Distribution Volume of Three $^{99m}$Tc-Labeled Compounds in the Rat Liver with Time after Intraportal and Intravenous Injections

Junko Nishigaki,* Satoshi Suzuki, Joji Yui, and Akiyo Shigematsu

Institute of Whole Body Metabolism, 340-2 Nauchi, Shiroi, Inba, Chiba 270-14, Japan.
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With the aim of making direct investigational studies of various liver functions of sustained healthy animals, we devised operative procedures which are not only simple and reproducible but also adaptable to experiments even over the course of one-week without anesthesia.

Using rats which have been operated upon, three kinds of tracers, $^{99m}$Tc-pool scinti, $^{99m}$Tc-pertechnetate and $^{99m}$Tc-phytate, were recorded by a scintillation camera with a single-photon system immediately after intraportal administration. The results revealed that the discharge of these radioactive components from the hepatic vein began 0.2 s after the administration, that the maximum intrahepatic concentration was reached between 2 and 5 s thereafter, and that 10 s later, these radioactive components were returned to the liver after their systemic circulation. Intrahepatic uptake of these three tracers was about 20% for all radioactivities administered.

Key words first pass; rat liver distribution volume; $^{99m}$Tc-labeled compounds; liver vessel cannulation; nonanesthetic condition

With advances in research on the kinetics of drugs and toxic substances, the importance of in vivo experiments has been attracting renewed attention. Exogenous drugs and toxic substances taken into the body exert different influences on the internal organ tissues depending on the route of administration.1,2) Hence, studies are required regarding the amount of first pass and absorbed dose, as well as the metabolic rate and metabolism of the drugs and toxicant in terms of the administered amount per organ or tissue, in addition to the dose relative to total body weight.

The most typical target of study in first-pass experiments has been the liver, and representative of the in situ method has been liver perfusion.3–7) However, the liver perfusion method has many problems yet to be solved. That is, (1) the interruption of control by the central nervous system, (2) the perfusate, which is artificial and alters the liver condition from the normal physiological state, (3) physiological conditions which differ largely depending on the duration of the pretreatment prior to experiment, (4) serious damage of the cells due to the artificial perfusate which results in the activation of enzymes different from those in normal metabolism.

Meanwhile, there have been many reports on the methodology of in vivo experiments. Sugiyama8) has reported experimental results on $^{125}$I-EGF excretion from the liver at the time of first pass, and a renal clearance value was obtained using rats in a semivigilant state. The deterioration of liver function due to anesthesia or postoperative stress has not been prevented. Huang9) reported an ingenious operation method involving cannulation of a blood vessel. This study verified, using $^{3}$H$_{2}$O, that the blood obtained through a tubule inserted into the hepatic vein illustrates a drug concentration which immediately reflects the function of the liver. However, many other studies have reported observation of the first pass in the liver more than one minute after administration. Also, there have been no reports on the time-radioactivity curve at the first pass, or the relationship between the area under the curve (AUC) dose.

* To whom correspondence should be addressed.

We have taken the above-described points into consideration and carried out an experiment devoting special attention to the change in radioactivity in the liver at the time of the first pass, using $^{99m}$Tc as a marker and a scintillation camera.10)

MATERIALS AND METHODS

Animals Male Wistar rats (Japan Clea), purchased at 6 weeks of age and acclimatized for a week, were used at the age of 7 weeks. They were raised at 22±1°C with 60±10% RH, and were fed CE-2 (Japan Clea) with fresh tap water ad libitum.

