Quest for the Perfect Hepatobiliary Radiopharmaceutical

In this issue of the *Journal* the development of another potentially superior Tc-99m-labeled hepatobiliary radiopharmaceutical, Tc-99m(Sn)-N-pyridoxyl-5-methyltryptophan (Tc-PHMT) is reported (*1*). The current stage in expansion of this class of radiopharmaceutical suggests the need to consider several questions with regard to the superior radiopharmaceutical. What are the properties of the perfect radiopharmaceutical? How do these properties translate to the perfect hepatobiliary radiopharmaceutical? What have the major advances in hepatobiliary agent development been? How will the latest agent, Tc-PHMT, compare with others that have been developed?

In general, the ideal radiopharmaceutical is one that passes rapidly through the physiologic or pathophysiologic pathway of interest and is not simultaneously cleared by other pathways. Such a radiopharmaceutical gives the highest number of counts from the pathway of interest for a given injected dose at all levels of function. The initial biodistribution of any radiopharmaceutical depends upon clearance or flow times extraction efficiency. Since flow is independent of the radiopharmaceutical, maximum clearance will be achieved if the extraction efficiency of the pathway of interest approaches 100% while the extraction efficiencies of all other pathways approach 0%. If the pathway of interest involves further steps beyond an initial organ clearance, such as additional membrane transport, excretion, or metabolism, the radiopharmaceutical should rapidly pass through these steps as much like a bolus as possible to allow relatively independent, sequential evaluation of each step. The high extraction efficiency in the pathway of interest provides high sensitivity for the visualization and evaluation of the pathway at all levels of function. The low extraction efficiency for other pathways results in high specificity for the desired pathway and minimizes the possibility of interfering or confusing radioactivity.

These considerations can be applied to the hepatobiliary pathway (*2*). Intravenous injection of the radiopharmaceutical places it in the vascular system where it is made available to the liver by passage through the sinusoid level of blood vessels in the liver. The next desired step is hepatocellular uptake across the sinusoidal membrane with high extraction efficiency. Subsequent steps are transcellular movement and then excretion into the bile canaliculi across the canalicular membrane. Bile flow carries the radioactivity into the major portions of the biliary tree and then into the duodenum and intestines. If the hepatocyte transit and canalicular transport steps are rapid, the radioactivity moves as a bolus, and anatomic details such as intrahepatic ducts are well visualized in patients with good hepatocyte function.

A final consideration is enterohepatic cycling. The reabsorption of radioactivity from the intestines and then subsequent hepatic uptake is undesirable, since more complicated pharmacokinetics and eventual visual interferences would result. There has been no evidence for significant amounts of enterohepatic cycling for hepatobiliary agents that have been clinically evaluated. If a class of imaging agents based on bile acids were developed, such complications would be anticipated (*3*).

The synthesis of radiopharmaceuticals that use radiolabeled hormones and antibodies for visualization of tumors (*4*) and organs such as the adrenals (*5*) has been based on the concept of receptor proteins. The receptors involved in recognizing naturally occurring hormones can be thought of as being highly specific, since they need to recognize at most a single or a few related compounds. Excretory pathways such as the hepatobiliary and renal tubular system can also be considered in a conceptually similar manner even though the specificity requirements are not of the same level. With respect to hepatobiliary excretion, membrane transport proteins discriminate between classes of organic compounds—including dye anions and bilirubin, bile acids, organic cations, and neutral compounds; within these classes differences in binding affinities for such compounds as reflected by transport maxima (Tm) and maximum velocity (V_{max}) values are observed (*2*).

Thus, the differences in specificities and rates of hepatobiliary excretion of radiopharmaceuticals can be considered to be the result of differences in affinities for membrane proteins or receptors in the sinusoidal and canalicular membranes as well as for intracellular proteins such as ligandin or glutathione transferase-B (*6*).

As early as 1923 rose bengal was used for the measurement of liver function (*7*), and in 1955 it was subsequently labeled with I-131 as a radiotracer for hepatic function (*8*). Since the report of hepatobiliary excretion of Tc-99m penicillamine in 1972 (*9*), at least 30 articles have appeared describing new, improved Tc-99m-labeled hepatobiliary agents (*10*).

Two major classes of Tc-99m hepatobiliary radiopharmaceuticals have emerged. The initial complex of the earlier series, Tc-99m pyridoxylidene glutamate (Tc-PG), was the product of Tc-99m binding to the Schiff base of pyridoxal and glutamic acid (*11*). Tc-PG was excreted into the bile at moderate rates, but in normal animals to an extent of only about 45% of the injected dose. Subsequent reports (*12,13*) showed that specificity could be improved by substituting other amino acids, and later, for ease of labeling, stannous ion was added as the reducing agent (*14,15*). In the latest report (*1*), the imine link ($R_2C{=}N{-}R'$) that results from the condensation of the aldehyde group of pyridoxal and the amino group of the amino acid have been reduced to a stable amine linkage ($R_2HC{-}NHR'$). This procedure eliminates the need for large amounts of pyridoxal and 5-methyltryptophan in the kit and reduces the potential for competing chelating compounds. In addition to the chemical advantages, Tc-PHMT demonstrates rapid hepatobiliary kinetics with high specificity in animals.

