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New Approach to the Hepatic First-Pass Effect by Whole-Body Autoradiography

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Whole-body autoradiography was used to investigate the influence of the route of administration and dose on the distribution of drugs in the rat. Propranolol and imipramine were used as model drugs with a relatively high hepatic extraction ratio, and salicylic acid and antipyrine were used as model drugs with a relatively low hepatic extraction ratio. Each drug was administered at the same radioactivity level to rats by intravenous infusion or portal venous infusion over 30 s, regardless of the total dose and route of administration. An autoradiogram obtained 3 min after portal venous infusion was compared with that obtained 3 min after intravenous infusion. The radioactivity of propranolol that reached the systemic circulation was highly dependent on both the route of administration and dose, while that of imipramine was dependent only on the route in the dose range studied. The radioactivity of salicylic acid and antipyrine was distributed in the whole body independently of the route of administration and dose. The two drugs having a higher hepatic extraction ratio showed an uneven distribution in the liver after portal venous administration, but the two drugs having a lower hepatic extraction ratio did not. It was shown that the technique of portal venous administration for whole-body autoradiography is useful to visualize differences in drug distribution depending on the route of administration and dose and to study hepatic tissue binding in the first-pass extraction of drugs.

Keywords——first-pass effect; whole-body autoradiography; propranolol; imipramine; salicylic acid; antipyrine; rats; route of administration and dose; hepatic uptake of drug; uneven drug distribution in the liver

The disposition of drugs may be markedly dependent on the route of administration.[1] Drugs having a high hepatic extraction ratio undergo extensive biotransformation in the first pass through the liver following oral administration. Thus the blood concentrations achieved following oral doses are much lower than those achieved after intravenous doses, even if gastrointestinal absorption is complete. This phenomenon is referred to as the first-pass effect and is a major physiological factor influencing the bioavailability of drugs.[1a] Studies on the first-pass effect have been directed toward the time course of the blood drug concentrations, and not toward the drug distribution in the body, since the first-pass effect of drug is evaluated as one minus the AUC (area under the blood concentration time-curve) ratio for oral and intravenous administration.[1] However, the drug distribution presumably depends greatly upon the route of administration and dose.

A significant advance in the techniques of autoradiography and syntheses of radioactive compounds has brought about the frequent use of whole-body autoradiography in the fields of pharmacology and toxicology.[2] Since unchanged drug and its metabolites cannot be differentiated on an autoradiogram, a whole-body autoradiogram prepared shortly after intravenous administration is useful to estimate the distribution of unchanged drug in the body.[2a] On the other hand, an autoradiogram prepared after oral administration is less useful, since it generally takes a longer time for a drug to reach the systemic circulation from the gastroinstestinal tract, and biotransformation may occur simultaneously with gastrointestinal absorption. Furthermore, a whole-body autoradiogram of orally administered drug cannot

cast light on the rate and amount of drug absorption. If a drug is given directly into the hepatic portal vein instead of orally, the rate and extent to which the administered dose reaches the liver can be easily controlled. As a result, it becomes possible to investigate the influence of the route of administration and the size of oral dose on drug distribution by using whole-body autoradiography if different doses containing the same redioactivity are administered to animals by different routes.

The aim of this study was to develop a technique of portal venous infusion for whole-body autoradiographic studies in the rat instead of using oral administration, and to visualize differences in drug distribution depending on the route of administration and dose. Propranolol[3] and imipramine[4] were chosen as model compounds having a high hepatic extraction ratio, and salicylic acid[5] and antipyrine[3] were chosen as model compounds having a low hepatic extraction ratio.

Experimental

Animals——Male rats of the Wistar strain weighing 250—300 g were used. Animals were fasted for about 16 hr before use in the experiments but were allowed water *ad libitum*.

