A Structure-Distribution-Relationship Approach Leading to the Development of Tc-99m Mebrofenin: An Improved Cholescintigraphic Agent

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Thirty-three HIDA (hepatobiliary IDA) derivatives were tested and correlations drawn between physicochemical parameters, structural effects, and in vivo characteristics. Capacity factors of the ligands on reverse-phase HPLC were used as a measure of lipophilicity, and to predict protein binding and in vivo distribution of the complexes. Fragmentary π values were used to derive theoretical lipophilicities, which showed that ortho substituents have reduced lipophilic activity, probably because of self-shielding. Ortho substitution was found to affect hepatocellular transit times. Various combinations of substituents with the desired overall lipophilicity were tested. The best compound, Tc-3-bromo-2,4,6-trimethyl HIDA, possessed high hepatic specificity, and rapid hepatocellular transit; it was also resistant to competition for hepatobiliary excretion from bilirubin.

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The discovery in 1975 of 2,6-dimethyl HIDA (hepatobiliary IDA) established a new class of hepatobiliary agents and stimulated interest in cholescintigraphy. In common with other new classes of radiopharmaceuticals, the HIDAs have undergone several stages of refinement. Also in common with other radiopharmaceuticals, the early development of the HIDAs followed a "random walk" process because the physicochemical factors governing hepatobiliary elimination were not precisely known. Although a large number of the substituted aniline precursors required for synthesis of the ligand are commercially available, the range of their physicochemical properties is not great. Despite this limited choice and weak theoretical direction, many researchers were able to synthesize second-generation HIDAs with improved in vivo characteristics (*1–3*).

This paper reports preliminary correlations and simple structure-distribution relationships (SDRs) that we have developed for hepatobiliary agents of the HIDA class. We report the use of these correlations in the development of a third generation HIDA. Physicochemical data were acquired on over thirty HIDA derivatives and the data correlated to the in vivo characteristics of the technetium complexes. Where necessary, new aniline derivatives were synthesized and used to make the HIDAs that were needed to improve the correlations. This work resulted in the synthesis of 3-bromo-2,4,6-trimethylphenylcarbamoylmethyliminodiacetic acid (mebrofenin) (*4*). Tc-99m mebrofenin exhibits: (a) high specificity for the hepatobiliary system, (b) rapid transit through the hepatobiliary system, (c) high resistance to competition from compounds such as bilirubin and, (d) rapid radiolabeling with high radiochemical purity and stability.

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MATERIALS AND METHODS

Synthesis of ligands. The ligands were synthesized using the general method reported by Burns et al. (*5*). This involves reaction of the appropriate aniline derivative with the anhydride of nitrilotriacetic acid prepared in situ. Those anilines that were commercially unavailable, included that leading to mebrofenin, were synthesized using standard procedures, to be reported in detail elsewhere. After recrystallization, the product was analyzed by IR, NMR, elemental analysis, and other accepted analytical methods, with satisfactory results.

Formulation of the technetium-99m complexes. These were made either from aqueous solution or by reconstitution of lyophilized kits. In all cases, stannous fluoride was used as the reductant and the molar ratio of the stannous fluoride to ligand was approximately 1:150. The solution contained 30–50 mg of ligand at pH 5–6. Up to 200 mCi of $^{99m}TcO_4^-$ in 1–10 ml of 0.9% saline were added to 1 ml of either an aqueous preparation or a lyophilized powder corresponding to 1 ml of solution.

