RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Hepatic Clearance Mechanism of Tc-99m-HIDA and Its Effect on Quantitation of Hepatobiliary Function: Concise Communication

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Parameters affecting the hepatobiliary clearance of Tc-99m N(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (Tc-HIDA) were evaluated in dogs. Competitive clearance studies were performed with Tc-HIDA after infusion to plasma saturation levels of an anion, sodium sulfobromophthalein (BSP), and a cation, oxyphenonium. The results demonstrated that Tc-HIDA is transported through hepatocytes by a carrier-mediated organic-anion pathway. The data are consistent with an alteration of the elimination kinetics of Tc-HIDA induced by elevations in the serum bilirubin level, and it is predicted that serum bilirubin at some increased concentration will dominate the distribution and elimination kinetics of Tc-HIDA independently of hepatobiliary status. A quantitative description of liver function in terms of regional distribution and elimination rate constants will require either a pharmacokinetic model that expressly includes the effects of bilirubin, the development of new anionic hepatobiliary agents capable of displacing endogenous bilirubin from transport binding sites, or the development of new hepatobiliary agents that use a different clearance mechanism from that used by bilirubin.

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Technetium-99m *N*(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (Tc-HIDA) has been developed recently as an intended replacement for I-131 rose bengal (RB) (*1,2*) and has demonstrated superior kinetic properties for hepatobiliary imaging (*3*). Its value in the clinical evaluation of jaundice in man was shown by Ryan et al. (*4*) through the use of sequential scintigraphy. An additional clinical goal for this new agent is the quantitation of certain aspects of liver function through the development of a pharmacokinetic model that relates Tc-HIDA's in vivo time-activity curves to regional distribution and elimination rate constants. For a pharmacokinetic approach to the quantification of liver function to be successful, the in vivo distribution of the radiopharmaceutical should be solely a function of a partient's hepatocellular status. Of the four known carrier-mediated mechanisms of hepatobiliary clearance, the general anionic clearance mechanism used by bilirubin is used by most, if not all, of the existing radiotracers (*5,6*). This suggests that in the jaundiced patient bilirubin may competitively displace existing radiopharma-

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ceuticals from carrier-mediated binding sites, and that at some point the concentration of serum bilirubin may become the determinant of radiopharmaceutical distribution and elimination kinetics independently of other clinical parameters. To determine the mechanism of the hepatobiliary clearance of Tc-HIDA, competitive clearance studies were performed in dogs with an anion, sulfobromophthalein, and a cation, oxyphenonium.

METHODS

Anionic clearance mechanism. In three female dogs the distribution and elimination kinetics of I-131RB and Tc-HIDA were simultaneously evaluated before and after inducing plasma saturation levels of sulfobromophthalein (BSP) (*7*). The animal model was prepared for blood, urine, and bile collection after i.v. administration of pentobarbital (30 mg/kg): a Cournand needle was inserted upstream into a femoral artery for blood collection; a Foley catheter was inserted into the bladder; and a midline abdominal incision (approximately 15 cm) was made so that the gallbladder and biliary tree could be isolated. The cystic duct was ligated to prevent bile flow into the gallbladder. A polyethylene catheter was introduced through a small incision into the common bile duct and advanced to the junction of the two hepatic ducts to ensure total bile drainage into the catheter.

After preparation of the basic model, each animal was given 1 mCi of Tc-HIDA intravenously into a front cephalic vein. This injection also contained 7 μCi of I-131 RB to ensure that initial kinetic values would correlate with literature values for the blood clearance and biliary uptake of RB. One-cubic centimeter blood samples were collected at 5, 10, 15, 30, 45, and 60 min postinjection. Cumulative bile samples of known volume were collected at four 15-min intervals, and a cumulative urine sample was obtained 1 hr postinjection. At 1½ hr postinjection, blood, bile, and urine samples were again taken to ascertain baseline values for the second part of the experiment. At this point, each animal received a 50-mg bolus injection of BSP followed by an i.v. infusion of BSP in saline (7.5 mg/ml), which continued throughout the remainder of the experiment at a rate of 1 ml per minute. Blood samples were taken before infusion and ½-hr postinfusion and were analyzed for plasma BSP levels and for the percentage of Tc-HIDA that was protein bound. Sodium sulfobromophthalein levels were determined using the standard colorimetric method (*8*). The extent of protein binding was measured by addition of Tc-HIDA to the plasma samples followed by equilibrium dialysis for 18 hr. After a 30-min infusion of BSP, each animal was administered a 10-mCi dose of Tc-HIDA (15 mg) mixed with 70 μCi of I-131 RB. Blood, bile, and urine were collected at the same time intervals as in the first phase of the experiment.