Materials——Propranolol and labelled propranolol ([1-^{14}C]-1-isopropylamino-3-(1-naphthoxy)propane-2-ol, specific activity 12.9 μCi/mg)[6] were kindly furnished by Sumitomo Chemical Co., Ltd., Osaka, as their hydrochloride salts and racemic forms. Labelled imipramine (N-(3-dimethylaminopropyl)iminodi[methylene-^{14}C]benzyl hydrochloride, specific activity 33 μCi/mg) and labelled salicylic acid ([carboxy-^{14}C]salicylic acid, specific activity 421 μCi/mg) were purchased from the Radiochemical Centre, Amersham, UK. Labelled antipyrine ([N-methyl-^{14}C]antipyrine, specific activity 258 μCi/mg) was purchased from New England Nuclear Corp., Boston, USA. The radiochemical purity of each labelled drug was tested by thin-layer chromatography on Kieselgel 60 F_{254}-precoated plates (Merck) and was found to be more than 98% except in the case of imipramine (at least 95%). The solvent systems used were: BuOH–AcOH–water (8: 2: 1, v/v) and EtOH–conc. ammonia–water (80: 4: 5, v/v) for propranolol; PrOH–$CHCl_3$–conc. ammonia (25: 25: 1, v/v) and $CHCl_3$–EtOH (4: 1, v/v) for imipramine; cyclohexane–$CHCl_3$–AcOH (2: 8: 1, v/v) and toluene–dioxane–AcOH (90: 24: 4, v/v) for salicylic acid; AcOH–isoPrOH–conc. ammonia (45: 35: 20, v/v) and MeOH–$CHCl_3$ (1: 9. v/v) for antipyrine. Unlabelled imipramine hydrochloride, salicylic acid and antipyrine were of JP IX grade. Other chemicals were all of reagent grade.

Drug Administration—— Each labelled drug was appropriately diluted with unlabelled drug. 3.0 μCi of ^{14}C-propranolol, 3.5 μCi of ^{14}C-imipramine, 5.0 μCi of ^{14}C-salicylic acid or 6.0 μCi of ^{14}C-antipyrine, unless otherwise stated, was given to an animal, regardless of the route of administration and dose. Doses of 1 and 10 mg/kg with the specified radioactivity in 0.3 ml of physiological saline solution were infused either through a cannula inserted into the hepatic portal vein *via* the pyloric vein[7] over 30 s or through a cannula inserted into the femoral vein over the same time period. A dose of 10 mg/kg ^{14}C-propranolol in 0.3 ml of aqueous solution was passed into the stomach by means of a stomach tube.

Preparation of Whole-body Autoradiograms——After administration of each drug, the animal was anesthetized with ether 1 min before sacrifice. The abdominal incision closed with a nylon suture was covered with thick adhesive-tape to prevent loss of the body fluids. Aqueous carboxymethyl cellulose gel (5%) was applied to the mouth, nose and the places which were operated on for cannulation, then the animal was quickly frozen by immersion into acetone cooled with solid carbon dioxide. The frozen animal was transferred to a refrigerator at about −20°C.

Whole-body autoradiography was performed by the method described by Ullberg.[8] Sagittal sections of 60 μ thickness were taken and applied to adhesive tape. The sections were dried and exposed to Sakura N-type X-ray films (Konishiroku) for about 1 to 2 months unless otherwise stated. The films were developed, fixed and printed. For comparison of autoradiograms corresponding to the different routes of administration and doses, the autoradiography of each drug was carried out under rigorously identical conditions. The animals were sacrificed 3 min after administration, regardless of the route of administration and dose. When 1 and 10 mg/kg of ^{14}C-propranolol were given into the hepatic portal vein, animals were sacrificed 3 and 7 min after administration. When 10 mg/kg of ^{14}C-propranolol was given orally, the animal was sacrificed 30 min after administration. The sagittal sections of liver after oral administration of ^{14}C-propranolol were exposed to films for 2 weeks.

In order to investigate the distribution of propranolol in the liver, 0.1, 0.4 and 0.7 mg/kg of ^{14}C-propranolol (0.3 μCi for each animal) were administered through the hepatic portal vein and then sections were taken at right angles to the spinal cord. The autoradiograms of these sections were prepared as described above. One of the sections of the liver at right angles to the spinal cord was stained with hematoxylin-eosin after exposure.

