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*Radiopharmaceuticals for the Scintigraphic Assessment
of
Hepatobiliary Function*

by:

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Radiopharmaceuticals for the Scintigraphic Assessment of Hepatobiliary Function

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RADIOPHARMACEUTICALS FOR THE SCINTIGRAPHIC ASSESSMENT OF HEPATOBIILIARY FUNCTION

STATEMENT OF OBJECTIVES

The goal of this correspondence lesson is to increase the reader's knowledge and understanding of current hepatobiliary radiopharmaceuticals. To that end, this course discusses hepatobiliary anatomy and function, hepatocyte uptake, metabolism, and excretion of endogenous and exogenous compounds, and the pathway of bile excretion. In addition, gallstone formation, composition, and treatment is reviewed. The course presents information related to the development of current hepatobiliary radiopharmaceuticals, optimal characteristics of such agents, and the *in vivo* and *in vitro* properties of agents available for routine use in the U.S. This is followed by a discussion of the clinical use of hepatobiliary radiopharmaceuticals, including augmented cholescintigraphy, and a review of quantitative methods for the assessment of hepatocyte function.

Upon successful completion of this material, the reader should be able to:

1. Describe hepatobiliary anatomy and function.
2. Explain the major functions of the hepatocyte.
3. Describe the biliary uptake and excretion pathways for bile acids and organic anions.
4. List three major components of bile.
5. Explain the origin, conjugation, enterohepatic circulation and function of bile acids.
6. Describe the origin and excretion pathway of bilirubin and how it affects hepatocyte excretion of other organic anions.
7. Discuss the factors that affect gallbladder filling and emptying.
8. Explain the current understanding of the underlying causes for gallstone formation.
9. Explain methods used in the treatment of gallstone disease.
10. List the required chemical and pharmacologic characteristics of an ideal hepatobiliary radiopharmaceutical.
11. Discuss the development of current hepatobiliary radiopharmaceuticals.
12. Explain the role that analysis of structure-activity relationships has played in the development of improved cholescintigraphic agents.
13. List hepatobiliary radiopharmaceuticals currently available for routine use in the U.S.
14. Describe the preparation and radiochemical quality control of Tc-99m iminodiacetic acid (IDA) analogs.
15. Compare the *in vivo* characteristics in humans of routinely used Tc-99m IDA radiopharmaceuticals.
16. List the four organs that receive the highest absorbed radiation doses from Tc-99m iminodiacetic acid (IDA) radiopharmaceuticals.
17. Understand how drug therapy can interfere with the movement of cholescintigraphic agents through the hepatobiliary system.
18. List the principal applications for cholescintigraphy.
19. Describe how hepatobiliary imaging studies are performed.
20. Explain the three types of pharmacologic interventions used in augmented cholescintigraphy.
21. Discuss the various causes of cholecystitis.
22. Explain the two most common causes of neonatal jaundice and the role of cholescintigraphy in the differential diagnosis.
23. Discuss the current status of methods for quantitation of hepatocellular function.
24. Describe the hepatocyte uptake and handling of Tc-99m galactosyl-neoglycoalbumin.

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- III. HEPATOBILIARY PHYSIOLOGY
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 - C. Organic Anions
 - D. Organic Cations
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- D. Sphincter of Oddi Dysfunction
- E. Biliary Obstruction
- F. Bile Leakage and Reflux
- G. Neonatal Jaundice

X. QUANTITATION OF HEPATIC FUNCTION

- A. Measurement of Hepatic Extraction Fraction (HEF) Using Tc-99m IDA Analogs
- B. Quantitation of Hepatic Binding Protein Receptors

RADIOPHARMACEUTICALS FOR THE SCINTIGRAPHIC ASSESSMENT OF HEPATOBILIARY FUNCTION

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INTRODUCTION

Hepatobiliary radiopharmaceuticals are an important adjunct in the differential diagnosis of acute and chronic abdominal pain. Due to the excellent physical and biological properties of current Tc-99m labeled radiopharmaceuticals, cholescintigraphy is considered to be both a noninvasive and very sensitive test for evaluation of the functional status of the cystic duct and the gallbladder. Hepatobiliary imaging can also be helpful in the differentiation between surgical

and medical jaundice, and it is an excellent method for visualizing the pathway of bile following surgery or trauma. The greatest impact has been on the diagnostic evaluation of suspected acute cholecystitis (1). More than 90% of cases of acute cholecystitis result from cystic duct obstruction by gallstones, which cause stasis in the gallbladder followed by chemically-induced inflammation and mucosal injury (2). Biliary tract disease caused by gallstones (cholelithiasis) is a major medical and surgical problem in many parts of the world. It is estimated that 20 million individuals in the United States have gallstones. Although gallstones do not cause symptoms in the majority of individuals, gallstone-related disease afflicts up to two million Americans annually, leading to direct health care costs exceeding \$2 billion per year. Cholelithiasis is the most common single indication for abdominal surgery in the United States, with around 500,000 cholecystectomies performed annually (2). The epidemiology, symptoms, diagnostic workup, and available treatment for cholecystitis and some other abnormalities of the hepatobiliary system will be reviewed in the following sections.

HEPATOBIILIARY ANATOMY AND FUNCTION

The liver is the largest organ in the body, weighing 1200-1600 g in normal adults. The hepatic parenchyma contains a complex network of blood vessels and excretory ducts. The liver has dual blood supply with about 80% coming from the G.I. tract via the portal vein, and the rest being supplied by the hepatic artery (2,3). The blood is mixed in a vast network of hepatic sinusoids lined by endothelial and Kupffer cells. These cells are part of the reticuloendothelial system and remove foreign particles from blood by phagocytosis. Pores or fenestrae between the endothelial cells allow plasma to flow into the space of Disse, where it comes in contact with the hepatic parenchymal cells. The parenchymal cells (hepatocytes) account for 60% of liver cells and 80% of organ volume. The hepatocytes are polygonal cells, 30 μm in diameter, and are organized in plates to form a continuous three-dimensional lattice. A network of exchange vessels nourishes each parenchymal cell on several sides. The hepatocyte is charged with the task of detoxifying and excreting harmful compounds and of regulating plasma levels of beneficial nutrients (3,4). The various transport pathways for hepatocyte uptake and excretion are discussed in the next section. The hepatocyte also performs the important function of bile formation. Biliary excretion is an essential pathway for the elimination of many substances removed from

blood by the hepatocytes, including bile acids and bilirubin. The alternate pathway of excretion is secretion back into plasma and subsequent clearance by the kidneys. The biliary excretory system begins with the bile canalicular network, which drains into a series of progressively larger ducts that eventually combine to form the common hepatic duct. This is joined by the cystic duct from the gallbladder to form the common bile duct, which empties into the duodenum through the sphincter of Oddi. Just prior to entering the duodenum, the common bile duct is usually joined by the pancreatic duct. During fasting, bile is diverted into the gallbladder where it is concentrated and stored. In response to a meal, the sphincter of Oddi relaxes, the gallbladder contracts, and concentrated bile empties into the duodenum.

HEPATOBIILIARY PHYSIOLOGY

The first step in hepatobiliary excretion is uptake across the hepatocyte membrane. Receptor binding sites on the sinusoidal plasma membrane of hepatocytes are responsible for selective uptake of a variety of endogenous and exogenous compounds. Specific carrier-mediated active transport systems appear to exist for various classes of compounds such as organic anions, bile acids, organic cations, and neutral organic compounds (3,5). Distinct transport mechanisms exist for inorganic ions as well; the transport of these ions in and out of hepatocytes is regulated by various "pumps" that maintain conditions required for optimal cellular function (2). Research within the last 10-15 years has provided a great deal of new knowledge regarding the mechanism by which hepatocytes handle electrolytes and other solutes (5). However, only a few of the carrier-mediated transport systems of the liver have been systematically studied (3). The liver is important for the elimination of weakly polar and non-polar compounds that are poorly excreted via the kidneys because of protein binding and tubular reabsorption. The chemical properties that determine if a compound is primarily excreted via the hepatobiliary pathway are discussed in the next section. When protein-ligand complexes enter the proximity of hepatocyte carriers with high ligand affinity, the ligand is removed from the protein and taken up by the hepatocyte. A primary function of the hepatocyte is to convert nonpolar drugs and toxins into more water-soluble compounds that can be effectively excreted. However, compounds that are relatively polar and are ionized at physiologic pH may be excreted unchanged into the biliary tract (3,5).

The hepatobiliary system produces approximately 600 ml of bile daily. Bile acids, water and electrolytes constitute the major components of bile.

Compared to other bodily secretions, bile is relatively rich in lipids, principally cholesterol and the phospholipid lecithin. Bile also contains a variety of other lipophilic substances, including vitamins and steroids. Bilirubin, which gives bile its yellow color, normally represents less than 1% of total biliary solids. Physiologic control of bile secretion is complex and incompletely understood. Hepatic bile flow is closely related to the size of the bile acid pool, determined by the rate at which bile acids are processed through the liver. Changes in the rate of bile acid delivery to the liver correspond to the feeding cycle. This is usually referred to as the bile acid dependent portion of canalicular bile formation. Other, bile-acid independent, factors are also believed to take part in the regulation of canalicular bile secretion, but these factors and their significance are not well understood (2,3,5,6).

Bile Acids

Bile acids (also referred to as bile salts) are the end-products of cholesterol degradation and the major organic constituents of human bile. The primary bile acids, cholic acid and chenodeoxycholic acid are synthesized in the liver. They are virtually completely conjugated, usually with glycine or taurin, before being excreted into bile. Bile acids are natural ionic detergents and play an important role in the absorption, transport and secretion of lipids, such as fat-soluble vitamins and cholesterol (2,5). Bile acids are effectively (95%) absorbed from the intestinal tract and returned to the liver in portal blood, bound to albumin. The removal of bile acids from sinusoidal blood is very efficient, allowing bile acids to circulate through the liver and intestines eight to 10 times per day (2,5). This enterohepatic circulation effectively preserves the bile acid pool.

The mechanisms responsible for hepatocyte uptake and secretion of bile acids are not completely understood, but are clearly different from the mechanisms responsible for handling other organic anions such as bilirubin. Different bile acids are not handled identically, and it is generally accepted that each bile acid undergoes its own enterohepatic circulation (6). Certain bile acids, for example taurocholate, enter the hepatocytes via a sodium-dependent, carrier-mediated active transport mechanism (3). More hydrophilic bile acids presumably enter hepatocytes by non-ionic diffusion (2,5). Following hepatocyte uptake, bile acids are transported across the cell toward the canalicular pole. There is evidence that cytosolic binding proteins and various hepatocyte organelles play a role in the intracellular storage and transport of bile acids. Glutathione S-transferases (ligandin) have been

demonstrated to bind bile acids (5,6). The secretion of conjugated primary bile acids into the canalicular lumen has been shown to take place via a carrier-mediated transport mechanism distinct from that of other organic anions (2,3). Different primary bile acids compete for biliary secretion, but sulfated bile acids appear to use the general organic anion transport mechanism for canalicular secretion (5,6). Evidence indicates that canalicular membrane transport is the rate-limiting step in overall hepatocyte bile acid transport (5), and that the excretion step is easily impaired by liver disease (3).

