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Extraction efficiency and biliary excretion of hepatobiliary imaging agents in the rat perfused liver

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A multitude of transitional metal complexes has recently been introduced into clinical practice as hepatobiliary imaging agents; the kinetics of these substances are often poorly understood. To gain better insight into characteristics of these different agents, we measured the extraction efficiency and mean biliary transit time of a variety of iminodiacetate derivatives in the in-situ rat perfused liver. First pass hepatic extraction efficiency averaged 59% for ^{99m}Tc *N*-(*p*-isopropylacetanilide) iminodiacetate, 73% for ^{99m}Tc *N*-(2,6-diethylacetanilide) iminodiacetate, 74% for ^{99m}Tc *N*-(3-bromo-2,4,6-trimethylacetanilide) iminodiacetate, 90% for ^{99m}Tc *N*-(*p*-butylacetanilide) iminodiacetate, and 93% for ^{99m}Tc *N*-pyridoxyl-5-methyltryptophan. By comparison, extraction of another organic anionic compound, ^{131}I Rose bengal, was only 12·4%. Mean hepatocyte transit times varied from 2·3 to 7·5 min. Shorter mean transit times were observed for di*ortho* substituted and longer mean transit times for *para* substituted metal complexes. Radioactivity was quantitatively recovered in bile, and excretion kinetics overall were consistent with data generated in whole animals. These studies demonstrate the value of the in-situ rat perfused liver as a screening tool to characterize hepatobiliary imaging agents.

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The ideal hepatobiliary imaging agent should meet the following criteria: (Chervu et al 1982) efficient hepatic uptake (Fritzberg & Klingensmith 1982), short biliary transit time (Wistow et al 1977), specificity for hepatobiliary excretion in patients with both normal and decreased hepatocellular function and (Fritzberg et al 1982) adequate properties as far as imaging is concerned (Chervu et al 1982; Fritzberg & Klingensmith 1982). In the early 70s, a class of chelating agents which met most of these criteria was described (Chervu et al 1982). These agents were found to form complexes with the transition metal, technetium, whose 99m isotope provides high quality gamma camera images with a low radiation dose to the patient because of its abundance of 140 keV photons, low particulate emission and short half life of 6 h. Characterization of these different ^{99m}Tc complexes have involved collection of blood, bile and urine in baboons (Wistow et al 1977), rats (Fritzberg et al 1982) or recording time activity curves over regions of interest in rabbits (Nunn et al 1981). These methods, however, usually do not provide a direct measurement of hepatic extraction efficiency

and therefore, do not allow prediction of the affinity of these compounds for the hepatic uptake and excretory transport system.

The in-situ rat perfused liver permits easy measurement of hepatic extraction efficiency as well as characterization of biliary mean transit times. Furthermore, such studies can be carried out without potential interference by endogenous materials such as bilirubin and bile acids. Therefore, we compared hepatic extraction efficiency and biliary excretory kinetics of a variety of ^{99m}Tc labelled iminodiacetate derivatives.

Materials and methods

Radiopharmaceuticals. Commercial kits were used for the preparation of ^{99m}Tc complexes of the following compounds: *N*-(*p*-isopropylacetanilide) iminodiacetate (PIPIDA, Medi-Physics, Emeryville, CA), *N*-(2,6-diethylacetanilide) iminodiacetate (DEIDA, Amersham, Arlington Heights, IL), *N*-(3-bromo-2,4,6-trimethylacetanilide) iminodiacetate (BTMIDA, E. R. Squibb and Sons, New Brunswick, NJ), and *N*-(2,6-diisopropylacetanilide) iminodiacetate (DIPIDA, New England Nuclear, Billerica, MA). Three ml of 100 μCi ml^{-1} of ^{99m}Tc pertechnetate, obtained from a generator (New England Nuclear) were added to each kit at least 30 min before use. ^{99m}Tc *N*-(*p*-butylacetanilide) iminodiacetate (BIDA, New England Nuclear), ^{99m}Tc *N*-pyridoxyl-5-methyltryptophan (PMT, Nihon Medi-Physics, Takarazuka City, Japan) were prepared from the chelating agents and stannous chloride using standard methods for the iminodiacetate technetium (Klingensmith et al 1979) or reported procedures for ^{99m}Tc *N*-pyridoxyl-5-methyltryptophan (Kato-Azuma 1982). PMT was found to be unstable during the preparation, therefore, it was subjected to high performance liquid chromatographic purification shortly before use. The column effluent containing the Tc-PMT complex was collected in a tube containing ca 2 mg ml^{-1} freshly dissolved ascorbic acid. Dilutions were made in saline containing 1·5 mg ml^{-1} ascorbic acid also prepared shortly before use. HPLC was carried out on a octodecylsilyl column (Beckman, Berkeley, CA) with a gradient of 20–70% from 0·01 M phosphate buffer pH 6 to acetonitrile at a flow rate of 1·0 ml min^{-1}. ^{131}I Rose bengal was obtained commercially (E. R. Squibb and Sons).