Measurement of Radioactivity——A liquid scintillation counter (Beckman LS100C) was used for the measurement of radioactivity. The scintillator was a dioxane-based cocktail (naphthalene 100 g, DPO 5 g, dimethyl POPOP 0.3 g, toluene 135 ml, methanol 35 ml and dioxane 730 ml). The correction for quenching was made by use of a correction curve based on the external standard method.

Results

Whole-body Autoradiograms of Drugs having a High Hepatic Extraction Ratio

^{14}C-Propranolol (3.0 μCi each) at doses of 1 and 10 mg/kg was administered into either the hepatic portal vein or the femoral vein of rats over 30 s. The distribution of radioactivity 3 min after administration is shown in Fig. 1. The radioactivity after intravenous administration of 1 mg/kg ^{14}C-propranolol was distributed widely in the body (Fig. 1a). High concentrations of radioactivity were observed in the lung and heart, and rather high uptake of radioactivity in the brain, intestine and liver was observed. On the other hand, most of the radioactivity was taken up by the liver when the same radioactivity of ^{14}C-propranolol at a dose of 1 mg/kg was given through the hepatic portal vein. The radioactivity that reached the systemic circulation was extremely low, judging from the slight accumulation of radioactivity in the lung and heart (Fig. 1b).

When a dose of 10 mg/kg ^{14}C-propranolol was given intravenously, the distribution of radioactivity was quite similar to that after intravenous administration of 1 mg/kg ^{14}C-propranolol (Fig. 1c). When the intraportal dose of ^{14}C-propranolol was increased to 10 mg/kg, most of the radioactivity was also taken up by the liver and its distribution was similar to that after intraportal administration of 1 mg/kg ^{14}C-propranolol (Fig. 1d). The radioactivity that reached the systemic circulation seems to be very low in comparison with that after intravenous

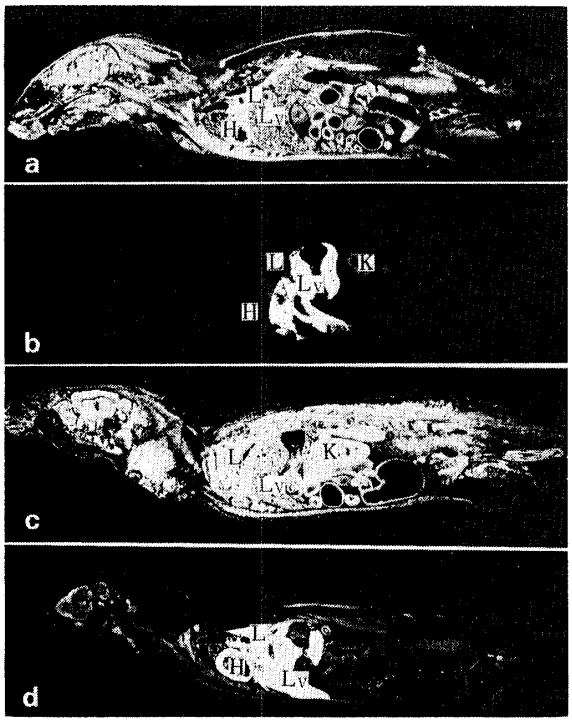

Fig. 1. Whole-Body Autoradiograms showing the Distribution of Radioactivity (White Areas) in Rats 3 min after Administration of ^{14}C-Propranolol (3.0 μCi each)

(a) an intravenous dose of 1 mg/kg, (b) an intraportal dose of 1 mg/kg, (c) an intravenous dose of 10 mg/kg, (d) an intraportal dose of 10 mg/kg. Abbreviations: H, heart; K, kidney; L, lung; Lv, liver.

