportional to dose.\(^{6,8}\) It has been presumed by \textit{in vitro} perfusion test of the rat liver that hepatic saturable uptake and metabolism of propranolol depending on the route of administration account for the above unusual AUC-dose relationship.\(^{21,22}\) There has been a
lack of solid experimental data regarding the hepatoporal concentrations of drugs after oral or intravenous administration. If the oral dose, absorption rate, and/or volume of distribution of a drug is large, the hepatoporal blood concentration of drug entering the liver after oral administration may exceed greatly that found when the drug is given intravenously. This is the case for the Fig. 1, in which the hepatoporal propranolol concentration found during the intraportal infusion was approximately 4-fold that obtained during the intravenous infusion of the same dose. The liver propranolol concentration immediately after 50 min intraportal infusion at a constant rate was also shown to be more than 6-fold that immediately after intravenous infusion by the same rate of administration. Under such circumstances, it is possible that the fraction of dose cleared during the first pass through the liver is dependent on dose and rate of absorption, i.e., nonlinear first-pass metabolism. Evidence in support of this presumption is shown in Fig. 2 indicating that the mean hepatic extraction ratio of propranolol is dependent on the concentration of drug entering the liver.

For a drug which has linear disposition kinetics, an intravenous bolus is commonly the standard procedure in bioavailability studies. Since such a drug given intravenously has 100% bioavailability, the AUC serves as a reference value to compare with the AUC following oral administration. Bioavailability is then simply defined as:

\[ F = \frac{(\text{AUC})_{\text{oral}}/(\text{Dose})_{\text{oral}}}{(\text{AUC})_{\text{iv}}/(\text{Dose})_{\text{iv}}} \]  
(Eq. 17)

For a drug which is completely absorbed through the gastrointestinal tract and is not subjected to intestinal metabolism, \( F \) represents the fraction of the administered dose which escapes the hepatic first-pass metabolism. However, for a drug which has nonlinear disposition kinetics, the AUC ratio per unit dose in Eq. 17 may be different from the true fraction of an oral dose which actually reaches the systemic circulation, because the mean systemic drug clearance for both routes of administration must be equal for Eq. 14 to be valid. For a drug which has linear kinetics for hepatic metabolism all the mean hepatic extraction ratios defined in THEORETICAL should be equal with each other, i.e., \( (\bar{E}\bar{R})_{iv} = (\bar{E}\bar{R})_{oral} = (\bar{E}\bar{R})_{oral} = (\bar{E}\bar{R})_{oral} \), since the fraction of the administered dose cleared by hepatic first-pass metabolism \( (\bar{E}\bar{R})_{oral} \) can be estimated by \( (\bar{E}\bar{R})_{iv} \).

However, the mean hepatic extraction ratio of propranolol \( (\bar{E}\bar{R})_{iv} \) following intraportal administration were shown to be dependent on rate of administration in the previous data, and to be \( (\bar{E}\bar{R})_{iv} \propto (\bar{E}\bar{R})_{iv} \propto (\bar{E}\bar{R})_{iv} \propto (\bar{E}\bar{R})_{iv} \) in higher doses in this study.

The AUC's of propranolol following intravenous and intraportal administration were not significantly different in a dose of 12.5 mg/kg. This seems to indicate no first-pass effect. However, propranolol can be presumed to undergo a considerable first-pass metabolism, since the mean hepatic extraction ratio \( (\bar{E}\bar{R})_{iv} \) was approximately 0.5. The present in vivo experiments have confirmed that the hepatic first-pass metabolism of propranolol was estimated more precisely by determining the mean hepatic extraction ratio \( (\bar{E}\bar{R})_{iv} \) in the first pass through the liver, and \( (\bar{E}\bar{R})_{iv} \) was shown to decrease with increasing dose. The mechanism responsible for dose-dependent bioavailability of propranolol could be explained on the basis of both the nonlinear hepatic first-pass metabolism and the nonlinear hepatic metabolism of drug surviving the hepatic first-pass metabolism.

Acknowledgement The authors wish to thank Dr. T. Rikihisa, University of Chiba, for helpful discussions throughout the course of this work.

REFERENCES
2) a) M. Gibaldi and S. Feldman: Pharmacokinetic basis for the influence of route of administration, in the area under the plasma concentration-time curve, J. Pharm.
Nonlinear First-Pass Metabolism of Propranolol