Lipids

Secretion of the two major lipids in bile, lecithin and cholesterol, is coupled with the secretion of bile salts. In humans, the liver is normally the most active site of cholesterol synthesis. Elimination of cholesterol from the body takes place by the catabolism of cholesterol to form bile salts, and also by direct secretion into bile. Bile salts, along with lecithin, solubilize cholesterol in bile in the form of mixed micelles and mixed lipid vesicles. The exact mechanism for transport of lipids through the hepatocyte is not well understood (3,5).

Organic Anions

Although organic anions make up a minor component of bile, biliary clearance is an important route of excretion for this group of compounds. Bilirubin, the principal end-product of heme degradation, belongs to this class of compounds. It is often used as a model compound for the transport of other organic anions such as certain dyes, drugs, and hormones (5). Examples of compounds that share the organic anion pathway for hepatobiliary excretion with bilirubin include the dyes sodium sulfobromophthalein (BSP) and indocyanine green (ICG), the radiographic contrast agent iopanoic acid, and several cholescintigraphic radiopharmaceuticals such as the iminodiacetic acid (IDA) derivatives (7). The transhepatic transport of these organic anions is rapid and may occur by several cellular pathways (3,5). Some compounds are metabolized in the liver prior to biliary secretion, whereas others are excreted unchanged. The normal liver has a very large capacity for excretion of organic anions and can process up to ten times the normal daily production of bilirubin (2).

As much as 80% of bilirubin is derived from hemoglobin following its release due to the destruction of senescent red blood cells in the reticuloendothelial system, primarily in the spleen (3,7). Bilirubin is poorly water-soluble and is a neurotoxic substance if allowed to accumulate in the body (2). Unconjugated

(indirect) bilirubin passes into plasma where it binds noncovalently to albumin. Because of the large binding capacity of albumin, large amounts of unbound, unconjugated bilirubin rarely occur clinically except in the neonate, in whom the capacity and affinity of albumin to bind bilirubin are less than in adults (7). From the spleen, bilirubin is transported to the liver sinusoids via portal venous blood. After dissociation from albumin, bilirubin is taken up by the hepatocytes. Special domains of the sinusoidal hepatocyte membrane have high affinity for organic anions such as bilirubin. Good evidence exists that hepatocyte uptake of bilirubin and related compounds involves a carrier-mediated, saturable process. Compounds in this class exhibit mutually competitive inhibition of uptake, but there is no competition between these anions and bile acids. Several transport proteins in the sinusoidal membrane appear to mediate the uptake process (3,5,7). In the hepatocyte, bilirubin is conjugated to form water-soluble derivatives suitable for excretion in bile. The process of conjugation and transport across the hepatocyte to the canalicular membrane involves a complex interaction with multiple intracellular hepatocyte components. The hepatocyte has a mechanism for temporary storage of bilirubin; this may be predominantly associated with the cytosolic protein ligandin (2,3,7). Ligandin may also act as a carrier protein to facilitate diffusion of bilirubin to the endoplasmic reticulum where esterification takes place (2,3). More than 85% of bilirubin is normally conjugated with glucuronic acid; the majority is in the form of diconjugates (2,3,7). Although it is not possible to sample bile at the point of canalicular excretion, evidence indicates that the transport of conjugated bilirubin and related organic anions across the canalicular membrane involves a saturable, carrier-mediated process. Different organic anions compete for excretion, but organic cation and unsulfated bile acid transport is not affected. More than one carrier system may be involved in the secretion step, which appears to be the rate-limiting step of overall transhepatic bilirubin transport (2,3,5). From the bile canaliculus, bilirubin conjugates pass with the bile into the small intestine. Very little reabsorption takes place; normally more than 90% of bilirubin is excreted in feces either unchanged or as intestinal metabolic products (7). Elevations in serum direct (conjugated) bilirubin above the normal range of 0.3-1.0 mg/dl may be of prehepatic (e.g., haemolysis), hepatic (hepatitis, cirrhosis), or cholestatic (obstruction of bile flow) origin (8). Because conjugated bilirubin is water-soluble, urinary excretion is observed in patients with direct hyperbilirubinemia (2).

Organic Cations

Despite the fact that most drugs are positively charged at physiologic pH, relatively little is known about the hepatic handling of organic cations. What is known is the result of work with model compounds such as procainamide, ethobromide, and chlorpromazine. The overall process appears to be similar to organic anion handling in many respects. For example, competition between different cations has been demonstrated. Organic anions do not compete for the uptake and secretion of cations, and biliary excretion of cations is not altered by bile acid infusion (5). No successful cholescintigraphic radiopharmaceutical belongs to the organic cation class.

Pathway of Bile Excretion

Hepatic bile, which is normally produced at the rate of about 600 ml per day, may enter the duodenum directly, or it may flow into the gallbladder where it is concentrated and stored (2,3,5). Gallbladder filling and emptying is affected by a number of factors, including the rate of hepatic bile secretion, sphincter of Oddi contraction, cystic duct pressure, serum levels of the hormone cholecystokinin (which stimulates gallbladder contraction), and possibly by cholinergic mechanisms. The gallbladder can store only 30-50 ml of bile, but due to its remarkable ability to concentrate bile by absorption of fluid through the gallbladder mucosa, it can accommodate the total volume of hepatic bile (2). In addition, it has been demonstrated that periods of gallbladder filling are punctuated by brief periods of partial emptying of concentrated bile, and that much of the bile secreted at night may bypass the gallbladder altogether (5). During gallbladder storage hepatic bile is concentrated by removal of an isotonic solution containing primarily Na^+ , HCO_3^- and Cl^- . The resulting bile has a very high concentration of bile salts and Na^+ as well as K^+ and Ca^{2+} (5).

Gallstones

The formation of gallstones represents a failure to maintain certain biliary solutes, principally cholesterol and calcium salts, in a solubilized state (5). Gallstones have traditionally been divided into two major types: cholesterol stones and pigment stones (2). In the United States, more than 75% of stones are considered to be cholesterol stones. These stones are formed in the gallbladder and contain more than 50% cholesterol by weight. Since cholesterol is not radiopaque, most of these stones are not visible on plain abdominal radiographs (2). Their formation is clearly related to supersaturation of bile with cholesterol, but this alone does not explain the

pathogenesis, since supersaturation is common in normal fasting subjects without stone formation (2,8). It is not well understood how other promoting factors are involved but this is an active area of research. Pigment stones can be subdivided into black and brown stones. Both types contain sufficient amounts of calcium salts to be radiopaque. Brown pigment stones are seen mainly in the Orient and are almost always associated with biliary bacterial infection. The primary calcium salt in brown stones is bilirubinate. Brown stones contain more cholesterol than black stones, and are usually formed in the bile ducts rather than in the gallbladder. Black pigment stones are composed of a mixture of calcium salts including bilirubinate, carbonate and phosphate salts, and are usually formed in the gallbladder. Black stones have been linked with chronic hemolytic disorders and cirrhosis (2,6).

Although this classification of gallstones may be useful for the purpose of selecting an appropriate course of therapy, in reality gallstones have been found to cover the full spectrum from "pure" cholesterol stones to stones of variable calcium and bile pigment content, leading to the classification of many stones as "mixed." Even so-called "pure" cholesterol stones contain calcium salts in the center, and current research suggests that the precipitation of calcium in the biliary tree is a critical event in the initial nucleation of both cholesterol and pigment stones (5,6).

Over the years, numerous epidemiological studies have been undertaken to try to uncover the causes for gallstone formation. This, in turn, would presumably lead to ways of preventing the formation of gallstones. Diet, sex, obesity, and hormonal status are some of the factors that have been implicated (6). Much work remains to be done to understand the factors that either promote or prevent cholelithiasis. A better understanding of the pathogenesis of gallstone formation is expected to lead to better methods to treat patients with gallstone disease. At the present time cholecystectomy is the only therapy proven to cure cholesterol gallstone disease (2,9). This type of surgery has recently become less traumatic due to refinement in technique. Laparoscopic cholecystectomy is now widely used in many parts of the world and has been found to reduce both postoperative pain and the recovery period, as well as cost (2).

Pharmacologic dissolution of cholesterol gallstones using orally administered bile acids has been extensively investigated. Two compounds have been found to be effective in clinical studies: chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA). UDCA has been reported to have less

side effects than CDCA, and combined therapy using both drugs is more effective than either drug alone. The optimal oral dose is 10 to 15 mg/kg/day (2,6,8). Not all patients with gallstone disease are suitable candidates for this type of therapy, however. The drugs are not effective for highly calcified stones, and work best for buoyant stones less than 15 mm in diameter (6). Presence of a functioning gallbladder is also required. Complete treatment may take as much as two years, and the drugs are relatively expensive. Even with a carefully selected patient population, the success rate for complete gallstone dissolution is only 40-60% (8), and recurrence rates of up to 50% in five years have been reported (6). The true success rate of stone dissolution is actually unknown, since current diagnostic methods have limited resolution. Tiny residual stones may be missed, and when bile reverts to its supersaturated state after the drug is discontinued these residual stones may start growing.

Extracorporeal shock wave lithotripsy (ESWL) is another non-surgical method for removal of gallstones (2,8). By sending focused shock waves from outside the body, stones can be reduced to 2-3 mm particles that can easily pass into the intestinal tract. The technique works best in patients with single small stones, and can be combined with dissolution therapy. Because these non-surgical treatments of cholesterol cholelithiasis have no lasting effect on the underlying cause of gallstone formation, and does not prevent recurrence, the overall therapeutic results have so far been disappointing.

CHARACTERISTICS OF OPTIMAL HEPATO-BILIARY RADIOPHARMACEUTICALS

Hepatobiliary radiopharmaceuticals are used to obtain scintigraphic images and functional data that can be used to evaluate hepatocyte function, the patency of intrahepatic ducts, the functional status of the cystic duct and the gallbladder, and also the patency of downstream portions of the biliary tract (10). The optimal or ideal radiopharmaceutical for evaluating the functional status of an excretory pathway such as the hepatobiliary system would be an agent that passes through rapidly in bolus form. The agent should not be partially cleared by other pathways or localized in areas outside the pathway of interest. In other words, the extraction efficiency should be 100%, and excretion steps such as membrane transport and metabolism should be rapid. There should be no reabsorption of the original compound or of any radiolabeled metabolites. In addition, the radiopharmaceutical must be labeled with a radionuclide with suitable half-life and radiation emissions. The labeling must be stable, and the

combination of half-life and clearance rate should be such that the radiation dose to the patient is not too high at dose levels required to obtain satisfactory images. If quantitation of function is desired, the clearance rate should only be affected by the functional status of the excretion pathway, and not by concentration changes of other substances that are handled by the same pathway.

Hepatobiliary imaging goes back to the introduction of I-131 rose bengal by Taplin and coworkers in 1955 (11). As will be explained further in the next section, I-131 Rose Bengal is not an ideal radiopharmaceutical for hepatobiliary imaging. It was replaced in the 1970s by Tc-99m-labeled compounds with more optimal characteristics. Investigations have continued to the present time with the aim of developing even better agents to study specific aspects of hepatocyte function.

