from the microsphere can be primarily attributed to dissolution of drug from the surface with a minor contribution of matrix diffusion of albumin. To slow the release rate to levels practical for long term therapy, the beads can be coated with polymer containing no drug. A sevenfold decrease in release rate was observed after coating insulin-containing slabs with 20% ethylene-vinyl acetate solution (9).

The microsphere preparation technique can be used for other drugs and polymers other than ethylene-vinyl acetate, but it is important that the polymer-solvent solutions have glass transition temperatures > −78°C. Nonsolvents for the polymer, other than ethanol, could be used as long as they do not freeze at −78°C. In addition, the low-temperature nature of the process makes it ideally suited for thermally labile compounds that cannot be processed at the higher temperatures of conventional prilling processes [*e.g.*, 60°C for gelatin encapsulation (18)].

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Comparison of ^{99m}Tc-*N*-Pyridoxyl-5-Methyltryptophan and ^{99m}Tc-*N*-(3-Bromo-2,4,6-trimethylacetanilide)-Iminodiacetate as Hepatobiliary Radiopharmaceuticals in Rats

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Abstract □ ^{99m}Tc - *N* - (3-bromo-2,4,6-trimethylacetanilide)iminodiacetate (I) and ^{99m}Tc-*N*-pyridoxyl-5-methyl-tryptophan (II) have been described as having optimal properties as hepatobiliary radiopharmaceuticals. This study compared specificity for hepatobiliary excretion, blood disappearance, rates of biliary appearance, and pharmacokinetic parameters including hepatic clearance, volumes of distribution, and mean residence times in normal and sulfobromophthalein-treated rats. The specificity of I was higher as indicated by 94% in the bile at 90 min compared to 91% for II in normal rats and a urine excretion of 0.3% for I compared with 1.9% for II. In sulfobromophthalein-treated animals, urine excretion increases were only to 0.5 and 3.0% for I and II, respectively. In control rats, blood disappearance was similar for both I and II, but II disappeared faster in treated animals. The clearance of II was 70 mL/min/kg in normal and 47 mL/min/kg in treated rats; clearance of I was 51 and 30 mL/min/kg in normal and treated rats, respectively. Volumes of distribution were larger for II. Compound I was superior in specificity while II was superior in clearance and excretion kinetics.

Keyphrases □ Hepatobiliary radiopharmaceuticals—^{99m}Tc-labeled compounds compared, sulfobromophthalein-treated and normal rats □ Radiopharmaceuticals—^{99m}Tc-labeled hepatobiliary compounds compared, sulfobromophthalein-treated and normal rats □ Sulfobromophthalein—effect on hepatobiliary radiopharmaceuticals, rats

Efforts to develop an optimal ^{99m}Tc-labeled hepatobiliary agent (I) have followed either a series beginning with ^{99m}Tc-labeled pyridoxylidene glutamate (2) or ^{99m}Tc-*N*-(2,6-dimethylacetanilide)iminodiacetate (3). These studies have resulted in improvements in hepatobiliary specificity, kinetics, and resistance to transport competition from elevated bilirubin levels. The currently "best" agents, based on animal and clinical studies, are ^{99m}Tc-*N*-(3-bromo-2,4,6-trimethylacetanilide)iminodiacetate (I) (4, 5) and ^{99m}Tc-*N*-pyridoxyl-5-methyltryptophan (II) (6).

To try to answer the question of which agent is superior, short of clinical paired comparisons in normal volunteers and patients, we have carried out detailed studies in rats. These studies were designed to compare rates of blood disappearance, rates of biliary appearance, specificity for hepatobiliary excretion, hepatic clearance of the complexes, and ability of the

agents to compete with sulfobromophthalein as a member of the dye anion and bilirubin pathway for hepatobiliary excretion.