The other class of Tc-99m hepatobiliary agents began with Tc-99m N-(2,6-dimethylacetanilide)iminodiacetate (Tc-HIDA, Tc-dimethyl-IDA) (*16*). This tracer has a rapid hepatic uptake and biliary excretion with a hepatobiliary specificity of about 80%. Subsequently, the alkyl substitution of the phenyl ring was varied to give a number of analogs: 2,6-diethyl substitution (Tc-diethyl-IDA, Tc-EHIDA), retained rapid kinetics with specificity increased to over 90% (*17*); *p*-isopropyl substitution (Tc-*p*-isopropyl-IDA, Tc-PIPIDA) resulted in similar specificity, but slower biliary excretion (*17*); *p*-butyl substitution (Tc-*p*-butyl-IDA, Tc-BIDA) had higher specificity with less than 2% renal excretion, but longer hepatocyte transit times (*17*); 2,6-diisopropyl substitution (Tc-diisopropyl-IDA, Tc-disofenin) demonstrated similar specificity to Tc-diethyl-IDA, but slightly faster biliary excretion in normal baboons (*18*); and finally, 3-bromo-2,4,6-trimethyl substitution (Tc-mebrofenin, Tc-SQ-26,962), provided a combination of high specificity and rapid hepatobiliary excretion kinetics (*19*). This partial listing of analogs represents those tracers that have had significant clinical evaluation.

Nunn and Loberg (*20*) recently reviewed the state of structure biodistribution relationships (SDRs) for hepatobiliary radiopharmaceuticals. The portions of the iminodiacetate chelating agent structure were subdivided into sections such as the phenylalkyl substitution pattern that modifies the receptor-type binding of the technetium complexes to plasma, hepatocyte membrane and intracellular hepatocyte proteins, and the carbamoyl group that modifies the imino nitrogen pKa values that in turn modify the technetium binding of the iminodiacetate group. Consideration of the structural changes that alter specificity and rates of hepatobiliary excretion should result in a recognition of requirements for optimal binding to the "receptor" proteins involved.

We have evaluated paired comparisons of these radiopharmaceuticals in patients and have observed gains in the parameters described for these agents. Technetium diethyl-IDA demonstrated higher specificity than Tc-PG with about 8% renal excretion in patients with normal hepatocyte function (*21*). The hepatic uptake and biliary excretion of Tc-diethyl-IDA were more rapid as well, but its specificity was not as high as that of I-131 rose bengal. Comparison of Tc-diethyl-IDA with Tc-PIPIDA showed both to have similar specificity, but Tc-diethyl-IDA had significantly shorter hepatocyte transit times that provided superior hepatic duct images (*22*). Comparison of Tc-diethyl-IDA with Tc-diisopropyl-IDA or Tc-disofenin demonstrated similar kinetics and specificity in patients with normal functions, but, compared with Tc-diethyl-IDA, specificity was relatively decreased in patients with reduced levels of hepatocellular function (*23*). Finally, comparison of Tc-diisopropyl-IDA with Tc-mebrofenin showed similar kinetics for both but superior specificity for Tc-mebrofenin in both normals ($1.1 \pm 0.6\%$ renal excretion) and in patients with increased bilirubin levels and, hence, reduced hepatocellular function levels (*24*). The higher specificity of Tc-mebrofenin was also reflected in the general absence of kidney visualization on the images.

Relative extraction efficiencies have been determined in these paired comparisons. Based on an absolute value of 48 to 56% determined for Tc-diethyl-IDA (*25*), Tc-diisopropyl-IDA has been estimated to be 60% (*23*) and Tc-mebrofenin 66% (*24*). In the series of comparisons, agents with high extraction efficiency also appeared to have short hepatocyte transit times (*22–24*).

Studies of the hepatobiliary radiopharmaceuticals have demonstrated that the differences observed can be translated into improvements in increased liver-to-background uptake because of higher extraction efficiencies, improved hepatic bile duct visualization because of rapid hepatocyte transit, and increased potential of demonstrating biliary excretion in patients with poor hepatocellular function because of improved specificity.

For new potential hepatobiliary radiopharmaceuticals the questions raised are where are we now and what improvements are possible? The specificity for hepatobiliary excretion reflected in the low renal excretion of Tc-mebrofenin is remarkable; however, the extraction efficiency of 66% suggests that room for improvement is still possible. Extraction efficiencies for bile acids, for example, have been determined as 86 ± 8% for glycocholic acid in humans (*26*) and 92 ± 5% for taurocholate in dogs (*27*).

The data presented for Tc-PHMT suggest that its extraction efficiency must be high since the 1-min images in the rabbit showed considerably higher liver-to-background uptake than did Tc-diethyl-IDA, and the hepatocyte transit times must be short, since the majority of the liver radioactivity appeared to have cleared by 5 min. The renal excretion in the presence of normal hepatocyte function was almost as low (2%) as that of Tc-mebrofenin. The animal data suggest that the new complex may be closer to the optimal combination of properties. It will be interesting to observe its behavior in humans with normal and decreased levels of hepatocyte function.

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