Determination of radiochemical purity. A reverse-phase HPLC system was used consisting of a 250-by-4.1-mm μBondapak C-18 column* (or equivalent) eluted with 0.025M phosphate buffer (pH 6.8) and methanol as modifier. Different columns gave good separations when the proportions of buffer to modifier were in the range 40:60 to 50:50. The system was run in an isocratic mode at 1.0 ml/min, 1000 psi. Two in-line detectors were coupled in series using low-volume tubing. The first was an absorbance detector† set at 235 nm and 0.4 absorbance units full scale. The second was a flow-through gamma detector having a 10-μl nominal active volume inside a shielded 3-by-3 inch NaI(Tl) well crystal. The output from the crystal was fed into both a multichannel analyzer (MCA) in multiscaler mode and a digital-to-analog converter. This allowed the simultaneous acquisition of analog and digital data. On occasion, the whole system was run automatically for 24 hr or more using a microprocessor‡ to command the pumps, injector, chart recorder, MCA, and printer. Between 2- and 20-μl samples were injected. Two ITLC systems were also developed to determine radiochemical purity. One system used a stationary medium prepared by dipping silicic acid strips (ITLC-SA)§ in a 10% sodium chloride solution, lightly blotting them to remove excess ligand, then drying at 75–80°C for 20 min. The pretreatment reduces fronting of the activity. The strips were spotted with 1–5 μl of sample and immediately developed with a saturated sodium chloride solution for approximately 10 cm. The strips were air-dried, cut in half, and counted in a well counter to determine free pertechnetate (R_f 1.0) and the sum of the reduced hydrolyzed technetium plus the technetium ligand complexes (R_f 0.0). Reduced hydrolyzed technetium was quantitated by placing 1–5 μl of sample on silica gel strips (ITLC-SG)§. The strips were immediately developed with a 3:1 acetonitrile:water solution for ~10 cm. The developed strips were treated as before to determine reduced hydrolyzed technetium (R_f 0.0) and the sum of the free pertechnetate plus the technetium ligand complexes (R_f 1.0).

Protein binding. The extent of protein binding was measured using a human serum albumin (HSA) affinity column consisting of HSA bound to an inert support (*6*). Briefly, 4 g of CNBr-Sepharose 4B were swollen and washed in 300 ml of 1 m*M* HCl. The acid was removed by washing with 300 ml of 0.025 *M* borate/1.0 *M* NaCl buffer (pH 8.0). Two ml of HSA (500 mg) in 75 ml of the borate/NaCl buffer were then added to the Sepharose and mixed gently for 2 hr at room temperature. After standing overnight at 4°C, the Sepharose was washed with 300 ml of the borate/NaCl buffer. Untreated CNBr groups were blocked by mixing the Sepharose with 100 ml of 1.0 *M* ethanolamine hydrochloride (pH 9) for 2 hr. Alternate washes of 3 × 100 ml of 0.1 *M* acetate/1 *M* NaCl buffer (pH 4), and 3 × 100 ml of 0.1 *M* borate/1 *M* NaCl buffer (pH 8) were then used to remove loosely bound HSA before loading the Sepharose into a glass column, 0.7-by-17-cm (6.5 ml). No attempt was made to measure the amount of HSA bound to the Sepharose; instead appropriate internal standards were used. No changes in the column performance were detected over a six-month period. Columns were eluated with 0.05 *M* borate/0.1 *M* NaCl buffer (pH 7.2) at 0.5 ml/min. Samples of 50 μl were chromatographed, and the eluted radioactivity detected using a flow-through gamma detector.

Ligand liphophilicity. The lipophilicity of the ligands was measured using a reverse-phase HPLC system similar to that described previously except that a 1% acetic acid in water/methanol eluant (60:40) was substituted for the phosphate buffer/methanol. The individual ligands were dissolved in acetone:methanol (2:1) at approximately 10 mg/ml, and 2–20 μl were injected.

Hepatobiliary specificity. Groups of at least six male Sprague-Dawley rats weighing 200–375 g were used after a 24-hr fast. Following penile ligation, a 0.25-ml dose was injected into an external jugular vein under ether anesthesia. The Tc-HIDA solution was reconstituted such that the injected dose contained ~1 mCi/ml of ligand. At sacrifice at 30 min after injection, various organs were removed and counted against suitable standards to determine the percentage of the injected dose per organ.

Intrahepatic kinetics. Male, nonfasted rabbits weighing ~4 kg were lightly restrained on the face of a gamma camera. Anesthesia was avoided to minimize any related variation in hepatic function. Each animal received 0.25 ml of a 5mCi/ml solution of the appropriate Tc complex via a marginal ear vein. Data were collected continuously at 30-sec intervals for 30 min using a computer-interfaced gamma camera. At the end of the study, regions of interest were delineated over various organs and used to obtain the time-activity curve from which the following parameters were obtained: (a) T_{max}, the time in minutes after injection until the maximum liver activity (b), T_{50}, the interval between T_{max} and the point at which the activity in the liver had dropped to 50% of its value at T_{max}.