Surgery 1) A rat was administered an i.p. injection of pentobarbital after hair was removed in the abdominal and groin areas using an electric clipper under anesthesia; the rat was then fixed on the operating table. 2) The skin was incised 2–3 cm along the median line, followed by another incision of the abdominal muscle along the median line. 3) The duodenum, drawn out from the incision, was fixed to render the portal vein. 4) The pancreatic-duodenal vein running toward the portal vein was stripped of mesenteron membrane, and two threads (No. 2) were set under the former vein for ligature. After ligation of the vein with a thread on the intestinal side, cannulation was done using Intramedic Polyethylene Tubing (PE10, Nippon Becton Dickinson Co.) as a cannula for the use of portal vein infusion toward the vein on the portal thread, and PE10 was fixed. 5) The duodenum left outside was returned through the incision, and the top of another side of the cannula tube was passed on subcutaneously to the back. 6) Abdominal muscle and the skin were sutured. 7) The top of the cannula was sealed with a small stopper and mounted and fixed in the subcutaneous space between the skin and back skeletal muscle. That is a good indicator to prevent any tampering by the experimental animal, since the animal is again free to eat and drink. In case of the experimental use, the cannula was connected with a short silicon tube following a relatively long PE10 for injection of the labeled fluid.

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Experiments Using $^{99m}$Tc Compounds 1) Radioactivity, Dose, and Dosage Regimen: $^{99m}$Tc, isolated as the daughter nucleus of $^{99m}$Mo, was charged with the following precursor of three $^{99m}$Tc specimens: (1) Sodium pertechnetate ($^{99m}$Tc) injection, Dinatiec, was purchased from Daiichi Radioisotopes Co., (2) Technetium-labelled human serum albumin ($^{99m}$Tc) injection, pool scinti, was purchased from Mediphys Co., (3) Technetium phytate ($^{99m}$Tc) injection, Technephytate kits, was purchased from Daiichi Radioisotopes Co. Each labelled injection fluid was administered into the portal vein (p.v.) or the vena cava (i.v.) at 1–3 mCi/0.05 ml/head.

2) Kinetic Analyses of the $^{99m}$Tc Compounds in the Liver: Using a gamma camera (Siemens, ZLC7500) and Scintpack 700 (Shimadzu), the time-radioactivity curve for 1 in the liver at 0.5-s intervals immediately after administration of the radioactive agent was obtained.

After correcting for the radioactive concentrations of the 3 compounds, each radioactivity was converted into the cpm value.

RESULTS

The changes in $^{99m}$Tc distribution in rats were recorded by using a single-photon emission probe of the scintillation camera immediately after the infusion of $^{99m}$Tc by use of a portal infusion cannula. The value of the radioactivity observed at each matrix was measured by the late radiometry system with every 0.2 s as a time constant. The integrated values for 1 m were converted to the images shown in Fig. 1. In this experiment, three kinds of tracers were used: $^{99m}$Tc-pool scinti, $^{99m}$Tc-pertechnetate, and $^{99m}$Tc-phytate. Highly dense localization was seen in the center of the upper one-third, the upper half of which corresponded to the heart and the lower to the liver. Radioactivity in the liver was seen to be significantly high. The rest of the animal showed a uniformly lower level of radioactivity. The images were taken every 0.2 s as a time

![Fig. 1. Scintigrams of $^{99m}$Tc Subjects after Intravenous Injection and Portal Vein Injection](image-url)
constant. In the region of interest (ROI) curve for the total body (Fig. 2), the value was almost constant after the 4th or 5th interval of 0.2 s. This indicates that approximately 1 s, the time required for the counting rate to reach the steady-state value, is the time required for the administered solution to become homogeneously mixed with the total body blood. As shown in Fig. 2, in scintigrams recorded more than 1 s after the administration of equal doses of $^{99m}$Tc-perchnetate, the counting rates (cps) of the total body ROI obtained in the case of i.v. administration agreed well with those obtained in the case of i.v. administration. However, when these two administration methods were compared specifically for ROI of the liver, the counting rates differed significantly. In the case of i.v. administration, a virtually constant counting rate of 4000 cps was observed 3 s after administration, whereas in the case of i.v. administration, it peaked at approximately 26000 cps 3–4 s after administration, and thereafter exhibited a gentle downward curve with approximately twice the half time of the upward curve, showing excretion from the liver. Upon reaching the order of 16000 cps approximately 5 s after the administration of $^{99m}$Tc-pool scinti, the rate of decrease in the counting rate of liver ROI approached a constant value and the downward curve became very mild. The half time was approximately 10 s, after which the counting rate was constant at around 8000 cps.