A number of chemical properties have been found to be of importance for a substance to be excreted efficiently by the hepatobiliary pathway. Although interrelated, these properties can be conveniently divided into three groups: molecular weight, polarity, and molecular structure. Compounds with molecular weight below 300 are preferentially eliminated in the urine, whereas bile is the dominant pathway above 500 Da (12). Animal studies have shown a marked species difference in the extent of biliary versus urinary excretion for organic anions in the 300 to 500 Da range (13). It has long been recognized that lipid solubility of a molecule is an important determinant of its biliary excretion (12). Lipophilic groups bind strongly to albumin, which prevents filtration by the kidneys and increases ligand solubility in circulation. Although molecules preferentially eliminated in bile have an overall hydrophobic structure, the presence of one or more polar groups (e.g., ionizable groups) is an essential requirement for quantitative biliary excretion to occur (3). That is why highly lipid-soluble molecules must be conjugated prior to excretion into bile canaliculi. In a review article, Firnau (14) compared the molecular structure of a number of compounds proposed as hepatobiliary radiopharmaceuticals. He concluded that for extensive biliary excretion to occur, a molecule may need to exhibit a certain balance between the polar and nonpolar aspects of its structure. Another feature of these compounds was that they all contained at least two cyclic structures arranged in two different planes. Although it is not fully understood how changes in molecular structure influences biliary excretion, it has been shown that introduction of certain groups affects biliary excretion rate without significantly altering lipid solubility or molecular weight (3,12).

Due to its optimal radiation properties, low cost,

and convenient availability, Tc-99m is widely regarded as the radionuclide of choice for diagnostic imaging (15). Technetium-99m is the decay product of Mo-99 ($t_{1/2} = 2.75$ days), and can be obtained as an isotonic, sterile and pyrogen-free solution of sodium pertechnetate from commercially available Mo-99/Tc-99m generator systems. The physical half-life of Tc-99m is 6.02 hours; this is generally well-suited to hepatobiliary excretion kinetics. It decays to Tc-99 by isomeric transition, emitting abundant (89%) 140 keV photons and minimal amounts of non-penetrating radiations. Labeling of suitable compounds with Tc-99m using well-established methods results in radiopharmaceuticals of high purity and stability. The photons from Tc-99m are optimal for detection by standard nuclear medicine imaging equipment and results in a low patient radiation dose per usable photon.

DEVELOPMENT OF CURRENT HEPATOBILIARY RADIOPHARMACEUTICALS

Historical Perspective

Despite its many shortcomings, I-131 rose bengal (11) was the principal radiopharmaceutical for studies of hepatobiliary function for many years. Rose bengal is a halogenated fluorescein dye, principally tetrachlorotetraiodo-fluorescein. It can be effectively radioiodinated by exchange labeling methods to yield a preparation of high specific activity. Iodine-131 is a beta emitter with a relatively long physical half-life (8 days). Its photon emission of 364 keV is not optimal for standard imaging equipment, and the administered dose was limited to about 300 μ Ci (111 MBq), resulting in poor quality images. Due to the relatively slow transit of I-131 rose bengal through the liver, it becomes difficult to visualize the gallbladder against the high background activity in the liver. Rose bengal is cleared through the same hepatocyte excretion pathway as bilirubin, but does not compete well for excretion at elevated bilirubin levels, adding another complication to its clinical usefulness. The physical disadvantages of I-131 could be overcome by replacing it with I-123, a cyclotron product with a principal gamma emission of 159 keV. Iodine-123, however, has a physical half-life of only 13 hours. Although I-123 rose bengal was successfully developed (16), its high cost and problematic supply logistics resulted in very limited clinical use.

Because of the excellent physical characteristics of Tc-99m, a large number of Tc-99m complexes have been investigated as potential hepatobiliary radiopharmaceuticals. The first agent to show promise, Tc-99m-D-penicillamine was introduced in 1972 (17). A discussion of all the agents that were

subsequently proposed is beyond the scope of this continuing education lesson. Several well-referenced review articles have been published (10,18). The two groups of cholescintigraphic agents that have undergone extensive clinical investigations are discussed below.

Tchnetium-99m Pyridoxylidene Amino Acid Complexes

Starting with Tc-99m Pyridoxylidene glutamate (Tc-PG) (19), a large number of similar complexes were investigated as hepatobiliary imaging agents. The condensation of pyridoxal and an amino acid such as glutamate results in formation of a complex known as a Schiff's base ligand (Figure 1), which is stabilized by binding to metallic cations (20,21). In the original preparation, Tc-99m pertechnetate was added to an equimolar mixture of pyridoxal and glutamate of pH 8-9 and autoclaved for 30 minutes at 120°C. In this method technetium is probably reduced by the aldehyde group on pyridoxal to a lower oxidation state that can readily form coordination complexes with Schiff's base ligands. This method resulted in a preparation containing multiple Tc-99m complexes as shown by electrophoresis and HPLC (19,22,23). However, Tc-PG showed significant promise as a cholescintigraphic radiopharmaceutical in several animal species (19,22,23,24). Blood clearance was rapid, and the biliary tract and gallbladder could be clearly visualized as early as 10-15 minutes after injection. There was no evidence of reabsorption or hepatocyte metabolism (23), and acute and chronic toxicity studies in animals showed a wide margin of safety for diagnostic purposes (19). The major disadvantage of Tc-PG was the high degree of urinary excretion, which was reported to be in the range of 20 to 50% of injected dose, depending on animal species. This may be partly due to a low degree of protein binding, which was estimated to be only 20% immediately following injection in rabbits (23).

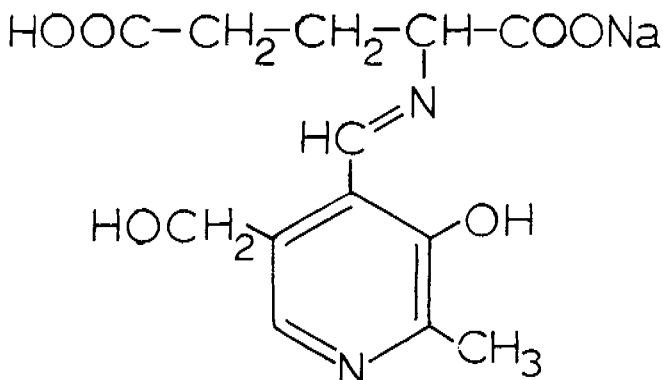


Figure 1. Structure of Pyridoxylidene glutamate.

Several clinical studies subsequently established the advantage of Tc-PG over I-131 rose bengal for hepatobiliary imaging (25,26). In normals, the common bile duct, gallbladder, and duodenum was seen 15-30 minutes post-injection, and renal retention did not interfere with scan interpretation. However, urinary excretion increased in jaundiced patients, and the biliary tract was not well-visualized with serum bilirubin levels above 4 mg/dl. The presence of activity in the gastrointestinal tract on late (18-24 hours) images still allowed differentiation between partial and complete obstruction.

Efforts to reduce the amount of urinary excretion initially centered on replacing glutamate with other amino acids (22,23,27), but this did not produce significant improvements. Some of the problems associated with the variable mixtures of complexes in the autoclaved products were solved by Kato and Hazue (28). They developed a new labeling method using stannous ion as the reducing agent in an alkaline medium with ascorbic acid as a stabilizer. Two-component kits using several different amino acids were prepared; in the final labeling step Tc-99m pertechnetate was incubated with aliquots of the two kit components for one hour at room temperature. Biliary excretion of this new series of compounds was found to correlate well with lipophilicity as determined by thin-layer and paper chromatography. Further improvements were realized when kits containing N-pyridoxylamino acids were introduced (29). This significantly increased the stability of the ligands, and (Sn)-N-pyridoxalaminates were prepared that could be efficiently labeled with Tc-99m in a brief boiling step. Of the amino acid complexes tested, Tc-99m (Sn)-N-pyridoxyl-5-methyltryptophan was the most promising. In rats, over 90% of the activity passed through the liver in the first 30 minutes after injection, and only 2% was excreted in urine. Continuous infusion of sulfobromophthalein (BSP), designed to simulate elevated bilirubin levels, had minimal effect on the rate of biliary clearance as well as the extent of urinary excretion. No hepatobiliary radiopharmaceutical based on pyridoxal and amino acids has been commercially available in the United States, but this group of agents has been used extensively in Asia and the Far East.

Tchnetium-99m Acetanilideiminodiacetates

The development of Tc-99m-N-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (Tc-dimethyl-IDA) was first reported by Loberg and coworkers in 1976 (30). The ligand was synthesized in a two-step procedure starting with 2,6-dimethylaniline and chloroacetic acid. The resulting intermediate was then reacted with iminodiacetic acid

to form the dimethyl-IDA complex (Figure 2). The iminodiacetic group has excellent chelating properties and forms a complex with stannous-reduced Tc-99m at room temperature. Analysis by paper and gel chromatography showed insignificant amounts of Tc-99m in the form of pertechnetate or colloids. Initial animal distribution studies in mice showed that Tc-dimethyl IDA was cleared rapidly from the blood by the liver, with more than 80% of the injected dose in bile after 50 minutes. Urinary excretion in dogs was 17% at 5 hours after injection (30). This agent clearly showed great promise, and extensive investigations of Tc-dimethyl-IDA and similar IDA complexes as potential cholescintigraphic radiopharmaceuticals followed. Studies of the chemical structure (31) indicated that the molar ratio of dimethyl-IDA to technetium is 2:1, suggesting a bis structural complex. Tin ions are not part of the final complex. Technetium is present in the +3 oxidation state, giving the complex an overall charge of -1. Mass spectroscopy studies (32) have subsequently confirmed a bis-structural anionic ligand complex of Tc +3, presumably octahedral in structure. A mass value of 685 was observed.

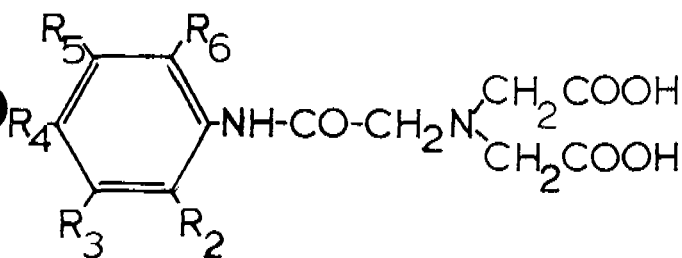


Figure 2. Structure of acetanilidoiminodiacetic acid.

Animal distribution and imaging studies using Tc-dimethyl IDA in a variety of animal species confirmed that this complex is primarily excreted in bile, with only moderate amounts of urinary excretion (27,33,34,35). Tc-dimethyl IDA is not extensively protein bound (36) or metabolically altered *in vivo* (30,31). Safety studies in mice (37) indicated that the LD₅₀ for dimethyl-IDA exceeded the proposed human dose by a factor of 1,000 on a per-weight basis. To determine the mechanism of hepatobiliary clearance of Tc-dimethyl-IDA, Harvey and coworkers (38) performed competitive clearance studies in dogs with BSP, an anion, and oxyphenonium, a cation. The administration of saturating levels of BSP resulted in a decrease in the one-hour cumulative biliary excretion from 55 to 1.5% of the injected Tc-dimethyl-IDA. The one-hour urinary excretion increased from 13% to 44% as a result of BSP infusion. No effect was observed with oxyphenonium, demonstrating that Tc-dimethyl-IDA is cleared through hepatocytes via the

organic anion pathway. This suggested that in the jaundiced patient, bilirubin may be able to prevent Tc-dimethyl-IDA and related radiopharmaceuticals from being efficiently excreted by the hepatobiliary system.