All fluid samples were counted in a NaI(T1) well counter with separate counting windows for Tc-99m and I-131. The radioactivity in each window was corrected for residual activity from the previous study, and the Tc-99m counts corrected for crossover from I-131. The counts were compared with those from a known aliquot of the injectate. The canine blood volume was calculated at 7% of body weight.

Cation clearance mechanism. The tracer cation, *N*([^{14}C]methyl) lidocaine, was synthesized by the reaction of $^{14}CH_3I$ with lidocaine (*9*) and was used to monitor saturation of the organic cation pathway (*10*). The cation clearance mechanism was studied in six female dogs that were prepared for serial bile collection as previously described except that the renal pedicles were ligated to prevent urinary excretion. Three of these dogs were used for a baseline kinetic study of blood and bile levels of *N*([^{14}C]methyl)lidocaine. In the other three dogs the same studies were done after the administration of oxyphenonium—a known saturator of the cationic pathway (*11*)—and saturation levels of oxyphenonium were obtained by i.v. administration of 7.9 mg/kg body weight over a 5-min period. After 15 min Tc-HIDA (1 mCi) and N([^{14}C]methyl)lidocaine (0.26 −0.82 μCi) were injected simultaneously and plasma and bile samples were collected over a 2-hr period. The samples from both groups of animals were prepared and counted according to a previously published method (*12*).

RESULTS

Table 1 shows the effect of i.v. infusion of BSP and oxyphenonium on the hepatobiliary clearance of Tc-HIDA in dogs. A mean cumulative total of 55.2% of Tc-HIDA was excreted into the bile before

TABLE 1. THE EFFECT OF BSP AND OXYPHENONIUM INFUSION ON BILIARY EXCRETION OF Tc-HIDA IN DOGS*

	BSP (1-hr cum. values)		Oxyphenonium (2-hr cum. values)	
Dog	Before	After	Before	After
1	42.5	0.5	90.03	92.03
2	65.2	1.6	84.36	92.94
3	58.0	2.3	88.22	73.98
Mean	55.2	1.5	87.50	86.30

* Percentage of injected dose of Tc-HIDA in bile.

BSP infusion, compared with only 1.5% after BSP infusion. Additional results, not included in Table 1, showed that the BSP infusion reduced the RB hepatobiliary excretion from 20% to 6% and increased the 1-hr urinary clearance of Tc-HIDA from 13.3% to 44.0%. Before BSP infusion, 2.7% of Tc-HIDA and 8.8% of RB remained in the blood at 30 min, and after infusion these values were elevated to 8.6% and 40%, respectively. Postinfusion serum BSP levels were always greater than 10 mg/dl, substantially exceeding the 4 mg/dl value quoted by Wheeler (*7*) as sufficient to saturate the carrier-mediated clearance of BSP. These saturation levels of BSP were found to exert a minimal effect on the protein binding of Tc-HIDA, decreasing it on the average from 64% initially to 56% postinfusion. The BSP levels achieved in these studies are capable of drastically altering the elimination pattern of Tc-HIDA without significant competitive displacement of Tc-HIDA from its protein binding sites.

In contrast, saturation levels of the cation oxyphenonium had no effect on the hepatobiliary clearance of Tc-HIDA. Ligation of the renal pedicles resulted in an increase in the hepatobiliary clearance of Tc-HIDA from 55.2% to 87.5%, and of *N*([^{14}C]methyl)lidocaine from 2% to 11% before oxyphenonium infusion. Table 1 shows that Tc-HIDA's biliary excretion was not changed by infusion of oxyphenonium, whereas the hepatobiliary clearance of *N*([^{14}C]methyl)lidocaine decreased from 11% to 2% under identical conditions.