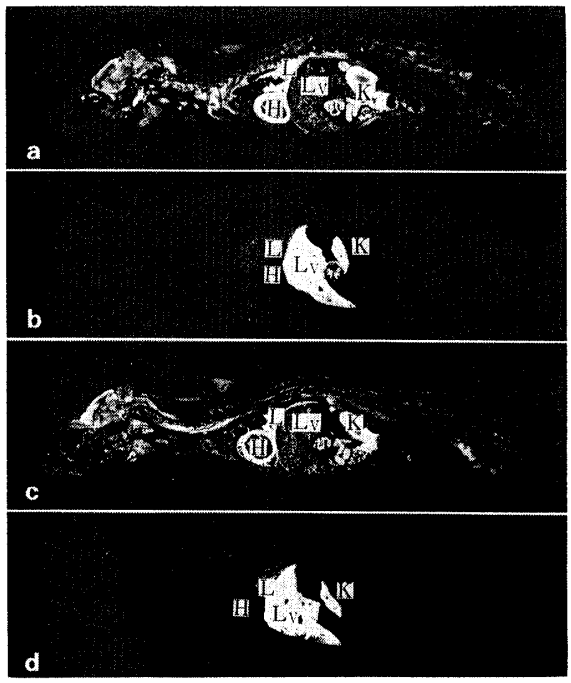

Fig. 2. Whole-Body Autoradiograms showing the Distribution of Radioactivity (White Areas) in Rats 3 min after Administration of ^{14}C-Imipramine (3.5 μCi each)

(a) an intravenous dose of 1 mg/kg, (b) an intraportal dose of 1 mg/kg, (c) an intravenous dose of 10 mg/kg, (d) an intraportal dose of 10 mg/kg. Abbreviations are the same as in Fig. 1.

administration of the same dose. However, the radioactivity observed in the lung, heart and kidney after intraportal administration of 10 mg/kg ^{14}C-propranolol was higher than that after an intraportal dose of 1 mg/kg. A low but distinct distribution of radioactivity was observed in the brain and intestine after intraportal administration of 10 mg/kg ^{14}C-propranolol, but not after an intraportal dose of 1 mg/kg. These observations indicate the occurrence of saturable hepatic uptake with increasing dose.

The distribution patterns of radioactivity 7 min after intraportal administration of 1 and 10 mg/kg ^{14}C-propranolol were similar to those 3 min after intraportal administration of corresponding doses.

The autoradiograms of ^{14}C-imipramine (3.5 μCi each) prepared in the manner described for ^{14}C-propranolol are shown in Fig. 2. The radioactivity after intraveous administration of 1 mg/kg ^{14}C-imipramine was distributed widely in the body (Fig. 2a). High concentrations of radioactivity were observed in the lung, heart, brain and intestine, and a rather high radioactivity was observed in the liver. When the same dose of ^{14}C-imipramine was given through the hepatic portal vein, most of the radioactivity was taken up by the liver, as in the case of ^{14}C-propranolol (Fig. 2b). The radioactivity that reached the systemic circulation was extremely low, judging from the slight distribution in the intestine, lung, heart and kidney.

When a dose of 10 mg/kg ^{14}C-imipramine was given intravenously, the distribution of radioactivity was quite similar to that after intravenous administration of 1 mg/kg ^{14}C-imipramine (Fig. 2c). When the intraportal dose of ^{14}C-imipramine was increased to 10 mg/kg, most of the radioactivity was taken up by the liver, and the radioactivity that reached the systemic circulation seemed to be very low (Fig. 2d). In contrast to ^{14}C-propranolol, saturable hepatic uptake of ^{14}C-imipramine was not found in the dose range used in this study.