Initial human studies with Tc-dimethyl-IDA (37) in normal subjects and non-jaundiced patients showed rapid concentration of tracer by the liver, with 14% of the injected dose excreted in urine by 90 minutes. Activity was present in the biliary tract after 10 to 20 minutes. In jaundiced patients as much as 53% of the injected dose was in the urine at 18-24 hours. Blood clearance was delayed and the biliary system was not visualized in patients with severe jaundice. However, intestinal activity, indicating biliary tract patency, was observed on late images in patients with incomplete common duct obstruction. Safety studies in the same group of patients did not detect any alterations in various hematological and biochemical tests after administration of Tc-dimethyl-IDA. Following the initial successful clinical use of Tc-dimethyl-IDA, many researchers attempted to prepare an even better radiopharmaceutical by manipulating various parts of the basic IDA molecule. The goal was to produce an agent with increased hepatobiliary specificity, decreased renal excretion, and improved competition for excretion in the presence of elevated serum bilirubin levels.

Over the next few years some 60 different Tc-99m labeled IDA analogs were tested in animals (27,34,35,39,40,41). Most of the new analogs had different chemical substitutions only on the aromatic ring of the basic IDA structure, and in general it was observed that hepatobiliary specificity improved with increased lipophilicity. Changes introduced in the side chain were less successful, sometimes resulting in unstable complexes or increased urinary excretion (39,42). The *in vivo* kinetics of only the more extensively investigated analogs will be reviewed here. When the methyl groups on the phenyl ring were replaced by ethyl, increased hepatobiliary specificity and decreased urinary excretion was observed in a baboon model (27). However, these two analogs (dimethyl and diethyl) performed essentially identical in normal humans (43). Increasing the substituent group in the ortho positions further, as in di-isopropyl IDA, did not reduce renal excretion significantly, but the hepatic excretion rate increased (33,43). Urinary excretion of less than 2% of the dose 30 minutes after injection was observed in animals with p-butyl-IDA (35) and 2,4,6-trimethyl-3-bromo-IDA (TMB-IDA) (41). The p-butyl analog cleared very slowly through the liver, a characteristic thought to make it particularly well-suited for use in jaundiced patients (33). The low degree of urinary clearance of TMB-IDA was later confirmed in normal humans; this was

combined with increased hepatocyte extraction efficiency compared to the di-isopropyl-IDA analog (43,44). During these investigations, several important observations were made, leading to an improved understanding of how chemical structure affects hepatobiliary vs. renal excretion of Tc-99m-IDA analogs.

Relationship Between Biodistribution and Chemical Structure

The development of new and improved radiopharmaceuticals has generally followed pathways similar to those used for other groups of drugs. The process must begin with the identification of a compound with interesting biological activity (45). A hypothesis must be arrived at regarding which chemical features are related to bioactivity. New compounds must be synthesized and tested in an appropriate biological assay to evaluate the hypothesis. Based on this analysis, several modifications to the original hypothesis may have to be developed and tested. This process is intended to disclose how changes in the molecular structure affect biological activity, i.e., a structure-activity relationship (SAR) is identified.

A number of methods for quantitative SAR (QSAR) analysis using computers have been developed during the last 30 years. QSAR methods utilize mathematical models which describe the structural dependence on biological activities either by physicochemical parameters (Hansch analysis), by indicator variables encoding different structural features (Free-Wilson analysis), or by three-dimensional molecular property profiles of the compounds (comparative molecular field analysis, CoMFA) (46). Hansch (47) examined QSAR between biological activity and the underlying chemical properties such as atomic charges, oil-water partition coefficients, and molecular volumes. The most frequently used approach involves the determination of the change (π) in the lipophilicity of a parent compound caused by the introduction of a substituent group. The value of π is defined as the difference in the log of the partition coefficient (log P) between octanol and water of the parent compound and that of the substituted analog. A basic assumption of this method is that the effect of an analog of the parent compound equals the effect of the parent compound plus the effect of one or more substituent groups, in other words, additivity is assumed. At least in the simplest model it is also assumed that activity of a substituent group is independent of other substituents. The Free-Wilson analysis (48), sometimes referred to as the *de novo* approach, was one of the earliest QSAR methods. It represents a statistical approach

for ranking substituent group contributions to biological behavior without specifying any physicochemical properties. One of the limitations of this method is that activity predictions cannot be made for a compound containing a substituent which was not included as an observation in the original analysis. It is, however, a useful technique in cases where additivity is a reasonable assumption and where only a limited number of substituents are involved (45). It should be pointed out that prediction of the most desirable compound is not the primary goal of QSAR analysis. Rather, it should be considered as a tool to rationalize and shorten the path from a lead structure to an analog with the desired biological activity.

Structure-activity analysis has been applied in the development of radiopharmaceuticals only recently (15). One of the first examples of the use of SAR in radiopharmaceutical development was for the Tc-99m labeled cholescintigraphic agents. Since radiopharmaceuticals generally have no pharmacologic action, and we are primarily interested in *in vivo* distribution, the terminology "structure-distribution relationship" (SDR) is frequently used instead of SAR.

In 1977, Burns and coworkers (49) reported on the preparation and testing of five different Tc-IDA analogs. Biodistribution studies in mice revealed a linear relationship between biliary excretion and the natural log of the molecular weight divided by the charge. A few years later Nunn, et al. (41) reported on correlations between physicochemical parameters, structural effects, and *in-vivo* characteristics of a total of 33 Tc-IDA derivatives with different combinations of substituent groups on the phenyl ring. Lipophilicity was measured using a reverse-phase HPLC system, and compared to theoretical π values from published reference tables. A linear relationship was apparent when the compounds were divided into groups based on the location of the substituents. The extent of protein binding was determined *in vitro* using a HSA-affinity column. Hepatic and renal excretion at 30 minutes were determined in rats, and imaging studies in rabbits were used to measure intrahepatic kinetics. This body of data was correlated to gain a better understanding of the relative importance of the size, type, and location of substituent groups. No attempt was made to do a QSAR analysis. Increased lipophilicity generally resulted in increased hepatic specificity and reduced renal excretion. Increased protein binding of the para substituted analogs correlated with increasing theoretical lipophilicity. This was not observed for substituents in the ortho positions; no correlation was observed with either protein binding or theoretical lipophilicity. When a single lipophilic group was present in para position, hepatocyte transit time increased significantly, whereas

diortho substitution resulted in decreased hepatocyte transit time. Initial studies led the investigators to test additional compounds based on what they had learned. When a group of IDA analogs with various arrangements of methyl and bromo substituents was tested, TMB-IDA was found to have the best overall *in vivo* characteristics in rats and rabbits.

The structure-activity relationship for Tc-PG analogs has also been investigated (50) using derivatives of Tc-99m(Sn) pyridoxylidene-phenylalanine. Fluoro-, chloro-, and six different alkyl substituents were introduced in various positions on the phenyl group of the phenylalanine moiety. The lipophilicity of the analogs was determined by measuring the octanol/buffer partition coefficient. *In vivo* distribution studies in rats showed that increased lipophilicity correlated well with a decrease in renal excretion. Generally, many of the observed effects of substituent groups on the phenyl ring corresponded to what had been observed with the Tc-99m IDA analogs. Preliminary SDR work with a different group of potential hepatobiliary radiopharmaceuticals related to indocyanine green has also been reported (51). Indotricarbocyanine, a symmetrical compound with two phenyl rings, was synthesized and monoiodinated with I-131. Dihalogenated compounds were also prepared by introducing F, Cl, Br or I on the second phenyl ring. The *in vivo* characteristics were studied in several animal models. All the analogs had rapid blood clearance and hepatic uptake, and minimal urinary clearance. Liver clearance rate was significantly affected by the second halogen group, and a correlation was observed between liver clearance rate and the polarity of the halogen substituent.

The QSAR method of Free and Wilson (48) has been used to investigate the effect of various alkyl substituents on the phenyl ring of Tc-99m IDA complexes (52). Seven analogs, including unsubstituted Tc-99m IDA, were initially used. The analogs were substituted in either the para or both ortho positions. The following *in vivo* parameters were determined in rabbits: activity in urine at 30 minutes, time to reach maximum liver uptake, and liver clearance half-time. The response measures used in this QSAR model were the log of the experimentally measured values. A computer model allowed calculation of the contribution to biological activity for each of the substituent groups (para or diortho), as well as the activity of the unsubstituted Tc-IDA. It then combined this data to give predicted numbers for a total of 16 related Tc-IDA analogs (including those tested). An additional analog was subsequently tested and added to the data set. Within the range of experimental errors associated with

animal studies, good agreement was seen between predicted and experimental values.

COMMERCIALY AVAILABLE HEPATO-BILIARY RADIOPHARMACEUTICALS

Agents Available in the U.S.

At the present time, three radiopharmaceutical kits for the preparation of Tc-99m cholescintigraphic agents are available in the United States. They are all derivatives of Tc-IDA, and are available as lyophilized mixtures of the IDA analog and stannous ions sealed under nitrogen to prevent oxidation. The Tc-99m complex is formed by simply adding Tc-99m pertechnetate solution followed by a brief incubation period at room temperature. During their development, these IDA analogs have been referred to in the literature by a variety of names and acronyms. The generic names of commercially available IDA derivatives are as follows:

dimethyl-IDA: lidofenin
 diisopropyl-IDA: disofenin
 trimethyl-bromo-IDA: mebrofenin

These are the names adopted by the United States Adopted Names (USAN) Council. In addition, the various kit manufacturers have given their product a trade name (Table 1). The formulation, as given in current package inserts (53,54,55), of commercial kits for the preparation of these Tc-99m labeled IDA analogs are given in Table 1.

Table 1. Formulation of commercially available Tc-99m hepatobiliary radiopharmaceuticals.

Trade Name (Generic Name)	Manufacturer	Kit Contents	Preparation and Storage
Technescan (Lidofenin)	HIDAMerck Frosst Canada for Mallinckrodt, Inc.	Lidofenin 10 mg SnCl ₂ • 2H ₂ O 0.8-1.1 mg HCl or NaOH to adjust pH to 3.9-4.1.	Add 2-10 ml Tc-99m pertechnetate, up to 100 mCi (3.7 GBq). Incubation time: 15 minutes. Use within 6 hours. Store at 2-8°C.
Hepatolite (Disofenin)	DuPont Pharma	Disofenin 20 mg SnCl ₂ • 2H ₂ O 0.24-0.6 mg HCl and/or NaOH to adjust pH to 4-5.	Add 4-5 ml Tc-99m pertechnetate, 12-100 mCi (444-3700 MBq). Incubation time: 5 minutes. Use within 6 hours. Store at 15-30°C.
Choletec (Mebrofenin)	Squibb Diagnostics	Mebrofenin 45 mg SnF ₂ • 2H ₂ O 0.54 to 1.03 mg. Methylparaben up to 5.2 mg Propylparaben up to 0.58 mg HCl or NaOH to adjust pH to give a pH of 4.2-5.7 in the reconstituted product.	Add 1-5 ml Tc-99m pertechnetate, up to 100 mCi (3700 MBq). Incubation time: 15 minutes. Use within 18 hours. Store at 15-30°C.