With regard to the scintigrams of the $^{99m}$Tc-pool scinti shown in Fig. 3, the change with time in the total body ROI was in good agreement with the results in Fig. 2, and the change with time in the liver ROI value also agreed well with the results in Fig. 2 in the case of i.v. administration. However, in the case of i.v. administration, liver ROI values peaked at 16000–18000 cps for a relatively long period within 4–6 s after administration, which were much lower values than the peak value in the case of $^{99m}$Tc-perchnetate administration. After reaching the peak value, the curve turned downward showing drug excretion from the liver and exhibited a very mild slope, with the half time increased to approximately three to four times that of the upward curve. However, the ROI value settled at approximately 8000 cps, the same as in the case in Fig. 2, approximately 40 s after administration.

Figure 4 shows an example of the case of $^{99m}$Tc-phytate administration. In this case also, the peak value of the total body ROI of approximately 40000 cps agreed well with those of the other two specimens. The curve for liver ROI after i.v. administration also agreed well with those in Figs. 2 and 3. The curve of liver ROI after i.p. administration was similar to that in Fig. 2. The slope of the upward curve was steep: a peak value of approximately 22000 cps was reached within 3–4 s after administration, and the slope in terms of half time of the downward curve was approximately twice that of the upward curve. The counting rate of ROI remained constant on the order of 15000 cps from 10 s after administration, and thereafter it increased slightly.

The counting rate vs. time curves obtained on the basis of scintigram analysis of total body and liver ROIs of rats after i.v. and i.p. administration of three kinds of $^{99m}$Tc
specimens showed that after i.p. administration, most of the radioactive components administered to the liver circulated through the entire body in the bloodstream. Therefore, we calculated the ratio of radioactivity absorbed by the liver after the first i.p. administration to radioactivity supplied to the liver by i.v. administration. The curve showing the change in ratio with time is presented in Fig. 5. This figure shows that the highest percentage of radioactivity absorbed by the liver during the first circulation of the specimen through the liver after i.p. administration was observed in the case of $^{99m}$Tc-pertechnetate administration, followed by $^{99m}$Tc-pool scinti and $^{99m}$Tc-phytate administration. With all three specimens, most (approximately 80%) of the administered dose flowed out of the liver into the circulation within approximately 10 s after i.p. administration, indicating that the first-uptake rate was approximately 20%.

DISCUSSION

If drugs are directly administered to the target organ and the influence of the drug on the organ can be elucidated in terms of its medical, pharmacological or toxicological effect, the most direct information can be obtained. Cannulation into an artery or vein as a means of obtaining immediate information has recently become a common method due to the further development of surgical operation technology.\(^{11-14}\) In this experiment, we used rats whose vena cava and portal vein were cannulated for i.v. and p.v. administration of drugs, respectively. Rats on which cannulation is performed can be bred under regular conditions without restraints for approximately one month, depending on the objective of the experiment.

In the present experiments, single photon emission images were recorded every 0.2 s and the counting rates were determined using a rate meter. However, since the solution administered moves very rapidly in blood vessels within 1–2 s after entering the bloodstream, it cannot be considered to be stationary in the 0.2 s corresponding to each pixel. Therefore, the counting rates recorded in this manner are only apparent counting rates. If the rate meter has sufficiently high sensitivity so that the effect of the blood velocity can be ignored, true values can be obtained as follows using the counting rate of 0.2 s as a time constant.

$$\text{true counting rate} \times \left( \frac{0.2 \text{s}}{\text{time required for point in blood stream to pass through one pixel (≈0.02 s)}} \right)$$

This means that when the administered solution and circulating blood are mixed essentially to homogeneity, the counting rate recorded for each pixel is constant at 0.2 s. In the case of the p.v. administration, the radioactivity of each compound in the liver showed a maximum value within the first several seconds after the administration, and then fell to approximately one-half of the maximum value within several seconds.