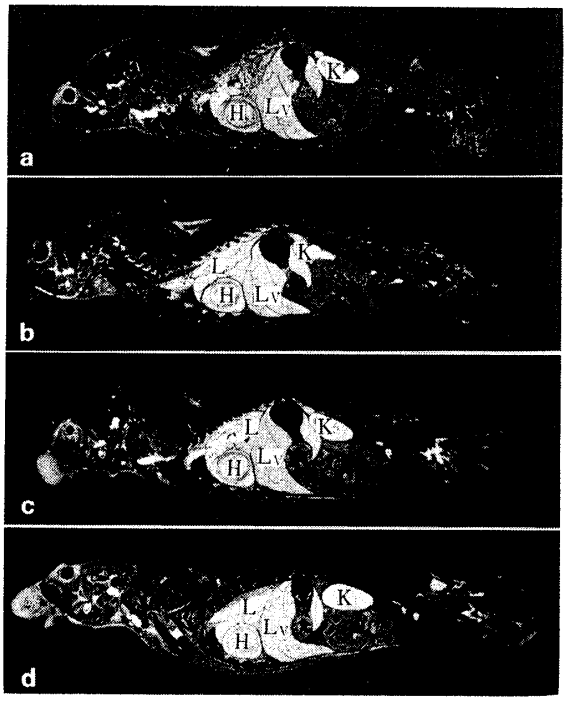

Fig. 3. Whole-Body Autoradiograms showing the Distribution of Radioactivity (White Areas) in Rats 3 min after Administration of ^{14}C-Salicylic Acid (5.0 μCi each)

(a) an intravenous dose of 1 mg/kg, (b) an intraportal dose of 1 mg/kg, (c) an intravenous dose of 10 mg/kg, (d) an intraportal dose of 10 mg/kg. Abbreviations are the same as in Fig. 1.

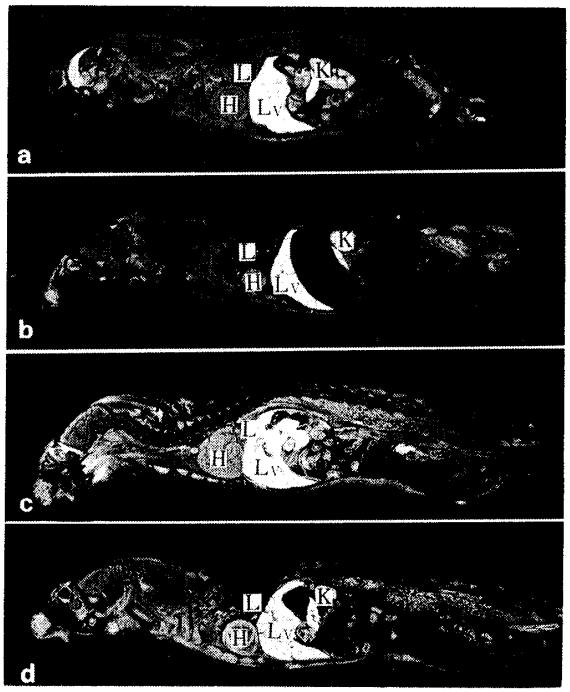

Fig. 4. Whole-Body Autoradiograms showing the Distribution of Radioactivity (White Areas) in Rats 3 min after Administration of ^{14}C-Antipyrine (6.0 μCi each)

(a) an intravenous dose of 1 mg/kg, (b) an intraportal dose of 1 mg/kg, (c) an intravenous dose of 10 mg/kg, (d) an intraportal dose of 10 mg/kg. Abbreviations are the same as in Fig. 1.

Whole-body Autoradiograms of Drugs Having a Low Hepatic Extraction Ratio

^{14}C-Salicyclic acid and ^{14}C-antipyrine were administered into the hepatic portal vein or the femoral vein of rats over 30 s. The whole-body autoradiograms of ^{14}C-salicylic acid (5.0 μCi each) 3 min after administration are shown in Fig. 3. The radioactivity after intravenous administration of 1 mg/kg ^{14}C-salicylic acid was distributed widely in the body (Fig. 3a). High concentrations of radioactivity were observed in the lung, heart, liver and kidney, and a rather high radioactivity was observed in the blood, but the radioactivity in the brain was low. When the same dose of ^{14}C-salicylic acid was given through the hepatic portal vein, the distribution of radioactivity was quite similar to that after intravenous administration (Fig. 3b). When a dose of 10 mg/kg ^{14}C-salicylic acid was given intravenously or through the hepatic portal vein, no appreciable difference due to the route of administration and dose was found in the distribution of radioactivity (Figs. 3c and 3d).