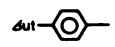
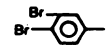
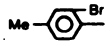
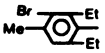
Isolated hepatocyte studies. Isolated hepatocytes were prepared using established methods (*7*) to give a sus-

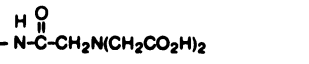
TABLE 1. THE LIGANDS USED IN THIS STUDY AND A SUMMARY OF THE RESULTS

	Substitution of Phenyl Ring[a]	Log k′	Σπ	HSA[b] retention	%[c] Renal extraction	%[d,e] Hepatic extraction	T_{max} (min)	T_{50}[e] (min)
I.		−0.37	0.00	−				
II.	4-F	−0.30	0.14					
III.	3,4-Me	−0.24	1.12	−	15.1 ± 4.1	63.4 ± 3.2(12)	4.7 ± 0.6	9.6 ± 2.0(10)
IV.	3,4-Cl	−0.24	1.42	−				
V.	4-F	−0.22	0.14					
VI.	4-EtO	−0.05	0.38					
VII.	4-Me	−0.04	0.56					
VIII.	2,4,6-Me	0.01	1.68	+	6.8 ± 0.9	88.1 ± 4.9(12)	3.9	8.1
IX.	4-Cl	0.18	0.71					
X.	3,4-Et	0.21	2.04	−	8.2 ± 1.4	83.8 ± 7.6(6)	5.3 ± 0.9	9.1 ± 2.0(5)
XI.	4-Et	0.28	1.02	+				
XII.	4-Br	0.29	0.86					
XIII.	3,4-Cl	0.29	2.13					
XIV.	2,4,6-Me	0.32	1.68		17.6 ± 1.5	72.6 ± 3.7(6)	2.5	4.8
XV.	4-I	0.43	1.12					
XVI.	2-Cl, 4-Et	0.49	1.73		17.9 ± 2.5	69.9 ± 5.2(6)	4.3	7.1
XVII.	2-Cl, 4-Me	0.50	1.27		7.0 ± 0.8	77.3 ± 8.3(6)	4.3	10.0
XVIII.	2-Et, 4-Cl	0.53	1.73		11.3 ± 1.7	83.2 ± 3.2(6)	4.9	8.0
XIX.	4-Pr	0.56	1.53	++				
XX.	2-Me, 4-Br	0.61	1.42		4.8 ± 0.9	91.9 ± 7.1(7)	4.6 ± 0.7	8.0 ± 0.8(5)
XXI.	2-Br, 4-Me		1.42		6.4 ± 0.8	86.2 ± 3.9(6)	5.0	15.3
XXII.	3,4-Cl	0.62	2.84					
XXIII.	2,4,6-Br,Me	0.63	2.54		1.2 ± 0.6	94.8 ± 5.6(77)	3.5 ± 0.6	5.4 ± 0.8(16)
XXIV.	2,4,6-Me,Br		2.54		1.0 ± 0.2	94.6 ± 4.0(6)	4.1	5.5
XXV.	3,4-Et	0.66	2.75		2.7 ± 0.5	90.1 ± 3.5(7)	4.4	21.6
XXVI.	3,4-Pr	0.66	3.06	−	7.4 ± 1.6	89.6 ± 4.1(6)	5.9	26.7
XXVII.	2-I, 4-Me	0.69	1.68		4.7 ± 1.1	86.8 ± 4.5(9)	4.6	10.2
XXVIII.	2,4-Cl	0.80	1.42		28.6	50.4	8.0	37.0
XXIX.	2-CF_3, 4-Cl	0.86	1.58		3.6	89.1	5.3	22.0

(continued)

TABLE 1. *(continued)*

	Substitution of Phenyl Ring[a]	Log k′	Σπ	HSA[b] retention	%[c] Renal extraction	%[d,e] Hepatic extraction	T_{max} (min)	T_{50}[e] (min)
XXX.		0.98	2.13	+++	3.6 ± 0.5	86.0 ± 4.9(5)	3.0	18.0
XXXI.			1.72		2.4 ± 0.2	87.6 ± 4.3(6)	5.9	17.0
XXXII.			1.42		22.7 ± 7.9	64.6 ± 4.2(6)	–	–
XXXIII.			3.44		1.0 ± 0.1	95.7 ± 5.5(6)	5.0	13.9

a. 