Rat liver perfusion. Male, Sprague-Dawley rats, 250–350 g, were prepared under pentobarbitone (50 mg kg^{-1} i.p.) anaesthesia. The common bile duct was cannulated with PE 50 tubing. Perfusions were carried out as previously described (Reichen & Le 1983) using a semi-synthetic perfusion medium consisting of washed human erythrocytes (20% v/v) in Krebs-Ringer-bicarbonate buffer containing bovine serum albumin (2% w/v) and dextrose (0·1% w/v). The pH was monitored periodically and adjusted with 0·1 M $NaHCO_3$ as needed. All reported experiments met the following viability criteria: portal pressure (<11 cm H_2O), portal flow (>1·5 ml min^{-1} g^{-1}), oxygen consumption (>2 μmol g^{-1} min^{-1}), SGOT release (<10 iu h^{-1}), potassium release (<0·2 mequiv h^{-1}) and haemolysis rate (<0·2% h^{-1}). To maintain physiological bile flow rates, taurocholate sodium, >98% pure by thin layer chromatography (Hofmann 1962), was infused at a rate of 0·2 μmol min^{-1}/100 g body weight.

Determination of extraction efficiency and biliary excretion kinetics. 25 nCi of the ^{99m}Tc complex in 0·25 ml of 0·9% NaCl (saline) were rapidly injected into the portal vein cannula. Hepatic venous outflow was collected for 5 min after injection. Virtually all non-extracted radioactivity was washed out of the liver within the first 3 min after injection; less than 1% of the injected dose appeared in the hepatic venous outflow between 3 and 5 min after the injection. The collected perfusate was mixed and an aliquot taken for counting. In some experiments, 10 nCi of ^{131}I Rose bengal were administered together with the ^{99m}Tc complex and its extraction efficiency determined simultaneously. A dual channel well counter with appropriate correction for ^{131}I (364 keV) crossover into the ^{99m}Tc channel was used for sample counting. The extraction efficiency (E) was determined as:

$$E = \frac{\text{counts injected} - \text{counts recovered}}{\text{counts injected}}$$

Mean transit time was calculated from biliary dilution curves as the ratio of the first moment of the frequency function over the area under the curve (Meier & Zierler 1954). Biliary mean transit time was corrected for the common bile duct cannula transit time according to standard techniques (Goresky & Silverman 1964). Total biliary excretion was expressed as percentage of the dose excreted within 30 min after administration.

All results are expressed as mean ± one standard deviation.

Results

All experiments reported met the viability criteria set forth in the method section; in addition, adequate bile flow and bile salt secretion rates documented appropriate function of the perfused organs (Table 1). The extraction efficiencies are also reported in Table 1. All ^{99m}Tc complexes were efficiently taken up, extraction efficiency ranging from a low of 59% for PIPIDA to a high of 93% for PMT. Virtually all material taken up was excreted within 30 min in the case of PIPIDA, DEIDA, BTMIDA and DIPIDA. Only 84 and 89% of the dose of BIDA and PMT taken up was excreted, however (Table 1). By contrast, ^{131}I Rose bengal extraction efficiency averaged only 12·4%.

Mean biliary transit times are also reported in Table 1. They range from a short transit time of 2·3 min for DEIDA to a maximum of 7·5 min for BTMIDA. As a rule, the biliary mean transit time was shorter for

Table 1. Parameters of rat perfused liver hepatobiliary radio-pharmaceutical studies.[a]

99m-Tc complex	Bile flow ($\mu l\ min^{-1}\ g$)[b]	Bile salts excretion (nmol/ $100\ g\ min^{-1}$)[b]	Total bile excretion %	Extraction efficiency %	Mean transit time (min)
PIPIDA	1·7 ± 0·3	171 ± 51	58·2 ± 4·4	58·9 ± 3·8	5·3 ± 2·0
DEIDA	2·0 ± 0·1	171 ± 53	72·0 ± 5·9	72·8 ± 5·1	2·3 ± 1·0
BTMIDA	1·5 ± 0·1	202 ± 62	68·0 ± 9·0	74·1 ± 8·4	7·5 ± 1·0
DIPIDA	1·7 ± 0·3	174 ± 42	79·1 ± 6·4	79·6 ± 6·6	2·8 ± 0·7
BIDA	1·5 ± 0·0	243 ± 152	75·9 ± 7·6	90·5 ± 0·8	6·3 ± 1·6
PMT	2·0 ± 0·4	210 ± 58	82·5 ± 12·7	92·9 ± 2·3	2·8 ± 0·3
^{131}I Rose bengal				12·4 ± 4·9	

[a] Values are mean ± s.d.
[b] Bile flow values are based on liver weights and bile salts excretion values on rat body weights.

di*ortho*- and longer for *para*-substituted ^{99m}Tc acetanilideiminodiacetates (Table 1). Mean transit time for Rose bengal could not be calculated, since only 25% of the extracted fraction could be recovered in bile within the 30 min study.