EXPERIMENTAL SECTION

Radiopharmaceutical Preparation—Kits for the preparation of I for animal use were obtained as a gift[1]. They were prepared by the addition of 2–3 mL of generator eluate saline containing [^{99m}Tc]pertechnetate at a concentration of ~5 mCi/mL. The percent of ^{99m}Tc-radioactivity bound to the ligand was determined by TLC[2]. Saturated sodium chloride was used to develop silicic acid strips (R_f of pertechnetate = 1, R_f of I = 0–0.2) for the determination of free [^{99m}Tc]pertechnetate. An acetonitrile–water mixture (3:1) was used to develop silica gel strips (R_f of pertechnetate and I = 1, R_f of insoluble radioactive material = 0) for the determination of insoluble activity. The percent bound was calculated as 100 − (percent [^{99m}Tc]pertechnetate + percent insoluble radioactivity). Values were ≥98%. The ligand of II was supplied[3]. The preparation of kits for II was performed essentially as described (6). Radioactivity was added to a thawed kit (~30–50 mCi to 1.0 mL of kit volume) and the vial was heated in a boiling water bath for 30 min. At that time, small amounts of free pertechnetate were sometimes present. A 250-μL aliquot was therefore purified by HPLC to remove any free pertechnetate or insoluble radioactive material. Chromatographic conditions were: 0.01 M sodium phosphate buffer (pH 6) and acetonitrile with a gradient of 20–70% acetonitrile programmed over 20 min at a flow rate of 1.0 mL/min on an ODS column[4]. Fractions from three different peaks were collected without fractionation at 8–10 mL eluate volume [these fractions were shown to contain stereoisomeric chelate isomers that were only slightly different in their biological properties (7)]. The eluate was collected in a tube containing 1.5 mg of freshly dissolved ascorbic acid, as an antioxidant stabilizer, in 1 mL of water. Fifteen- to twenty-fold dilutions were made with saline containing 1 mg/mL ascorbic acid. The final injection volume of 0.25 mL contained ~50 μCi.

Animal Studies—Male Sprague–Dawley rats (300–430 g) were anesthetized with sodium pentobarbital. Catheters were placed in the femoral vein for injections, hydration, and infusions and in the carotid artery for blood sampling. The common bile duct was cannulated and bile was collected in tubes in a fraction collector. The body temperature of the rats was maintained at 37°C with external heat from a lamp. For competitive studies, sulfobromophthalein was infused at a transport maximum level of 2.5 μmol/min/kg for 15 min prior to and for 60 min postinjection of the radiopharmaceutical (8). The infusion flow rate was 0.1 mL/min. Urine normally was retained in the bladder during the 90-min studies. At that time the bladder was tied off, removed, and counted together with portions of the urinary tract that might contain urine. Bile and urine samples were counted in a well counter with appropriate dose standards. Total blood volume was assumed to be 7% of body weight (9). Blood samples of 0.3–0.4 mL were taken at 1, 2, 3, 4, 5, 6, 9, 12, 15, 20, 25, 45, 60, 75, and 90 min postinjection. Bile samples were collected at 0 and 2 min and then at 4-min intervals to 90 min postinjection.

Pharmacokinetic Calculations—Blood radioactivity concentration (cpm/mL of blood) *versus* time curves for each animal were used to calculate distribution volume, blood clearance, and mean residence time using standard methods (10, 11). All comparisons were made using Student's *t* test (12).

RESULTS

The preparations of II were purified by HPLC to remove unbound label as pertechnetate. This was necessary because the presence of small amounts of oxygen during the heating period decomposed some of the chelate and reoxidized the technetium. The isomeric chelated forms were not separated since they have been shown to have small *in vivo* differences (7) and ultimate use of this preparation in clinical situations would not involve HPLC purification (hence no possibility for separation of chelate forms).

Blood disappearance curves for the radioactivities of I and II under normal and sulfobromophthalein conditions are shown in Figs. 1 and 2. In control rats, II levels were somewhat lower in the first 5 min while I levels were slightly lower afterwards. Both compounds are rapidly cleared with <1% of the injected dose remaining in the blood at 14 min for I and at 18 min for II. In rats treated with sulfobromophthalein the blood disappearance of both compounds was slowed; however, the disappearance of II was faster than that of I at all times.