b. – = eluted in void volume; +, ++, +++ increasing retention.

c. Kidneys + bladder and contents.

d. Liver + gastrointestinal tract.

e. Numbers in parenthesis after the rabbit and/or rat data are the individual determinations used to obtain the data. When no standard deviation or number of determinations is indicated, less than five determinations were performed.

pension of 4 million cells/ml. The percent uptake into the cells for each Tc-99m complex was studied as a function of time and at various concentrations of bromosulphthalein (BSP) and bilirubin. The uptake of C-14 taurocholate was used as a positive control.

RESULTS

The ligands studied are listed in Table 1, together with a summary of the results obtained with both ligands and their technetium complexes. HPLC demonstrated that a radioactive reaction intermediate was present during the formation of the final product (*8*). In agreement with Fritzberg et al (*9*) we found that the reaction was normally complete in 15–30 min after reconstitution at room temperature. No ITLC system used was able to separate the intermediate from the final product. Once equilibrium had been reached, the radiochemical purity normally exceeded 90% when measured by HPLC or ITLC. We have not been able to determine the exact structure of the intermediate or the final product, nor can we substantiate the suggestion that the intermediate and final product are mono and bis ligand structures respectively (*10*). All animal studies were performed using samples that had reached equilibrium.

The Tc complexes examined by affinity chromatography could be divided into two groups depending upon the position of substitution on the phenyl ring of the ligand (*6,11*). Complexes of derivatives with ortho substituents (I, III, IV, X, XXVI, Table 1) passed through the column with no significant retention, whereas passage of those with para substituted derivatives (VIII, XI, XIX, XXX) was delayed. The degree of retention of the complexes increased as the chain length of the substituent in the para position increased (Table 1).

Reversed-phase HPLC was used to measure the lipophilicity of the ligands. This was taken as the capacity factor (k′) for each of the ligands under identical reverse-phase chromatographic conditions where:

$$\log k' = \log\left(\frac{V_R - V_M}{V_M}\right),$$

where V_M = retention time of an unretained peak, and V_R = retention time of the ligand.

Theoretical lipophilicities (Σπ) were obtained using the tabulations of Hansch and Leo (*12*). The theoretical lipophilicity is the sum of the fragmentary π values of the substituents on the phenyl ring of the ligand. It does not include the lipophilicity of the remainder of the ligand, which is assumed to be constant. The theoretical and measured lipophilicities for each of the ligands are recorded in Table 1.

Three groups of ligands result when the measured lipophilicity is plotted against the theoretical lipophilicity (Fig. 1). The membership of a group is determined by whether the ligand has 0, 1 or 2 ortho substituents. The slopes of the lines joining the members of the two main groups are similar.

Distribution studies in rats were limited to a single time point at 30 min after injection. At this time, distribution of injected radioactivity is essentially complete in normal rats and the slopes of the time-activity curves for the various organs are small. Hepatic extraction and renal extraction are listed in Table 1 for selected complexes. As expected, those complexes with low renal extraction also had high hepatic extraction, with little or no distribution of radioactivity elsewhere in the body. In most cases the sum of the recovered radioactivity exceeded 90% of the injected dose.