Discussion

Our studies in the in-situ rat perfused liver demonstrate that transition metal complexes of iminodiacetate derivatives are removed very efficiently by the liver. Uptake of these compounds is inhibited by neither taurocholate nor procaineamidoethobromide (Klingensmith et al 1984). Bromosulphophthalein and indocyanine green, in contrast, efficiently inhibit their hepatic transport (Klingensmith et al 1984; Fritzberg et al 1984). It is assumed, therefore, that these compounds are transported by the anionic dye transporting system. The extraction efficiency of ^{99m}Tc-IDA complexes considerably exceed that of other organic anionic dyes such as indocyanine green and Rose bengal (Iga & Klaassen 1977) but is comparable to the efficiency of 95% reported for taurocholate (Pries et al 1981).

Both the acetanilideiminodiacetate and pyridoxylamino acid groups are known to exist in a 2 : 1 ratio of ligand to metal (Henriksen et al 1978). Thus the molecular weights range from 700 to 800 depending on the substituents; this is in the molecular weight range preferentially transported into bile (Reichen & Paumgartner 1979). The former complexes are monanionic while the class to which PMT belongs is neutral (Klingensmith et al 1981). Extraction efficiencies in man have been determined directly only for DEIDA; efficiencies between 0·48 to 0·56 were found in patients with normal liver function (Klingensmith et al 1980). The order of efficiency, however, seems to be quite similar in man to what is reported in the present study. Thus extrapolated estimates of the extraction efficiency of PIPIDA were lowest at 24% (Klingensmith et al 1982) as compared to 60 and 66% for DIPIDA and BTMIDA respectively (Fritzberg et al 1979). The exception to this rule is BIDA; in a comparison a higher extraction efficiency for DEIDA than for BIDA was inferred (Wistow et al 1977). This is in contrast to the present study where BIDA at 91% was one of the most efficiently transported derivatives.

Biliary transit times varied according to the phenyl substitution pattern. Thus, di*ortho*-substituted compounds exhibited short biliary mean transit times while *para*-substituted analogues had relatively long transit times. The only exception to this rule was BTMIDA which has both di*ortho*- and *para*-substitution. In our study, it had the longest period of mean transit times which is in contrast to the rabbit where it has a short transit time (Wistow et al 1977).

Organic anionic dyes have been shown to have extraction efficiencies ranging from 7·5% for indocyanine green and 9·9% for Rose bengal to 45·6% for bromsulphophthalein (Klingensmith et al 1982). In contrast, bile acids are extracted 90–95% at similar blood flow rates (Pries et al 1981). Thus, our study demonstrates that the organic anionic dye pathway can be used at extraction efficiencies approximating those observed for bile acids.

The excretory kinetics are less easily available from studies in-vivo; our study shows that marked differences exist for substances with similar extraction efficiencies. This may have important clinical implications. In general, the correlation of our studies with clinical studies is quite good (Klingensmith et al 1980, 1981, 1982). A notable exception to this rule is the behaviour of BTMIDA which had relatively slow hepatocellular transit in our study but has been reported to be excreted rapidly in rabbits (Nunn et al 1981).

In conclusion, the in-situ rat perfused liver is an adequate model to screen hepatobiliary imaging agents for their kinetic behaviour, since extraction efficiency, completeness of biliary excretion and biliary mean transit times can be determined directly.

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Amphetamine-induced circling behaviour in MPTP-lesioned mice

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This study was designed to examine the effect of intrastriatal administration of MPTP on (+)-amphetamine-induced circling behaviour in mice. The results demonstrate that (+)-amphetamine elicits circling behaviour in MPTP-lesioned animals. MPTP produced a 70% depletion of striatal dopamine concentration.

In response to amphetamine administration, mice, lesioned unilaterally in one striatum with 6-hydroxydopamine, will circle to the damaged side (Ungerstedt 1968, 1971; Von Voigtlander & Moore 1973; Costall & Naylor 1975). Amphetamine is believed to elicit this circling behaviour by releasing a reserpine-insensitive, newly synthesized pool of dopamine (DA) from DA nerve terminals in the intact striatum (Weissman et al 1966; Chiueh & Moore 1973; Fung & Uretsky 1982). This mouse circling model has been widely used to study compounds with striatal dopaminergic activity.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has recently been recognized as a neurotoxin that destroys dopaminergic nigrostriatal pathways in man (Davis et al 1979; Langston et al 1983) and several animal species (Hallman et al 1984; Heikkila et al 1985; Fuller & Steranka 1985; Langston 1985). It has been shown that repeated systemic administration of MPTP (11 injections) to mice caused a 90% reduction in striatal DA content (Heikkila et al 1985). The present study was designed to determine if a single intrastriatal administration of various doses of MPTP would induce significant nigrostriatal damage in mice to elicit circling behaviour in reponse to the systemic administration of amphetamine.

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Methods

MPTP-induced lesions of the right striatum in mice. Male ICR mice (Sasco, Omaha), 25–32 g, were anaesthetized with chloral hydrate (430 mg kg^{-1} i.p.). Chilled NaCl 0·9% (saline) (4 μl), containing 10, 20 or 30 μg of MPTP hydrochloride (Research Biochemicals, Inc.) was injected into the right striatum of the mouse over 4 min, using a stereotaxic instrument (David Kopf) with a 10 μl Hamilton syringe. Saline was injected into the control animals. The solution was injected into the