Radiochemical Quality Control Methods

The most common radiochemical impurities in Tc-99m radiopharmaceuticals prepared with stannous ion reductant are Tc-99m pertechnetate (TcO₄⁻) and reduced, hydrolyzed Tc-99m (R-Tc) species. A number of rapid thin-layer

chromatographic methods have been described for the separation of Tc-99m complexes from these two impurities (56). In order to separate the Tc-99m IDA complex (Tc-IDA) from TcO_4^- and R-Tc, two instant thin-layer chromatographic (ITLC) systems must be used. Silicic acid (SA) ITLC strips in 20% methanol can be used to separate TcO_4^- ($R_f = 1.0$) from the other components ($R_f = 0$) (57). The amount of R-Tc can be determined using silica gel (SG) ITLC strips and either water or acetonitrile:water (3:1) as the solvent (41,57). In these systems both TcO_4^- and Tc-IDA move with the solvent front, and only R-Tc remains at the origin. More time-consuming, but reliable, gel chromatographic and paper electrophoretic methods can also be used (42,57).

Several reports have been published regarding the presence of an intermediate reaction product during the formation of Tc-99m IDA complexes (41,58). This can be observed during HPLC analysis, but separation is insufficient using ITLC methods. This does not appear to be a problem as long as sufficient incubation time is allowed following Tc-99m pertechnetate addition.

In Vitro Stability

Majewski, et al. (59) studied the *in vitro* stability of several Tc-99m IDA analogs. The amounts of Tc-99m in the form of TcO_4^- and R-Tc were determined by ITLC methods (57) up to 24 hours following preparation. All Tc-99m lidofenin preparations contained <4% TcO_4^- and < 1% R-Tc up to 8 hours after preparation. The corresponding numbers for Tc-99m disofenin were < 2% and < 1%. There was no significant change in percent R-Tc for either agent up to 24 hours. Some increase was observed for TcO_4^- , but it did not significantly exceed 10% in any preparation. Technetium-99m mebrofenin was not included in this analysis. It should be noted that Tc-99m mebrofenin can be used up to 18 hours after preparation, whereas the other products should not be used past six hours (Table 1). The difference is due to the presence of bacteriostatic additives in the mebrofenin kit, but it also indicates that the manufacturer has been able to show that the agent retains high radiochemical purity.

Pharmacokinetics in Humans

The *in vivo* behavior of current hepatobiliary imaging agents in humans generally reflects what was observed in animal studies, although some species differences have been observed. The scintigraphic delineation of hepatobiliary anatomy is primarily determined by the degree of hepatic uptake as well as the rate of elimination from the liver into bile. All current agents are rapidly cleared from the blood and

taken up by the hepatocytes, with relatively little renal excretion in normals (Table 2). Of the three agents, Tc-99m mebrofenin reaches maximum liver uptake sooner than the others, and also has the fastest liver transit time as expressed by hepatic excretion half-time (time from maximum liver uptake to 50% of maximum). All the agents reach maximum liver uptake within 10-15 minutes after injection, and activity in the common bile duct is usually seen within 10 minutes. The gallbladder is usually visualized within 30 minutes, and intestinal activity is apparent by 60 minutes in 80% of patients (43).

Table 2. Pharmacokinetics of hepatobiliary radiopharmaceuticals in normals (43)

Agent	% Dose in Blood at 60 Minutes	% Dose in Urine		Hepatic Excretion $t_{1/2}$ (Min.)
		60 Min.	24 Hr.	
Tc-99m lidofenin	3.0 ± 0.5	7.6 ± 1.0	15.5 ± 2.7	42 ± 5
Tc-99m disofenin	2.0 ± 0.6	6.1 ± 1.0	11.1 ± 1.5	19 ± 3
Tc-99m mebrofenin	0.8 ± 0.1	0.6 ± 0.1	1.5 ± 0.3	17 ± 1

In patients with increased serum bilirubin levels, blood clearance and hepatic transit of Tc-99m IDA analogs is usually delayed, and a larger proportion of the dose is excreted in urine. However, extensive clinical use has shown that both Tc-99m mebrofenin and Tc-99m disofenin can be used successfully in jaundiced patients (10,43,44,62-64), whereas lidofenin is more severely affected and should not be used. The effect of BSP and bilirubin on the hepatocyte uptake of Tc-99m mebrofenin was studied *in vitro* using isolated rat hepatocytes (41). Uptake decreased somewhat with increasing concentrations of both BSP and bilirubin, but the decrease was only about 25% at a bilirubin level of 20 mg/dl. Similarly, bilirubinemia induced by bilirubin infusion in rabbits with normal liver function did not significantly alter pharmacokinetics of four different Tc-IDA analogs (35). It has been suggested by Popescu (60) that it is not high serum bilirubin levels *per se*, but rather altered hepatocellular function due to liver cell damage, that limits the biliary excretion of Tc-IDA analogs. This view is supported by Sarkar (61), who reported normal hepatic uptake and excretion of Tc-IDA in a patient with a plasma bilirubin level of 36 mg/dl. This patient suffered from a hereditary biliary conjugation disorder, but did not have parenchymal hepatic disease.

RADIATION DOSIMETRY

The recommended injected dose for hepatobiliary scintigraphy in non-jaundiced patients is 5 mCi (185 MBq) or less; in jaundiced patients a higher dose, up to 10 mCi (370 MBq) can be used. The estimated absorbed radiation doses according to the manufacturer's package insert (53,54,55) are given in

Table 3 for normal and jaundiced individuals. The MIRD scheme for dose calculations using published "S" values for an average 70 kg man was used for these calculations. Different assumptions may result in different dose estimates, and large individual differences in weight, organ size, and clearance kinetics may result in actual doses that deviate by more than an order of magnitude from published estimates. The effect of a fatty meal given at the end of hepatobiliary scintigraphy on the absorbed radiation dose has been reported by Wu and coworkers (65). A scheme for estimating absorbed radiation dose to newborns from Tc-99m diethyl-IDA has also been reported (66).

Table 3. Estimated absorbed radiation doses from 10 mCi (370 MBq)

ORGAN	Tc-99m Lidofenin		Tc-99m Disofenin*		Tc-99m Mebrofenin**	
	Normal rads (mCy)	Jaundice rads (mCy)	Normal rads (mCy)	Jaundice rads (mCy)	Normal rads (mCy)	Jaundice rads (mCy)
Total Body	0.14 (1.4)	0.10 (1.0)	0.16 (1.6)	0.15 (1.5)	0.2 (2.0)	0.17 (1.7)
Liver	0.29 (2.9)	0.45 (4.5)	0.38 (3.8)	0.63 (6.3)	0.47 (4.7)	0.81 (8.1)
Gallbladder Wall	1.33 (13.3)	0.79 (7.9)	1.2 (12.0)	1.40 (14.0)	1.37 (13.7)	1.25 (12.5)
Small Intestine	2.08 (20.8)	0.18 (1.8)	2.2 (22.0)	1.63 (16.3)	2.99 (29.9)	1.60 (16.0)
Upper Large Intestine	3.79 (37.9)	0.22 (2.2)	3.8 (38.0)	3.25 (32.5)	4.74 (47.4)	2.48 (24.8)
Lower Large Intestine	1.58 (15.8)	0.24 (2.4)	2.8 (28.0)	2.25 (22.5)	3.64 (36.4)	1.97 (19.7)
Kidney	0.20 (2.0)	0.26 (2.6)	---	---	0.22 (2.2)	0.19 (1.9)
Urinary Bladder Wall	0.86 (8.6)	3.05 (30.5)	0.92 (9.2)	0.88 (8.8)	0.29 (2.9)	2.42 (24.2)
Ovaries	0.39 (3.9)	0.21 (2.1)	0.82 (8.2)	0.75 (7.5)	1.01 (10.1)	0.64 (6.4)
Testes	0.06 (0.6)	0.13 (1.3)	0.06 (0.6)	0.06 (0.6)	0.05 (0.5)	0.11 (1.1)
Red Marrow			0.28 (2.8)	0.15 (1.5)	0.34 (3.4)	0.25 (2.5)

* Assumes that 80% of activity localizes in the liver, and that 20% of liver activity is transferred to gallbladder.
** Normal = bilirubin of < 1.5 mg/dl and 98% liver uptake; data for jaundiced patients assume 66% liver uptake.

PRECAUTIONS

Drug Interference

A variety of drugs have been reported to interfere with the movement of radiopharmaceuticals through the hepatobiliary system (67,68).

Narcotic Analgesics. Morphine in particular and opiates in general cause impaired emptying of bile into the small intestine because they induce spasm in the sphincter of Oddi (69). Cholescintigraphy in the presence of narcotic analgesics will show prolonged visualization of the gallbladder and the common bile duct and no movement of activity into the bowel. This could result in the erroneous diagnosis of biliary duct obstruction. This is significant, since narcotic analgesics are often the treatment of choice for patients presenting with severe abdominal pain. Ideally, hepatobiliary imaging should not be undertaken until the effect of the drug dissipates, which may require 6-12 hours (70).

Nicotinic Acid. Large doses of nicotinic acid have been known to cause significant hepatic dysfunction, resulting in poor hepatocyte uptake of

cholescintigraphic radiopharmaceuticals (71). This appears to be a dose-dependent toxic effect, and liver function returns to normal after nicotinic acid intake is stopped or reduced.

Total Parenteral Nutrition (TPN) Therapy.

Absent or delayed visualization of the gallbladder has been reported in patients who are receiving TPN therapy (72). This may result in false-positive images in patients without gallbladder disease. During TPN therapy, the gallbladder is relatively inactive, which is thought to result in bile stasis and the formation of highly viscous gallbladder bile that impedes the flow of additional bile into the gallbladder.

Miscellaneous Drugs. The effect on gallbladder contractility of a number of drugs including atropine, calcium channel blockers, and progesterone has been reviewed by Kloiber, et al (73). This is of particular concern in gallbladder ejection fraction studies, a topic that will be discussed later.

Adverse Reactions

Exceedingly few incidents of adverse reactions attributed to current hepatobiliary radiopharmaceuticals have been reported. Single cases of chills and nausea have been observed following injection of Tc-99m lidofenin (54). A few cases of itching at the injection site and development of hypersensitivity reactions such as skin eruptions have also been reported (53,55).

Use During Pregnancy and in Nursing Mothers

According to the manufacturers, it is not known if hepatobiliary radiopharmaceuticals could cause fetal harm (53,54,55). These radiopharmaceuticals should not be given to pregnant or possibly pregnant women unless a nuclear medicine study is clearly needed. Package inserts also state that it is not known if these hepatobiliary agents are excreted in human milk. However, Tc-99m pertechnetate, which may be present in small amounts, is known to be excreted in breast milk during lactation and the manufacturers therefore recommend that formula feedings should temporarily be substituted for breast feeding. Actual measurements of Tc-99m disofenin excretion have recently been performed in a group of six lactating women (74). Up to at least 24 hours following Tc-99m disofenin administration, less than 0.3% of the injected dose was found in breast milk, and the authors conclude that interruption of breast feeding is not essential. If interruption is preferred, and it is possible to plan in advance, patients should be encouraged to express and save extra milk prior to administration of radiotracer; this can then be used during the interruption period.

HEPATOBIILIARY IMAGING

Biliary disease is highly prevalent in our society, and is seen in a variety of settings ranging from biliary colic in otherwise healthy young women to life-threatening biliary sepsis in elderly patients with severe intercurrent disease. The initial assessment of patients with symptoms suggestive of biliary disease must include a full clinical examination and history. In patients with gallstones, the symptoms may be sufficiently typical to diagnose gallbladder disease from historic information only. Other patients may present with vague and ill-defined symptoms that could be caused by a variety of pathological conditions (5,9). Various laboratory tests and imaging modalities other than cholescintigraphy add valuable diagnostic information to the workup of these patients, but will not be reviewed here. Hepatobiliary imaging is an important diagnostic modality in the differential diagnosis of many hepatobiliary abnormalities, which will be discussed in the following sections.