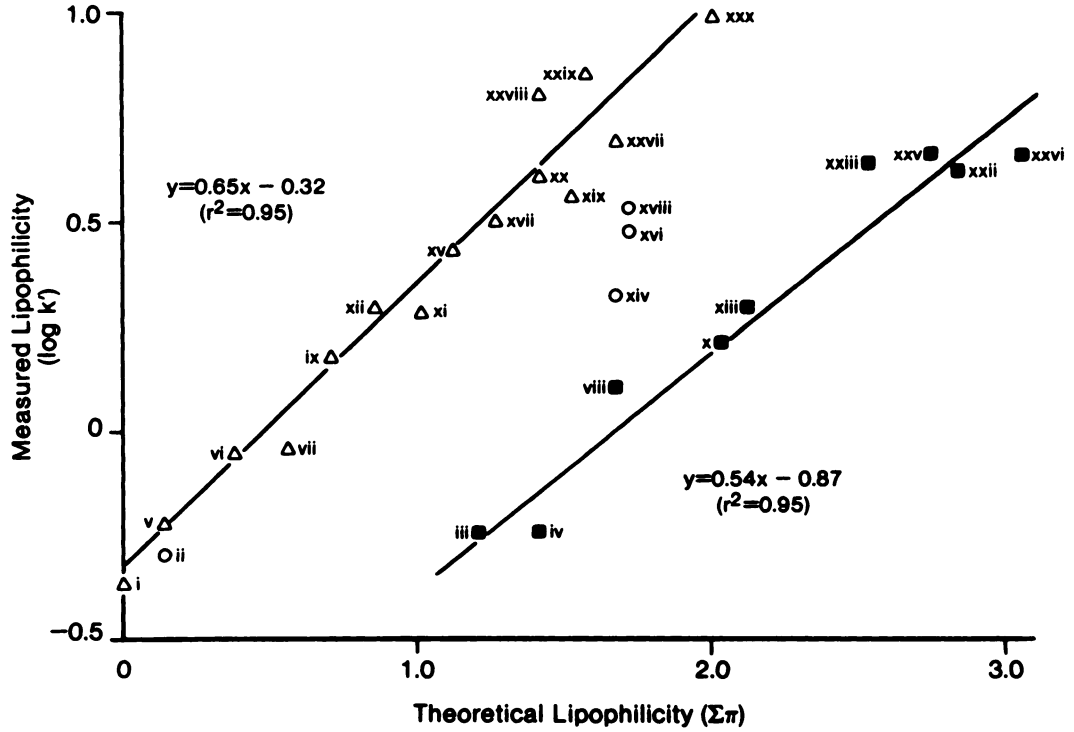


FIG. 1. Relationship between the measured lipophilicity and theoretical lipophilicity of ligands tested. Numbers correspond to ligand numbers in Table 1. (Δ) = no ortho substituents, O = one ortho substituent, (■) = two ortho substituents.

Fig. 2 shows a plot of the renal extraction of the complexes (where available) against the measured lipophilicity for the corresponding ligand. A straight line can be drawn through the data encompassing the two main groups of ligands. Mono ortho substituted ligands fall off this line.

T_{max} and T_{50} values for a selected group of ligands are listed in Table 1. Where standard deviations are given, five or more repeat studies were performed in the same or different animals.

The results of isolated hepatocyte studies using Tc-99m diethyl HIDA and Tc-99m mebrofenin are shown

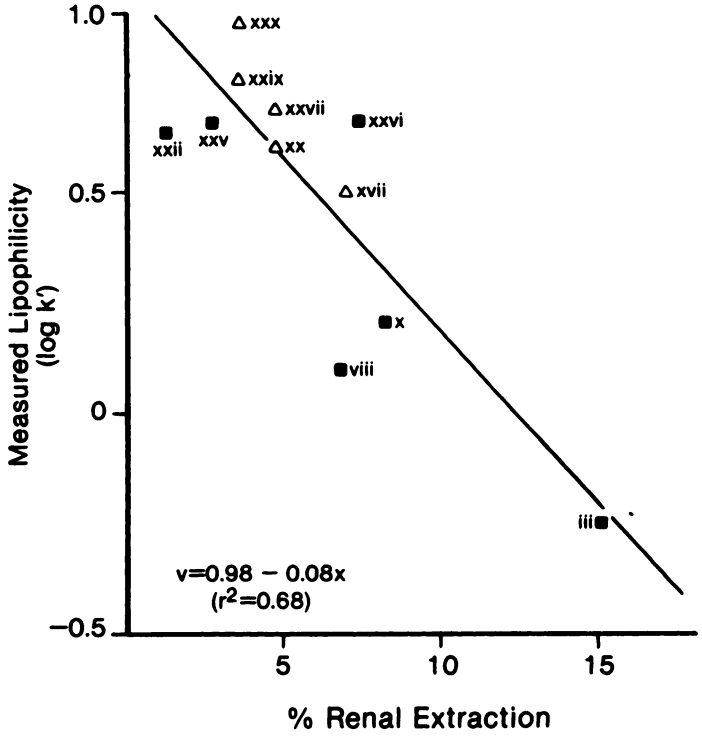


FIG. 2. Correlation between measured lipophilicity of ligand and renal extraction at 30 min of its Tc-99m complex in rat. Symbols as in Fig. 1.