The whole-body autoradiograms of ^{14}C-antipyrine (6.0 μCi each) 3 min after administration are shown in Fig. 4. As in the case of ^{14}C-salicylic acid, no appreciable difference depending on the route of administration and dose was found in the distribution of radioactivity. The radioactivity was distributed almost uniformly in the whole body with the exception of the liver, where a relatively higher concentration of radioactivity was observed.

Unevenness of Drug Distribution in the Liver Depending on the Route of Administration

As shown in Figs. 1b and 1d, the radioactivity was distributed unevenly in the liver after intraportal administration of ^{14}C-propranolol, whereas it was uniformly distributed throughout the liver after intravenous administration (Figs. 1a and 1c). Particularly after an intraportal dose of 1 mg/kg ^{14}C-propranolol, there were areas in the liver where no radioactivity was observed. The radioactivity in the liver was also unevenly distributed after intraportal administration of ^{14}C-imipramine (Figs. 2b and 2d), although the uneven drug distribution was not as marked as that of ^{14}C-propranolol.

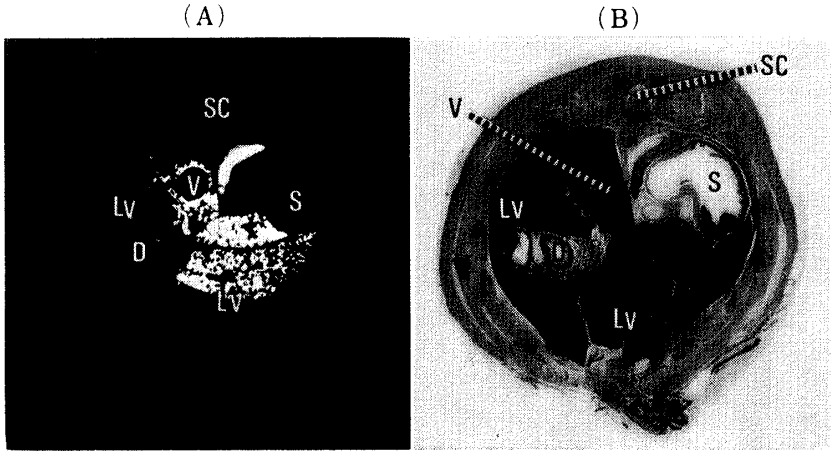

Fig. 5. Autoradiogram showing the Distribution of Radioactivity (White Areas) in the Rat Liver (A) and a Photograph of the Same Section stained with Hematoxylin-Eosin (B) 3 min after Intraportal Administration of 0.1 mg/kg ^{14}C-Propranolol (0.3 μCi)

Abbreviations: D, duodenum; Lv, liver; S, stomach; SC, spinal cord; V, vena cava.

To investigate the uneven distribution of radioactivity in the liver, 0.1, 0.4 and 0.7 mg/kg of ^{14}C-propranolol (0.3 μCi each) were given through the hepatic portal vein, and then sections at right angles to the spinal cord were taken. The autoradiogram of the liver 3 min after intraportal administration of 0.1 mg/kg ^{14}C-propranolol and a photograph of the same section stained with hematoxylin–eosin are shown in Fig. 5. The existence of areas where no radioactivity was present could be seen more clearly than after a dose of 1 mg/kg. After doses of 0.4

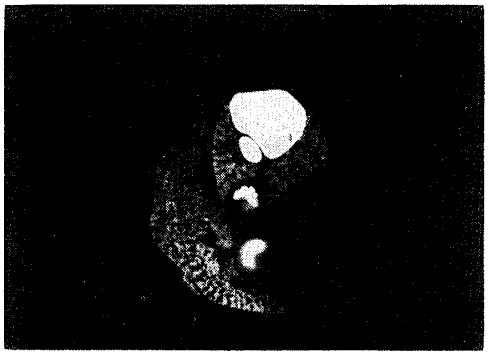

Fig. 6. Autoradiogram showing the Distribution of Radioactivity (White Areas) 30 min after Oral Administration of 10 mg/kg ^{14}C-Propranolol (3.0 μCi)

and 0.7 mg/kg, the autoradiograms were similar to that after a dose of 0.1 mg/kg.