Imaging Methods and Analysis

Patient Preparation. Patients are fasted for at least two hours before the study, preferably overnight, but not for longer than 24 hours (1,2,70,75). In the presence of food in the upper gastrointestinal tract the gallbladder may be contracting, which interferes with inflow of radioactive bile and may lead to a false-positive study. Prolonged fasting should be avoided as it may also cause non-visualization of a normal gallbladder. Narcotics or sedatives that act on the sphincter of Oddi should be stopped six to 12 hours before the examination (70).

Imaging Technique and Analysis. The usual dose range for Tc-99m cholescintigraphic agents is 3-10 mCi (100-370 MBq) for adult patients. In children the dose is adjusted according to age and weight. Cholescintigraphy is best performed with a large field-of-view scintillation camera equipped with a low-energy, high-resolution collimator. The patient is placed under the camera in the supine position such that the liver, gallbladder, and duodenum is within the field of view. Sequential images are obtained using a 20% energy window centered at 140 keV. Imaging protocols vary between institutions and depend on what information is to be obtained. Typically, serial anterior images are obtained at five- to 15-minute intervals up to 60 minutes after injection. The first image is acquired for 750,000 to one million counts and subsequent images are acquired for the same time as the first image (1,70,75). Simultaneously, a dynamic study can be obtained at a rate of one frame per minute and stored in a computer. Time-activity curves may be generated from this data by placing

regions of interest over liver parenchyma, common bile duct, or gallbladder. Functional parameters such as hepatic extraction fraction or gallbladder ejection fraction can then be evaluated (70). Additional images in the lateral or oblique projections may help define the anatomic location of collections of radioactivity. For example, a lateral view may help distinguish activity in the gallbladder (anterior) from renal excretion (posterior). Delayed images are obtained if all the desired information is not obtained during the first hour. The normal appearance rate of cholescintigraphic tracers in the various parts of the hepatobiliary system has already been discussed. In the evaluation of hepatobiliary images, the physician considers the various functional steps leading from radio-tracer delivery to the liver to the appearance of activity in the gut as well as hepatobiliary morphology (75).

Augmented Cholescintigraphy

Cholecystokinetic Agents. In humans, gallbladder emptying in response to a fatty meal is primarily due to the release of endogenous cholecystokinin (CCK). This hormone, as well as its synthetic C-terminal octapeptide analog, sincalide, have been used as adjuncts in the evaluation of hepatobiliary disease. Their effects include gallbladder contraction, relaxation of the sphincter of Oddi, and increased bile secretion (73,76). A fatty meal or a standardized preparation such as Neo-Cholex, a 50% emulsion of corn oil in water, will have the same effect. Xynos, et al (77) reported on the reproducibility of gallbladder emptying studies with Tc-99m diethyl-IDA following ingestion of 250 ml milk. Thirty individuals were studied twice, two to five weeks apart. The milk was given 30 minutes after the radioactive tracer dose. Good reproducibility was observed for both gallbladder ejection fraction and for the lag time between milk ingestion and start of emptying. However, use of CCK or sincalide does provide control of both dose and infusion rate, which may make it easier to compare studies from different institutions. Sincalide for intravenous administration is available in 5 μg vials from Squibb Diagnostics under the trade name Kinevac. Reported side effects include nausea, abdominal pain, and the urge to defecate. Transient dizziness and flushing have also been reported. The only contraindication to its use is sensitivity to sincalide. The usual intravenous dose of sincalide is 0.02 $\mu\text{g}/\text{kg}$ to be infused at a uniform rate over a three- to five-minute period (70,76,78,79). The dose must not be injected as a bolus, and some reports advocate a slower infusion rate of up to 15 minutes (73,75) in order to reduce the incidence of abdominal pain.

Gallbladder emptying can be utilized in two

different ways in cholescintigraphy: (a) to empty the gallbladder prior to radiopharmaceutical injection in order to optimize subsequent filling, and (b) to study the contractile function of the gallbladder. The first approach may be useful in patients who have been fasting for a prolonged (> 24 hours) period of time, or who are on total parenteral nutrition (70,76). As previously mentioned, the presence of very viscous gallbladder bile in these patients may prevent radiotracer flow through the cystic duct. Sincalide should be given 30 minutes prior to radiotracer injection (76). It has been suggested that rather than routinely pretreating this group of patients, it is better to use this technique only when the gallbladder is not visualized within one to two hours of tracer injection (80). At this point, sincalide can be injected, followed by a second radiotracer dose 30 minutes later.

In gallbladder ejection fraction studies, sincalide is not infused until after the gallbladder is visualized. A dynamic study of gallbladder emptying is obtained and used to determine the ejection fraction. Assuming complete mixing of radiotracer with gallbladder contents, the percent decrease in activity corresponds to the volume being evacuated (73). Although there is no universal agreement regarding what a normal ejection fraction should be, most investigators feel that an ejection fraction below 35-40% is clearly abnormal (75,78,79). It should, however, be noted that ejection fraction can vary significantly depending on both dose and duration of sincalide infusion (64). Various medications may either inhibit or stimulate gallbladder emptying, and should be discontinued prior to an ejection fraction study. Care is also required in patients with systemic diseases that may have an effect on gallbladder contraction (73). The inability of the gallbladder to contract and eject bile normally is a helpful piece of information in the evaluation of patients with suspected chronic acalculous biliary disease, which will be discussed later.

Morphine. Cholescintigraphy is considered diagnostic for acute cholecystitis if the gallbladder is not visualized within four hours of radiopharmaceutical injection. Markedly delayed visualization may be observed in patients with chronic cholecystitis, bile stasis secondary to prolonged fasting, and alcoholic liver disease (81). Morphine augmented cholescintigraphy can be utilized to speed up the differentiation of patients with complete cystic duct obstruction from those who for other reasons have impaired flow of tracer into the gallbladder (82). Morphine acts by constricting the sphincter of Oddi, resulting in a prompt elevation of biliary tract pressure that may cause radionuclide to flow into the gallbladder (76,81). The usual protocol is to administer morphine if, after 40 to 60 minutes the

radiotracer is visualized in the common bile duct and small bowel, but not in the gallbladder. There must be sufficient activity left in the hepatobiliary tree to permit gallbladder visualization. Imaging is continued for another 30 minutes; persistent non-visualization is considered to indicate acute cholecystitis (76,82).

The usual dose is 0.04 mg/kg (up to 4 mg) of morphine sulfate diluted to 10 ml with saline for injection. Administration is by slow I.V. push over two to three minutes (76). The increased intraductal pressure caused by morphine can result in symptoms ranging from epigastric distress to typical biliary colic. In Choy's initial study (81) about 40% of patients reported transient cramps. If very severe pain should occur, the effect of morphine can be reversed with naloxene. Morphine may produce a wide spectrum of unwanted effects such as respiratory depression, nausea, and vomiting. Administration of a single dose as in these augmentation studies appears to be well tolerated, however (81). There are few contraindications to the use of morphine. Allergic reactions may occur, and it may not be advisable to use this approach in patients with a history of morphine addiction. False-positive studies can occur secondary to many causes, the most common being chronic cholecystitis (76,82,83). False-negative studies are less common. Studies comparing delayed imaging to morphine augmentation have shown that the false-positive rate can be decreased using morphine (84,85).

Phenobarbital. Phenobarbital has been used in the pediatric population in conjunction with cholescintigraphy to improve the accuracy of differentiating neonatal hepatitis from biliary atresia (76,86). The general stimulating effect of phenobarbital on the liver is well known. Phenobarbital increases bilirubin conjugation and excretion, enhances bile flow, and increases the hepatic uptake of other organic anions such as the common hepatobiliary imaging agents (76,86). Increased uptake and visualization of the liver leads to a more reliable determination of the underlying etiology of neonatal jaundice (to be discussed later). In phenobarbital-augmented cholescintigraphy, the patient is premedicated orally with 5 mg/kg per day of phenobarbital for three to seven consecutive days. The radiotracer, usually around 1 mCi (37 MBq) Tc-99m disofenin, is then injected (76,86). Serial images are obtained and computer-generated time-activity curves can also be generated. Lateral images can help to separate activity in the bowel from renal excretion. Delayed images are obtained up to 24 hours after injection if the bowel is not seen on earlier images. If any radiotracer passes into the bowel, the diagnosis of biliary atresia is excluded. Phenobarbital is

available in a variety of dosage forms, including liquids. The safety of phenobarbital therapy in children and neonates has been established (86). Allergic reactions such as swelling have been observed in individuals who are hypersensitive to barbiturates.

Cholecystitis

Cholecystitis, or inflammation of the gallbladder, may be acute or chronic in its presentation. In acute cholecystitis the cystic duct is completely obstructed, which incites an inflammatory response in the gallbladder. The acute attack is most often an exacerbation of underlying chronic cholecystitis, and although it may be convenient to view the two as separate entities, cholecystitis frequently represents a continuum of disease (1,8). Acute cholecystitis is nearly always associated with gallstone disease. In acute acalculous cholecystitis the cystic duct obstruction is caused by edema from the inflamed gallbladder. The factors that induce gallbladder inflammation are not well understood, but a number of factors have been implicated, such as lithogenic bile, trapped bile acids, regurgitated pancreatic juice, prostaglandins, and others (8,9).

Cholecystitis is one of the most common abdominal emergencies and must be distinguished from other abdominal conditions with similar symptoms. Prompt diagnosis and treatment is of the essence, particularly in patients who are both very ill and elderly. Patients may present with a wide variety of complaints and physical findings. Symptoms vary from vague, poorly-defined abdominal discomfort to short spasms of biliary colic or severe right upper quadrant pain that may last for several days (9). The rapid and intense pain of biliary colic is typically caused by a stone passing through the cystic duct, but if the stone becomes lodged in the cystic duct the pain will continue until the stone passes through (2,9). Acute calculous cholecystitis is self-limiting in 85% of patients, but recurrence may eventually follow unless the gallbladder is removed (8). If a stone remains lodged in the cystic duct the patient will develop serious complications such as complete necrosis of the gallbladder wall and perforation (5,8,9). As previously discussed, cholecystectomy remains the only definitive cure for gallstone disease, and may take place as soon as the diagnosis has been established, or after several weeks, when the patient is stabilized and the inflammatory process has resolved (2,8,9). The epidemiology of acalculous cholecystitis differs from cholecystitis associated with stones (2). Acute acalculous cholecystitis has been linked to previous surgery, massive trauma, extensive burns, cardiovascular disorders, and malignancy (5,8). Removal of the gallbladder is warranted in patients

diagnosed with acute acalculous cholecystitis (5,9).

Cholescintigraphy is a non-invasive and highly sensitive diagnostic test for evaluation of cystic duct obstruction. Failure to visualize the gallbladder within 4 hours of radiotracer injection has been 98% accurate in the diagnosis of acute cholecystitis (63). Calculous and acalculous acute cholecystitis present with identical imaging patterns. As discussed earlier, a number of conditions are associated with delayed as well as non-visualization of the gallbladder in the absence of complete cystic duct obstruction. The use of sincalide or morphine augmentation as well as delayed imaging up to 24 hours results in increased sensitivity in these patients. Depending on the patient population and the imaging protocol, the diagnosis of acute cholecystitis by Tc-99m IDA scintigraphy has an overall sensitivity of 92-100% and specificity of 95-100% (63,64,70,76,81).