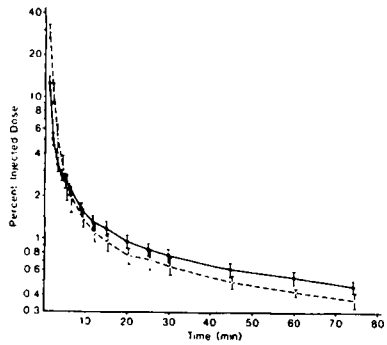

Figure 1—*Disappearance of radioactivity from I (- -○- -) and II (-●-) from blood of normal rats. Data are plotted as mean* ± SEM (n = 5) *and represent percent of injected dose remaining in total blood.*

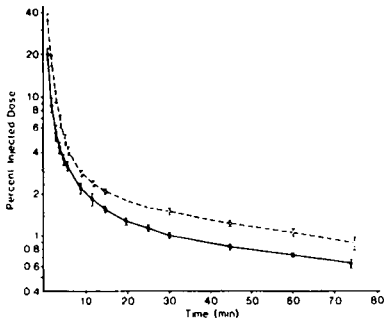

Figure 2—*Disappearance of radioactivity from I (- -○- -) and II (-●-) from the blood of rats treated with sulfobromophthalein. Data are plotted as mean* ± SEM (n = 5) *and represent percent of injected dose remaining in total blood.*

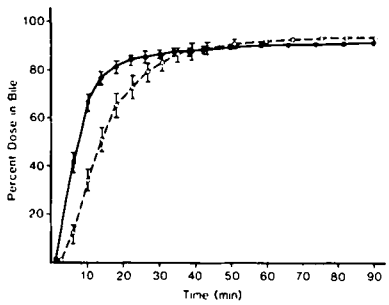

Figure 3—*Comparative rates of appearance of radioactivity from I (- -○- -) and II (-●-) in bile in normal rats. Data are plotted as mean* ± SEM *of percent of injected dose.*

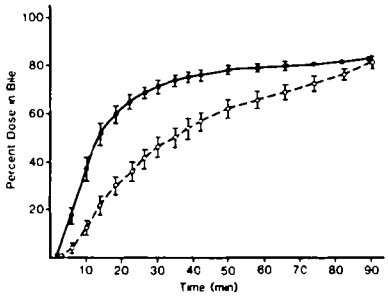

Figure 4—*Comparative rates of appearance of radioactivity of I (- -○- -) and II (-●-) in bile of rats treated with sulfobromophthalein. Data are mean* ± SEM (n = 5) *percent of injected dose.*

Bile cumulative radioactivity curves for the radiopharmaceuticals are shown in Figs. 3 and 4. In normal rats II appeared significantly faster. Forty-one percent of the dose of II was found in the bile by 6 min compared with 12%

[1] E. R. Squibb & Sons, New Brunswick, N.J.
[2] Gelman ITLC, Ann Arbor, Mich.
[3] Nihon Medi-Physics, Takarazuka, Japan.
[4] Beckman-Altex gradient system with 5-μm ultrasphere ODS column, Berkeley, Calif.

Table I—Excretion and Pharmacokinetic Parameters of Hepatobiliary Agents in Control and Treated Rats [a]

Radiopharmaceutical	Urine Excretion, %[b]	Biliary Excretion, %[b]	CL_t, mL/min/kg	Vd, mL/kg	Mean Residence Time, min
I					
Control	0.27 ± 0.08	93.86 ± 1.13	50.8 ± 4.6	743 ± 73[c]	14.6 ± 0.56[c]
Sulfobromophthalein	0.47 ± 0.08	81.02 ± 1.48	29.5 ± 0.93[c]	564 ± 36	19.2 ± 1.00
II					
Control	1.89 ± 0.25	91.45 ● 1.30	70.0 ± 8.0	1695 ± 209	24.1 ± 0.51
Sulfobromophthalein	3.00 ± 0.39	81.98 ± 1.17	46.8 ± 2.7	1042 ± 88	22.1 ± 0.89

[a] Data are mean ± *SEM*. [b] Urine and biliary excretion are percent injected dose at 90 min postinjection. [c] Statistically significant difference ($p < 0.05$) from II (sulfobromophthalein).

for I. Comparative values at 10 min were 66 and 34%; by 40 min, equivalent percentages of each had been excreted. The high final values indicate the high specificity of both compounds.

In sulfobromophthalein-treated animals the rates of appearance of radioactivity in the bile were significantly decreased. Moreover, the differences between compounds I and II were more marked in treated animals than controls. Over the first 20 min II appeared at a lower rate than in the normal animals, but essentially at the same rate as I in normal animals.