A dose of 10 mg/kg ^{14}C-propranolol (3.0 μCi) was given orally and an autoradiogram was prepared 30 min after administration, since the blood levels of radioactivity reached maxima from 15 to 30 min after oral administration of 2.5 and 25 mg/kg ^{14}C-propranolol.[9] The uneven distribution of radioactivity in the liver was again observed, as shown in Fig. 6.

Discussion

Whole-body autoradiography permits the visualization of the distribution of radioactivity in various parts of the body, including tissues and organs that are often difficult to excise for measurement of the radioactivity. We attempted the visual estimation of the dependence of the amount of drug reaching the systemic circulation on the route of administration and dose by using an autoradiographic technique. For this purpose, the same radioactivity of a drug was always given to animals regardless of the route of administration and dose, and the process of preparing the autoradiograms was performed as consistently as possible. The autoradiograms were prepared shortly after administration to minimize the influence of metabolites on the distribution of radioactivity. Furthermore, drugs were given directly into the hepatic portal vein instead of orally since the rate and amount of drug given through the hepatic portal route could be easily controlled.

Propranolol is a well known drug subject to a first-pass effect.[10,11] It is eliminated almost entirely by metabolic transformation in the rat,[12] and the hepatic extraction ratio is remarkably high.[13] The AUC of propranolol was reported to be extremely small after an intraportal dose of less than 2 mg/kg.[11] The AUC after an intraportal dose of 10 mg/kg was, however, as large as that after intravenous administration of the same dose, indicating a reduction in hepatic extraction ratio with increasing dose.[11] In agreement with these observations, the radioactivity was almost completely taken up by the liver after intraportal administration of 1 mg/kg ^{14}C-propranolol (Fig. 1b). It can therefore be presumed that propranolol is subject to extensive first-pass metabolism. A reduction in hepatic extraction with increasing dose was also visualized on the autoradiogram after intraportal administration of 10 mg/kg ^{14}C-propranolol, but it should be noted that the radioactivity that reached the systemic circulation after an intraportal dose of 10 mg/kg was not as large as was expected from the ratio of 90% between the AUC's for intraportal and intravenous administration described in our previous paper.[11] This discrepancy is ascribed to an overestimation of bioavailability as calculated from the AUC ratio, because the mean hepatic extraction ratio is dependent on the route and rate of administration and the true bioavailability cannot be estimated simply from the AUC ratio.[14] Figures 1c and 1d reflect the finding[13] that the fraction of the intraportal propranolol dose reaching the systemic circulation is lower than that estimated from the AUC ratio.

Imipramine is also a drug subject to an extensive first-pass effect.[4,15] Most of the radioactivity given through the hepatic portal vein was taken up by the liver after administration of 1 mg/kg ^{14}C-imipramine and the high first-pass uptake is visualized in Fig. 2b. In contrast to the case of propranolol, the capacity of the hepatic uptake of imipramine was shown to be very large. The extent of hepatic uptake after a dose of 10 mg/kg was almost identical to that after a dose of 1 mg/kg. Stegmann *et al.*[16] examined the kinetics of imipramine in an isolated non-recirculating rat liver preparation perfused over a 2-min period. The hepatic

uptake process of imipramine was shown to be saturable, but even the highest concentration of 1000 μg/ml in the perfusate, calculated to represent an influx amount of about 290 mg/kg, was far from reaching saturation. The observations in our study are consistent with their results and suggest that the first-pass effect of imipramine is due to high hepatic uptake and retention.