As shown in Fig. 5, at 0 s after administration, the peak ratios were approximately 40.0, 47.0, and 45.6% of the initial maximum, respectively. Thus, it was apparent that a difference in the count values in the liver between that at 0 s and that at 1–2 s after administration was limited to approximately a two-fold increase. Such a difference was assumed to be close to the difference using the true value corrected from the apparent one of the time constant obtained by the scintillation camera, compared with the difference in peaks between that at 0 s and that 5 s later in the ascending curve obtained by the scintillation camera after administration into the portal vein.

When a radioactive tracer was injected from the portal vein, the differences in peaks between the count values in the liver and the whole body 60 s after administration, were equivalent to approximately 25.6, 20.0, and 37.5% with pertechnetate, pool scinti, and phytate, respectively, based on the data output from the scintillation camera, and differences in peaks of the count values in the case of i.v. were equivalent to approximately 7.7, 7.5, and 10.0%, respectively. According to the data of Sankyo Laboratory Service, the standard body weight of a male Wistar rat 10 weeks of age is about 243 g, and its liver weighs about 7 g. Blood circulating in the liver is about 1.3 g, assuming that 7.5% of $^{99m}$Tc-pool scinti is in the liver 60 s after the i.v. injection and the whole blood volume is about 7% of the body weight (243 g). It means that the content of blood in the liver is about 19% in weight. When blood in the liver is assumed to be about 1.3 g, 20.0% of which is found in the liver 60 s after the administration of pool scintillator via p.v., it indicates that 20.0% of blood was exchanged in 60 s. When such an exchange is made under the conditions of the one-compartment model, $\lambda = 0.02682$ can be obtained from the equation of $0.200 = 1 \times e^{-\lambda \times 60}$. Thus, it suggests that a flow rate in the liver is equivalent to approximately 2.09 g/60 s against that of blood at 1.3 g.

Huang devised a method which permits the fixation of a cannula for a long period of time in an animal in a awakened state, and reported a method which enables the observation of the reaction of the liver to drug under a condition free from mixture with blood from tissues other than the liver.\(^9\) His experiments compared the radioactive blood concentration in the hepatic vein after injection of

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**Fig. 5.** $^{99m}$Tc Concentration Ratio in Whole Liver of Rats with Time after i.v. and p.v. Injections
tritium water into the inferior vena cava and that at an upper position of the vena cava higher than the point of tritium injection, in terms of the time–radioactivity curve. The results showed that the $T_{\text{max}}$ in the hepatic vein tends to be longer than that in the vena cava. However, in both cases, $T_{\text{max}}$ was reached within approximately 1 m after injection, when the time difference due to the experimental procedures is corrected. Conversely, the results of the current experiment show that the $T_{\text{max}}$ of the radioactivity in the liver following p.v. administration was reached within only several seconds, irrespective of the kind of compound, and in case of i.v. injection, the radioactivity in the liver almost reached the plateau within 10 s.

The results obtained from the current experiments are important as basic data for in vivo experiments seeking to obtain the amount of first pass of a drug in the liver. It has been shown that a marker instantaneously injected into the portal vein is swiftly distributed in the liver, and a high concentration of marker passes through the hepatic vein in only several seconds. Therefore, the period of at least one minute after the injection of drugs into the portal vein is the most important duration for the in vivo observation of the first pass of drugs in the liver. The location of the cannula fixation and the reduction of the influence of blood drawing on the blood flow are important from the standpoint of performing experiments on animals under physiological conditions as close to normal as possible.

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REFERENCES