Chronic cholecystitis is the most common biliary disorder but also the most difficult to evaluate (2,8). It is most frequently associated with gallstone disease (8,87) and patients may have experienced episodes of biliary colic. Most patients have functional cystic ducts, and although many patients may have delayed gallbladder visualization, cholescintigrams are generally normal. Since biliary calculi can be detected using ultrasound, the usefulness of cholescintigraphy in this group of patients is limited (1,87). A subset of patients with chronic cholecystitis will not have calculi. These patients present with recurrent right upper quadrant pain, but blood chemistries, oral cholecystograms, and ultrasound studies are normal (8,87). Three major categories of abdominal disease can produce typical "biliary" pain in the absence of gallstones: (a) chronic acalculous cholecystitis or gallbladder dyskinesia, (b) cystic duct syndrome or sphincter of Oddi dysfunction, and (c) irritable bowel syndrome. If the symptoms are due to impaired gallbladder evacuation (as in cystic duct syndrome) and/or impaired gallbladder contraction (as in chronic acalculous cholecystitis and gallbladder dysfunction), the patient could benefit from cholecystectomy. A number of recent studies (73,76,79) support the use of gallbladder emptying studies using sincalide to predict which patients will become symptom-free following gallbladder removal.

Sphincter of Oddi Dysfunction

Sphincter of Oddi dysfunction is characterized by a physiologic obstruction to bile drainage from the common bile duct into the bowel. It is seen in patients with recurrent pain after cholecystectomy (70,75). Sincalide augmented cholescintigraphy has been proposed as a method to detect sphincter of Oddi dysfunction. The disorder can be identified if there is

a delay in biliary-to-bowel transit of radiotracer and a failure of the sphincter of Oddi to relax following sincalide administration (76,83). A scoring system that combines visual and quantitative criteria has shown improved sensitivity and specificity for the diagnosis of sphincter of Oddi dysfunction (88).

Biliary Obstruction

In the presence of good hepatocyte function, acute total bile duct obstruction by gallstones can be readily identified using cholescintigraphy. Hepatic uptake of tracer will be prompt, with no evidence of biliary excretion by four hours. In the later stages of total obstruction, hepatic uptake will decrease and differentiation of obstruction from hepatocellular disease becomes progressively more difficult. In partial bile duct obstruction, the biliary tree is visualized to the level of obstruction, filling defects or narrowing may be observed, and some radiotracer will be seen downstream from the area of obstruction (70,75). Patients with partial common bile duct obstruction also show reduction in the sincalide induced gallbladder ejection fraction and ejection rate (43).

Bile Leakage and Reflux

Hepatobiliary scintigraphy has proven to be a very sensitive method of disclosing and monitoring intra- and extrahepatic bile leaks. These are seen most commonly following biliary tract surgery, but can also be a result of abdominal trauma or inflammatory erosion of the gallbladder wall (75,87,89). The location of bile accumulation depends on the site of injury, and images in several different projections will be helpful in distinguishing free bile from normal areas of radiotracer localization. Delayed images should also be obtained. Cholescintigraphy is also an excellent technique for demonstrating patency and function of biliary-enteric anastomoses (75,89). The non-invasive detection and quantitation of enterogastric bile reflux can only be performed using cholescintigraphy (83). The technique is of value for the confirmation and follow-up of bile reflux after surgical interventions.

Neonatal Jaundice

In the pediatric population, cholescintigraphy is a valuable noninvasive technique for the evaluation of prolonged jaundice. Jaundice in the neonate can have numerous causes, but conjugated hyperbilirubinemia that persists beyond the first month of life is usually due to either neonatal hepatitis or biliary atresia (5). It is important to identify the cause as early as possible, because the treatment and prognosis of these two conditions are quite different. Extrahepatic bile

duct atresia is defined as the lack of lumen in part or all of the extrahepatic biliary tract, causing complete obstruction to bile flow. It is considered to be the result of a progressive, destructive inflammatory process of unknown etiology that eventually destroys the whole bile duct system and results in death from cirrhotic liver disease. Biliary atresia can be treated successfully by surgery only if performed at a very early age (5,86,87). Neonatal hepatitis is a clinical syndrome that is not associated with a specific viral infection in the majority of cases. There are many causes for prolonged conjugated hyperbilirubinemia in neonates, including metabolic disorders, infectious diseases, and intoxicants (78,87). In many cases cholestasis attributable to these causes is medically treatable and reversible (5). The clinical, biochemical and histologic features of these two disorders are often similar, making the differential diagnosis extremely difficult.

In biliary atresia, the radiotracer does not enter the bowel. In the absence of significant cirrhosis, hepatocyte function is preserved, and there is rapid blood clearance and hepatic uptake, resulting in early definition of liver boundaries, which persists throughout the study (76,86). Patients with neonatal hepatitis in the presence of a patent extrahepatic biliary tree may present with a more variable imaging pattern (86). Depending on the severity of cholestasis and hepatocellular disease, initial liver uptake may be normal or markedly decreased. The gallbladder is not always visualized. Hepatic clearance is delayed, resulting in delayed visualization of bowel activity. However, detection of even the slightest amount of radioactivity in the bowel in these patients identifies biliary patency and argues against surgical intervention (75). If hepatic uptake is extremely poor due to very high serum bilirubin levels, it may not be possible to identify bowel activity on images. The liver uptake pattern still makes it feasible to make a differential diagnosis. If hepatocyte function is severely compromised, the activity initially seen in the liver is primarily due to blood pool activity, and it can be observed that it will decrease over time at the same rate as cardiac activity, a pattern not seen in biliary atresia (86). As previously mentioned, pretreatment with phenobarbital significantly improves the accuracy of cholescintigraphy in differentiating extrahepatic biliary atresia from neonatal hepatitis, at least when the agent Tc-99m paraisopropyl-IDA was used (86). In a series of 27 patients with neonatal jaundice that did not receive phenobarbital pretreatment, Gerhold and his associates (90) reported that the most useful diagnostic criterion for biliary atresia was absence of intestinal radioactivity up to 24 hours after radiotracer injection. All nine patients who demonstrated

intestinal activity did so by three hours. These patients were studied with either Tc-99m disofenin or Tc-99m diethyl-IDA. The sensitivity and specificity for biliary atresia in their study were 97% and 82%, respectively.

QUANTITATION OF HEPATIC FUNCTION

In the early stages of liver disease, serial quantitative assessment of liver function would be helpful in monitoring therapy and in determining prognosis. However, current noninvasive methods are neither simple nor reliable (8,91). Due to the multitude of metabolic tasks performed by the liver, none of the liver function parameters that can be easily measured will reflect overall hepatic capacity. Another major use of such a method would be to help differentiate between biliary obstruction and hepatocellular disease in the jaundiced patient. Up to very recently, techniques using radiopharmaceuticals have met with limited success. The ideal radiopharmaceutical for quantitation of hepatocyte function should be highly specific for liver uptake, and should remain in the hepatocyte long enough to allow sufficient data acquisition. The *in-vivo* distribution should be solely a function of hepatocellular function, and should not be affected by factors such as elevated bilirubin levels (38). Attempts at quantitation of liver function have been made using Tc-99m sulfur colloid, however, colloids are taken up by reticuloendothelial cells and are not specific for the liver. Liver uptake often correlates poorly with hepatocellular function (5). Hepatobiliary radiopharmaceuticals have been studied extensively, going back to I-131 rose bengal. More recently, the Tc-99m IDA analogs have been used. Quantitative measurement of liver uptake of Tc-99m IDA analogs is complicated in the jaundiced patient by delayed blood clearance and simultaneous renal excretion. Recirculation of tracer and active hepatocyte uptake is prolonged, and this is further complicated by excretion into bile of tracer that was taken up by the liver early on. Multicompartmental analysis of blood pool and hepatic uptake using pharmacokinetic models of varying complexity have been attempted, but results have been discouraging (91).

Measurement of Hepatic Extraction Fraction (HEF) Using Tc-99m IDA Analogs

Estimation of the fraction of radiotracer that can be removed by the liver during a single pass, or extraction fraction, has been proposed as a quantitative measure of liver function (91,92). If the tracer could be presented as a bolus directly into the blood supply of the liver, hepatic extraction fraction (HEF) could

be measured directly. However, because of the liver's dual blood supply and the complication of simultaneous hepatocellular uptake and excretion, this is not possible using Tc-99m IDA analogs. Juni and coworkers have calculated HEF by an indirect method using deconvolution analysis (91,92,93). Deconvolution analysis is a computer-assisted mathematical technique which can correct an organ's time-activity curve for the dynamically changing pattern of blood activity (obtained from the heart's time-activity curve) being presented to that organ (92). Care must be taken so that no biliary, renal, or major vascular activity or scatter is included in the region(s) of interest used to generate the hepatic time-activity curve (93). In a group of 53 patients with acute jaundice, Juni et al. (91) found that this measure of HEF was most specific during the first few days after onset of jaundice, with fairly good separation between patients with normal livers (80-100%) or acute biliary obstruction (65-100%) versus those with acute hepatocellular dysfunction (14-77%). Another study (93) showed markedly decreased HEF in patients with alcoholic cirrhosis compared to patients with diseases confined primarily to the biliary tract. It appears that HEF is an improved quantitative measure of hepatic function compared to other parameters assessed using hepatobiliary radiopharmaceuticals. The deconvolution analysis is rapid and entirely automated and can be performed using standard commercial nuclear medicine computers (91). Its clinical utility will be tested further as more institutions add this analysis to their hepatobiliary imaging procedures.

Quantitation of Hepatic Binding Protein Receptors

In addition to the transport pathways for hepatocyte uptake discussed earlier, the liver also has the ability to selectively extract macromolecular ligands from blood by receptor-mediated endocytosis (2,5). Of the receptors on the hepatocyte surface, the asialoglycoprotein receptor is the best characterized. This receptor is found only on mammalian hepatocytes, and is often referred to as hepatic binding protein (HBP) receptor (94). The hepatic asialoglycoprotein receptor promotes the clearance of serum glycoproteins that have exposed galactosyl residues made terminal by removal of sialic acid. After binding at the hepatocyte membrane, the ligand-receptor complex is internalized and transported to hepatic lysosomes where the complex dissociates and the ligand is catabolized. The receptors recycle back to the hepatocyte membrane where the entire process is repeated. It has been postulated, but not proven, that the HBP receptor participates in the regulation of turnover of serum glycoproteins. Alterations in asialoglycoprotein receptor binding has been

demonstrated in specific physiologic and pathological states (5). Since the HBP receptor is highly specific for ligands containing exposed galactose residues, this type of ligand has been investigated as a potential radiopharmaceutical for quantitation of hepatocyte function. There is currently a great deal of interest in receptor-specific radiopharmaceuticals (15). The potential exists for the utilization of mathematical models to obtain *in vivo* measurements of receptor number and affinity from imaging observations using receptor-specific radiopharmaceuticals. It is anticipated that such data may provide a quantitative index for patient diagnosis and management (15,95).