DISCUSSION

The inhibition of Tc-HIDA's hepatobiliary clearance by BSP demonstrated that Tc-HIDA is eliminated through the liver's general anionic clearance pathway (*5*), and that this pathway is affected by a competing cation, oxyphenonium. Since both BSP and bilirubin are known to utilize this general anionic clearance mechanism (*5*), it follows that hyperbilirubinemia will also impede the clearance of Tc-HIDA by the liver.

Technetium-HIDA's observed carrier-mediated anionic clearance mechanism is consistent with either facilitated diffusion or active-transport clearance mechanisms and is also in agreement with previous data (*13*) indicating that Tc-HIDA exists as an anionic, bis structure with substantial lipophilicity and a molecular weight of approximately 683. These molecular features have been associated with rapid hepatobiliary clearance (*14*). Note that the anions, HIDA and Sn-HIDA, do not competitively inhibit the hepatobiliary clearance of Tc-HIDA, since their lesser lipophilic quality has been shown to favor renal clearance (*4*).

Since serum bilirubin levels may be elevated through several mechanisms in a variety of disease states, competition between Tc-HIDA and bilirubin in the transport pathway will complicate any pharmacokinetic method seeking to quantitate an aspect of liver function. Approaches to the quantitation of liver function, therefore, should expressly include the effects of bilirubin in the model; or, perhaps more practically, clinical studies should be undertaken to determine the serum level at which bilirubin dominates Tc-HIDA's in vivo clearance in man. At some serum bilirubin level the model-derived rate constants will be more indicative of competition with bilirubin than of the patient's hepatobilibary status. Ideally, new radiopharmaceuticals designed to measure hepatobiliary function should possess in vivo clearance patterns independent of serum bilirubin levels. This could be accomplished through the use of either radiolabeled cations or bile salts or through the development of new lipophilic, anionic agents that compete more effectively with bilirubin for hepatobiliary clearance than do either Tc-HIDA or I-131RB.

The difficulties in quantitating liver function using Tc-HIDA and other anionic hepatobiliary agents do not preclude their use in the clinical assessment of the jaundiced patient. Ryan et al (*4*) suggest that larger doses of Tc-HIDA should be administered to patients with elevated serum bilirubin levels. The increased dose appears necessary in order to keep relatively constant the millicurie quantity of Tc-HIDA entering the small intestine. Elevated serum bilirubin levels appear to delay the blood clearance of Tc-HIDA and to extend the interval after administration during which sequential scintiphotos must be obtained. It has been shown, however, that although the competition between Tc-HIDA and bilirubin increases the background radiation to nontarget tissues, it does not preclude imaging of the gallbladder up to bilirubin levels of 10 mg/dl nor does it preclude imaging of intestinal activity 18 hr postinjection if the biliary tract is patent. The latter is the major determinant of clinical significance in severely jaundiced patients. However, the use of Tc-99m as the radiotracer is inconvenient in these circumstances, since a long imaging time (½ to 1½ hr) is frequently required at 18 hr postinjection in order to visualize clearly the small amount of Tc-99m activity present in the intestinal tract, even when a substantial dose of Tc-99m is injected initially. Hepatobiliary tracers with significantly longer physical half-lives than 6 hr may be clinically useful in this situation, since imaging studies may be completed more rapidly at 18 hr and also may be performed at significantly longer intervals postinjection than with Tc-99m agents. An-

other approach to hepatobiliary imaging in the jaundiced patient would be the development of a Tc-99m hepatocyte imaging agent that does not compete with serum bilirubin for hepatic clearance. Our results suggest that imaging studies with this tracer in the jaundiced patient could be performed with a significantly smaller dose of injected radioactivity than is currently used and also could be completed in one day, even in the patient with significantly elevated serum bilirubin levels.

At present it appears that sequential scintigraphic data can provide more clinical information in the jaundiced patient than is readily obtainable through simple pharmacokinetic models and that the quantitation of hepatobiliary function should be restricted to patients with no more than modest elevations of serum bilirubin. A more valid quantitation of hepatobiliary function may require new hepatobiliary agents that either compete more effectively with serum bilirubin for transport binding sites or else employ totally different clearance mechanisms.

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