TABLE 2. EFFECT OF BSP AND BILIRUBIN ON UPTAKE OF Tc-99m-MEBROFENIN AND Tc-99m-ETIFENIN INTO ISOLATED RAT HEPATOCYTES

	% Uptake into isolated hepatocytes*	
BSP (m*M*)	Diethyl HIDA (Etifenin)	Mebrofenin
—	42.5	39.0
0.25	23.3	42.9
0.50	5.9	26.3
0.75	2.4	14.1
1.00	1.2	7.8
1.50	0.9	3.5
Bilirubin (mg%)	—	—
0	46.2	41.1
2.5	33.3	42.3
5.0	19.6	31.9
10.0	15.6	29.1
20.0	14.4	30.2

* At 30 min in the presence of 1% BSA.

in Table 2. The slight increase in uptake of Tc-99m mebrofenin on the first addition of competitor is thought to be due to displacement of bound complex from the albumin. This increase is not observed with the Tc-99m diethyl HIDA because it is less strongly protein-bound.

DISCUSSION

Previous attempts at optimization of the properties of HIDAs depended heavily on the molecular weight theories of hepatobiliary excretion (*13*). These held that a threshold molecular weight was necessary for efficient hepatobiliary clearance. An increase in the molecular weight above the threshold level should produce greater clearance up to a maximum level above which clearance decreases. In the field of cholescintigraphy, the rate of hepatic excretion is as important as the degree of extraction (*14*). A slow transit time is particularly detrimental, since it reduces the bile-to-liver radioactivity ratio and thereby degrades image quality. Although an increase in lipophilicity has been used to decrease renal extraction it is now quite clear that excessive lipophilicity achieves low renal extraction only at the expense of slow hepatocellular transit times (*2,3*). Previously constructed structure-distribution relationships (SDRs) have been helpful in determining the general activity of various parts of the molecule, and have demonstrated, in particular, that the type of substitution on the phenyl ring is the major determinant of in vivo distribution (*15*). Thus, evidence suggests that steric effects are more important than molecular weight, once this threshold has been reached (*16*). More precise SDRs could not be constructed without the acquisition of more data. The inherent variability of results arising from the use of animals models and the length of time required to obtain data make the use of more sophisticated chemical models attractive.

It is accepted that the main determinant of nonspecific binding to proteins is a compound's lipophilicity (*17*). The results reported here using affinity chromatography show that para substituents cause protein binding that increases with the theoretical lipophilicity of the complex. This is not the case for ortho-substituted HIDAs. Not only do they show no correlation between their theoretical lipophilicity and their protein binding, but they also bind less to albumin than para-substituted HIDAs of lower theoretical lipophilicity. For example, the o-diethyl HIDA (X, $\Sigma\pi = 2.04$), should be more protein bound than the p-ethyl IDA (XI, $\Sigma\pi = 1.02$). The opposite was observed, which could be attributed to either steric effects operating at the albumin binding site of the HIDAs or by a reduction in the actual lipophilicity from that predicted by theory due to self-shielding of the molecule (*18*).

The lipophilicity of a compound is commonly described by its organic/aqueous partition coefficient, determined using shake-flask methods (*19*). These methods have a number of drawbacks, so we chose to use the alternative method of determining a compound's lipophilicity by measuring its retention time on a reverse-phase HPLC system. In this method, the organic phase is irreversibly bound to an insoluble support and the aqueous phase is passed over it. The retention time of a compound is dependent upon its partitioning between the mobile aqueous phase and stationary organic phase. The relative figures obtained for a series of compounds are pertinent only to the system used, but they can be directly related to octanol/water partition coefficients and theoretical lipophilicities (*20*).