Quantitative comparisons of renal and hepatobiliary excretion of the complexes and pharmacokinetic parameters are shown in Table I. Both agents exhibited high specificity for hepatobiliary excretion. However, the percentage of I (94.9%) was higher than II (91.4%) in bile. This was consistent with a lower renal excretion of I (0.27% compared with 1.89% for II). In animals treated with sulfobromophthalein, biliary excretion was reduced for both radiopharmaceuticals to ~82% at 90 min. Conversely, renal excretion at 90 min was increased for both, but only by a small percentage (from 0.3 to 0.5% for I and from 1.9 to 3.0% of the injected dose for II).

Statistically, the blood clearances for I and II were not significantly different for the controls ($p < 0.05$). Since both agents are highly specific for hepatobiliary excretion, the correction of blood clearances for renal clearances was considered negligible. The magnitude of clearances for the two agents indicates efficient hepatobiliary excretion of both agents at rates which would depend primarily on hepatic blood flow. The clearances of both II and I were reduced in the presence of sulfobromophthalein. In this case the difference in clearance values was statistically significant ($p < 0.05$). The distribution volume for II was greater than that for I ($p < 0.05$) and the volumes of both compounds were decreased during the administration of sulfobromophthalein.

The mean residence time (MRT) for II was greater than the MRT for I ($p < 0.05$). The MRT is directly related to distribution volume and inversely related to blood clearance (MRT = distribution volume/blood clearance). Thus, although the hepatobiliary excretion (blood clearance) of II is more efficient than that of I, the II distribution volume is greater, resulting in a greater MRT for II. Caution should be exercised however, since interpretations of MRT values are difficult if the clearing organ (in this case the liver) is also responsible for significant distribution of the compound (13).

DISCUSSION

Both of the ^{99m}Tc-labeled hepatobiliary agents evaluated here are the result of the synthesis and biological study of a long series of chelating agents beginning with *N*-(2,6-dimethylacetanilide)iminodiacetate for I and pyridoxylidene glutamate for II. They both rate highly with regard to the clinical objectives of rapid hepatocyte uptake from the blood, short hepatocyte transit times, high specificity for hepatobiliary excretion, and ability to be taken up and excreted by the liver with high specificity in the presence of bilirubin or other competing transport pathway members. The questions of this study are whether or not one agent is significantly superior and whether or not a comparative evaluation in animals can indicate which will be superior in clinical use.

In this study sulfobromophthalein was used as a model for the determination of the ability of these technetium complexes to be taken up and excreted by the liver in the jaundiced or hyperbilirubinemic patient. A more direct way would have been to use bilirubin. However, it has been difficult to achieve sufficiently high serum bilirubin levels for significant effects on the transport of ^{99m}Tc-labeled iminodiacetate radiopharmaceuticals without causing toxic reactions (14, 15). The secretion of bilirubin is competitively inhibited by sulfobromophthalein (16) and many examples of similar effects on the transport of bilirubin and sulfobromophthalein are known (17). In addition, studies in isolated hepatocytes, in which sulfobromophthalein and bilirubin were separately added, both were reported to have comparable effects on the uptake of ^{99m}Tc-labeled iminodiacetate complexes by the hepatocytes (14).

Short hepatocyte transit times result in a "bolus" effect on the passage of radioactivity from liver to bile. This effect allows imaging of the hepatic ducts superimposed on the liver and often provides information on the location of problems in the biliary ductule network (14). The data from these studies demonstrate that with similar blood disappearance rates and thus hepatic uptake,the more rapid biliary excretion of II results in an advantage in bile-to-liver ratio comparisons.

Under normal liver function conditions the blood disappearance rates of both agents were similar. The bile appearance of II was significantly faster and indicates a shorter hepatocyte transit time for II. Final values of collected activity in the bile were consistent with their reported specificities for hepatobiliary excretion (4, 6).

Studies in which the transport capacity of the liver was stressed by sulfobromophthalein resulted in greater differences between the complexes. Under this condition the blood percent dose values for II were lower at all times and biliary excretion differences were more marked than in normal control studies. Thus, II was superior in its uptake and excretion in competition with sulfobromophthalein and, as such, appears to have a higher affinity for the transport proteins.

The results of these studies support a small specificity superiority for I and a significant kinetic superiority for II as hepatobiliary radiopharmaceuticals. However, species differences are well known (18) and it must be emphasized that there is still a need to compare these agents in humans.

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