On the other hand, salicylic acid is considered to have a much lower hepatic extraction ratio as compared with propranolol and imipramine.[5] This is clearly shown by the autoradiograms in Fig. 3, and the distribution pattern of radioactivity after intraportal administration was approximately the same as after intravenous administration throughout the dose range examined. The hepatic uptake was so low that considerable radioactivity was shown to reach the systemic circulation. Antipyrine is another drug having a low hepatic extraction ratio.[3] It was distributed almost evenly in the whole body, as in the case of salicylic acid (Fig. 4), and its distribution was independent of the route of administration and dose.

George *et al.*[17] found by needle biopsies that propranolol was unevenly distributed in the dog liver after intraportal administration, while it was distributed uniformly after intravenous administration. This report is consistent with the results obtained from autoradiograms in this study. In addition to the uneven distribution, a kind of "reticular" localization of radioactivity with a length of 0.5—1 mm was seen in every segment of the liver 3 min after intravenous administration of ^{14}C-propranolol and ^{14}C-imipramine. The "reticular" localization in the liver was also observed after intraportal administration of these two drugs in the areas where concentrations of radioactivity were low. However, when ^{14}C-salicylic acid or ^{14}C-antipyrine was given by both routes, the radioactivity was distributed uniformly in the liver and no "reticular" localization of radioactivity was observed. It has been well recognized that "reticular" localization in the liver could be observed shortly after intravenous administration of such weakly basic and lipophilic compounds as chlorpromazine,[18] diazepam[19] and chlordiazepoxide.[19]

It was shown in experimental animals[20,21] that a compound given from different tributaries of the hepatic portal vein reached the porta before it was well mixed with portal blood and entered specific different branches of the portal vein in the liver, whereas a compound given intravenously was well mixed with portal blood and hepatic arterial blood, and was distributed uniformly in the liver.[21] Thus, it is suggested that there are discrete channels of streamline flow from different parts of the gastrointestinal tract in the portal vein and these may enter specific different regions of the liver.

Since the initial concentration of ^{14}C-propranolol may not be uniform in portal blood, it is likely that ^{14}C-propranolol delivered by the hepatic route is concentrated at sites in the liver to which it is passed. The hepatic uptake of propranolol is extremely high, and so a small fraction of the dose recirculates through the liver. No difference could be observed between the autoradiograms 3 min after intraportal administration of ^{14}C-propranolol and those 7 min after intraportal administration. This indicates that the amount of ^{14}C-propranolol draining into the hepatic vein and recirculating through the liver after entering into the liver was minor during this period of time. Furthermore, the uneven distribution of ^{14}C-propranolol in the liver became clear with decreasing intraportal dose. Thus, it is suggested that both streamline flow of ^{14}C-propranolol in portal blood and its high hepatic extraction ratio may be responsible for the uneven distribution in the liver. On the other hand, the hepatic uptake of salicylic acid or antipyrine is very low. Most of each drug leaves the liver and recirculates through the liver, resulting in a less uneven distribution in the liver, even if the initial concentration of drug is not uniform in portal blood.

The unevenness of radioactivity after oral administration of ^{14}C-propranolol was not as marked as after intraportal administration, since it was apparent only on exposure to films for a shorter time period. However, it should be noted that the distribution of radioactivity in the liver was also uneven after oral administration of ^{14}C-propranolol. This uneven distribu-

tion in the liver, therefore, is probably a common phenomenon when drugs having a high affinity to the liver are given through the hepatic portal route.

The distribution data of drugs in the body are useful in understanding their therapeutic and toxicologic effects. The present study on the visualization of drug distribution by a whole-body autoradiographic technique provides a useful method for detecting visually a first-pass effect and also for visual characterization of the nature of the first-pass effect, *i.e.*, the significance of hepatic uptake and retention of drug in relation to the dose administered. The correlation between quantitative drug distribution and the present whole-body autoradiographic data will be presented in a subsequent paper.

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