Technetium-99m galactosyl-neoglycoalbumin (Tc-NGA) is a synthetic ligand that exhibits high specificity for HBP receptor binding (95,96,97,98). The NGA ligand is prepared by covalently attaching the galactosyl unit to human serum albumin (99). The number of galactose units per albumin molecule can be controlled, and since it is known that the ability of glycoproteins to bind to the HBP receptor increases dramatically as more galactose units are added (5), this gives control of both affinity and binding rate. NGA has commonly been radiolabeled with Tc-99m using an electrolytic method (99) for the reduction of pertechnetate. In Japan, Tc-99m-DTPA-galactosyl human serum albumin (Tc-GSA) is available as a stannous ion containing kit (100). Both methods result in stable preparations of high radiochemical purity. Animal studies have confirmed that Tc-NGA is highly specific for liver with a rapid uptake phase and a low rate of metabolism and liver exit (97,99). Both renal and gastrointestinal excretion does occur, but the excretion rate is too low to affect liver time-activity curves during the first 30 minutes after injection. Since Tc-NGA is not taken up by the general anion extraction mechanism, it is not affected by high serum bilirubin levels. Its rate of hepatocyte accumulation is dependent on the amount of ligand injected and is proportioned to the number of galactose units on the albumin (97). This radiopharmaceutical can be designed to have moderate receptor affinity so that the rate at which it binds is not governed solely by blood flow, but depends on the concentrations of both ligand and receptor (e.g., a second order process) (97). Since Tc-NGA has been shown to have relatively low toxicity (99), it can be used in a dose large enough to produce a second order response. Changes in liver and blood time-activity curves obtained with Tc-NGA in pigs were observed when either hepatic blood flow, ligand-receptor affinity, or molar dose of ligand were altered (101).

Tc-NGA appears to exhibit the unique properties of receptor-specific ligands: high ligand affinity, specificity, saturability, and distribution in relation to

physiologic response (15). The parameters that govern the rate of a receptor-binding process are the receptor and ligand concentrations, and the forward and reverse binding constants (102). Since the binding of Tc-NGA is highly irreversible (96), the reverse binding constant can be ignored (102). A mathematical model has been developed that permits estimation of kinetic parameters that represent receptor concentration and forward binding rate from imaging studies using 4-6 mCi (148-222 MBq) Tc-99m NGA. The kinetic model requires liver and heart time-activity data for the first 30 minutes after injection plus activity determination in a blood sample taken at three minutes (98,102). The kinetic model has been validated by independent *in vitro* assay of tissue obtained by liver biopsy (95). Multiple clinical studies using Tc-NGA and Tc-GSA have been reported from investigators in the United States, Europe, and Japan. A recent review article (98) provides an overview of the various liver disorders that have been studied, which include chronic liver disease, primary and secondary liver carcinoma, liver transplantation, and acute viral hepatitis. This technique for functional hepatic imaging shows promise as a sensitive measure of functioning hepatocyte mass, of particular interest when hepatectomy for hepatocellular carcinoma or liver transplantation is contemplated. Serial studies may help document changes in hepatocyte function in patients being treated for active viral hepatitis. This technique, while still undergoing validation, holds significant potential for obtaining information that is not readily available using other imaging techniques or conventional liver function tests.

CONCLUSIONS

Currently available Tc-99m labeled hepatobiliary radiopharmaceuticals have been developed based on what is known about the liver's organic anion clearance pathway. This group of radiopharmaceuticals serves as an example of the utilization of structure-activity relationship analysis during the development phase. Technetium-99m IDA analogs in use today satisfy most of the criteria for an ideal radiopharmaceutical to study the hepatobiliary excretion pathway. Although the clearance of these agents by the liver is reduced in the presence of high serum bilirubin levels, clinically useful images can be obtained in most situations. Cholescintigraphy with Tc-99m IDA analogs is a noninvasive, highly sensitive test for the evaluation of the functional status of hepatic and cystic and the gallbladder. It is also used to localize bile leaks and alterations in the bile pathway resulting from surgery or trauma, and to

differentiate between surgical and medical jaundice in the neonate. Improved diagnostic information may be obtained by utilizing drug-augmented cholescintigraphic methods. Technetium-99m IDA analogs do not possess ideal characteristics for quantitative assessment of liver function. However, hepatic extraction fraction estimates may be obtained using a computer-assisted technique that allows continuous correction for blood pool activity. Receptor-specific agents, Tc-99m labeled NGA and GSA, are currently being evaluated for quantitation of functional hepatocyte mass. These agents are not affected by elevated serum bilirubin levels, and are highly specific for the asialoglycoprotein receptor which is found only on mammalian hepatocytes.

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QUESTIONS

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1. A main function of the hepatocyte is:
- A. removal of foreign particles from circulation
 - B. breakdown of senescent blood cells
 - C. detoxification of harmful compounds
 - D. complexation to make polar compounds less water-soluble
2. The main function of the gallbladder is:
- A. degradation of cholesterol
 - B. storage and concentration of bile
 - C. absorption of unconjugated bile acids
 - D. regulation of canalicular bile secretion
3. The overall excretion rate of organic anions into bile depends primarily on:
- A. the carrier-mediated canalicular transport process
 - B. the number of available hepatocyte storage sites
 - C. the rate of bile formation and excretion
 - D. the number of available receptor sites on the sinusoidal membrane
4. There is no competition for biliary excretion between Tc-99m disofenin and
- A. bilirubin
 - B. chlorpromazine
 - C. indocyanine green
 - D. sulfobromophthalein
5. Compounds that are primarily excreted in urine rather than in bile are characterized by being
- A. of molecular weight above 600
 - B. highly protein bound
 - C. labeled with radioiodine
 - D. strongly polar
6. Which of the following statements pertaining to hepatic bile is false?
- A. secreted at an average rate of 25 ml per hour
 - B. bilirubin usually represents 10% of total bile solids
 - C. cholesterol is a major lipid component
 - D. flow is related to the bile acid pool

7. Which of the following statements pertaining to bile acids is true?
- primary bile acids are synthesized in reticuloendothelial cells
 - circulating bile acids are less than 5% protein bound
 - less than 10% of conjugated bile acids are reabsorbed in the gastrointestinal tract
 - bile acids are conjugated with glycine and taurine
8. The most important characteristic of bilirubin is that it:
- promotes G.I. tract absorption of lipids
 - is a degradation product of heme
 - keeps lipids in bile in solution
 - promotes bile formation and flow
9. The organic anion pathway for hepatocyte excretion is not followed by:
- asialoglycoprotein
 - indocyanine green
 - rose bengal
 - iopanoic acid
10. Acute cholecystitis is a result of cystic duct obstruction by gallstones in:
- more than 90% of cases
 - less than 50% of cases
 - most patients with supersaturated bile
 - patients treated long-term with opioid pain killers
11. The presence of calculi in the gallbladder cause symptoms in:
- patients with short cystic ducts
 - more than 80% of individuals
 - patients with bilirubin levels above 3 mg/dl
 - a minority of individuals
12. Gallstones classified as cholesterol stones:
- are common in patients with biliary tract infection
 - contains calcium in the center
 - are poorly suited for dissolution therapy with bile salts
 - are not usually visualized using ultrasound imaging
13. The best therapy to prevent recurrence of gallstones is:
- laparoscopic cholecystectomy
 - dissolution therapy with chenodeoxycholic acid
 - shock-wave lithotripsy
 - gallbladder ejection treatment
14. The major disadvantage of Tc-99m pyridoxylidene glutamate prepared by the autoclave method was:
- gastrointestinal absorption of radiochemical impurities
 - very slow blood clearance
 - high degree of urinary excretion
 - in vitro* instability
15. Which of the following statements best describes structure-activity relationship (SAR) analysis?
- SAR analysis is a method to reduce the number of related analogs that must be synthesized and tested
 - SAR analysis makes it possible to identify new chemical compounds with desired biological activity
 - All SAR methods require both physicochemical and biological measurements
 - Lipophilicity measurements used in SAR analysis are usually performed in an acetonitrile:water system
16. In the preparation of Tc-99m dimethyl-IDA,
- the final complex is a Tc-Sn-bis-complex with dimethyl-IDA
 - the oxidation state of technetium is +4
 - the overall charge of the complex is -1
 - the molecular weight of the complex is less than 600
17. For the preparation of Tc-99m IDA radiopharmaceuticals,
- kits are available that utilize either stannous ion or electrolysis for reduction of pertechnetate
 - the shelf-life following addition of Tc-99m pertechnetate is 18 hours
 - the agent is ready to use as soon as all solids are completely dissolved
 - the usual amount of Tc-99m to add is 100 mCi or less

18. Clinical studies with commercial Tc-99m IDA radiopharmaceuticals available in the U.S. have shown that
- increased serum bilirubin levels result in increased renal excretion for all agents
 - there is no significant change in blood clearance rate with increased bilirubin levels
 - of the three agents, Tc-99m disofenin has the fastest liver transit kinetics
 - in normals, the gallbladder can usually not be visualized until 45-60 minutes after injection
19. Which of the following statements related to drug effects on hepatobiliary kinetics is true?
- Nicotinic acid in large doses prolongs gallbladder contraction
 - Phenobarbital is used to speed up liver transit of hepatobiliary agents in patients with poor liver function
 - After administration of morphine, emptying of bile into the gastrointestinal tract is delayed
 - Morphine administration reduces the pressure within the gallbladder
20. In augmented hepatobiliary imaging studies with cholecystokinetic agents,
- a normal gallbladder should eject at least 75% of its content following an i.v. dose of 0.02 mg/kg of sincalide
 - viscous bile can be ejected from the gallbladder prior to scintigraphy to facilitate gallbladder filling with radiotracer
 - gallbladder emptying initiated by a fatty meal is not a reproducible technique
 - a gallbladder ejection fraction study is performed by injection of radiotracer 15-30 minutes following sincalide infusion
21. Which of the following statements pertaining to gallbladder filling and emptying is false?
- Partial gallbladder emptying takes place periodically during the filling phase
 - Delayed gallbladder filling may be associated with alcoholic liver disease
 - Gallbladder emptying cannot be induced by a fatty meal unless the gallbladder contains at least 50 ml of bile
 - The cystic duct syndrome is associated with impaired gallbladder emptying
22. Nonvisualization of a normal gallbladder with cholescintigraphy is not a result of:
- pain management using morphine
 - fasting in excess of 24 hours
 - fatty meal taken one hour prior to the study
 - total parenteral nutrition therapy
23. Which of the following statements related to biliary tract disease is true?
- Cholescintigraphy is a highly sensitive test for evaluation of cystic duct obstruction
 - Prolonged cystic duct obstruction leads to biliary duct inflammation and necrosis
 - Cholecystectomy is never indicated in patients with non-calculous gallbladder disease
 - Non-visualization of the gallbladder is not highly specific for acute cholecystitis
24. In neonatal jaundice,
- the gallbladder is usually visualized by four hours on cholescintigraphy
 - a dose of phenobarbital should be given 30-60 minutes prior to cholescintigraphy
 - evidence of Tc-99m disofenin in the bowel at four hours excludes biliary atresia
 - caused by hepatitis, the underlying disease process is usually a virus infection
25. Which of the following statements related to hepatic extraction fraction (HEF) studies is true?
- Deconvolution analysis is used to correct liver uptake for blood background activity
 - The blood time-activity curve is obtained from a region of interest over the hepatic artery
 - HEF provides a quantitative measure of hepatic binding protein receptor affinity
 - Correction for kidney activity is done by obtaining images in the lateral projection