If there are no modifying effects, a plot of the measured lipophilicity against the theoretical lipophilicity for a series of ligands should produce a straight line. The ligands we tested fall into three groups whose membership is determined by the degree of ortho substitution. The groups are similar to those previously noted in the protein-binding studies. Those ligands having a single ortho substituent lie between the two other groups (Fig. 1). (The limited number of compounds examined does not allow a statement to be made on this relationship). The two main groups are each linearly related, with similar slopes. This suggests that di-ortho substitution causes a constant drop in the measured lipophilicity of a ligand, whose magnitude is given by the difference in the intercept. In practical terms there appears to be a small negative contribution to the measured lipophilicity afforded by two ortho substituents (e.g., III, IV compared with VII, IX). This decrease is barely compensated for by the addition of a third substituent in the para-

position (e.g., VIII, XIII compared with VII, IX). Thus, the decreased protein binding of the ortho-substituted complexes can be explained by their lower measured lipophilicity. However, other effects must also operate, since complexes of ligands with similar measured lipophilicities do not exhibit the same degree of protein binding (e.g., XIX compared with XXVI).

Having obtained a relationship between lipophilicity and protein binding, we performed in vivo testing to determine whether lipophilicity could be used to predict the degree of renal extraction. The results, presented graphically in Fig. 2 demonstrate that there is indeed a single linear relationship between the measured lipophilicity and renal extraction for the two main groups. As the lipophilicity increases, the renal extraction decreases and the hepatic extraction increases (Table 1). Thus, contrary to the opinion of Molter and Kloss (*3*), lipophilicity and protein binding can be used to predict the in vivo distribution of Tc-HIDAs. The fact that the complexes with "mono ortho" ligands fall off the line passing through the two main groups in Fig. 2 suggests that additional factors may play a role in the in vivo distribution of this group of complexes.

The hepatocellular transit time could not be predicted from either the ligand's lipophilicity or the protein binding of the complex. However, previously published data (*3,21*) suggested that if the required lipophilicity is concentrated in a single substituent in the molecule, the complex's renal extraction will be low but its hepatocellular transit time will be excessively slow. We therefore chose to achieve the lipophilicity required for low renal extraction and high hepatobiliary specificity by using ligands containing both alkyl and halo substituents distributed throughout the phenyl ring. Our initial experiments were designed to select the best derivatives from a series of disubstituted HIDAs, substituted in the 3, 4, or 5 positions with a halogen or methyl group. It was evident that the dihalo HIDAs (XXVIII, XXXI) had in vivo characteristics inferior to the halomethyl HIDAs (XVII, XXI, XXVII). Furthermore the chloro-(XVII) or iodomethyl HIDA (XXVII) was inferior to those of the bromomethyl HIDA (XX). Although this bromomethyl HIDA (XXI) had excellent specificity, we considered its hepatobiliary transit time to be slow. This was thought to be due to the lack of substituents in the ortho positions. A similar steric effect of ortho substituents has been suggested as influencing the hepatobiliary transit times of Tc-labeled pyridoxylidenephenylalanine derivatives (*22*). Data published by Chiotellis, et al. (2,21) support the idea that ortho substituents influence the rate of hepatobiliary transit of Tc-HIDAs. Our previously documented effects of ortho substituents on the lipophilicity and protein binding of the Tc-HIDAs suggested that ortho substituents should be added to the HIDAs solely for their postulated kinetic effects and not as an additional means of obtaining the desired lipophilicity. We therefore chose to synthesize and test new HIDAs containing small alkyl derivatives in the ortho position together with the best substituents in the 3 and 4 positions. The results confirmed the beneficial effects on specificity of halogens in the meta position (XXIII compared with VIII) and also suggested that the ortho alkyl group should be restricted to methyl groups, (XXIII, XXIV compared with XXV, XXXIII and XXVI). After investigating the effect of various arrangements of the methyl and bromo groups in the meta and para positions (XX compared with XXI and XXIII compared with XXIV), Tc-mebrofenin (XXIII) was found to have the best overall in vivo characteristics in rats and rabbits.

Our rabbit studies show that the di-isopropyl derivative (XXVI) has a slower transit time (t_{50}) than the p-butyl derivative (XXX), which is in agreement with the data of Jansholt et al. (*23*) and Subramanian et al. (*1*). On the other hand, Molter et al. (*3*) report rabbit data which agree with data from a crossover study in humans (*24*) where the p-butyl derivative has a larger t_{50} than that of the di-isopropyl derivative. (They also report that the di-isopropyl derivative has a smaller t_{50} than the diethyl derivative (X) (*3*)). Although some difficulties have been reported in preparing radiochemically pure Tc-99m di-isopropyl HIDA (*8,9*) the discrepancy might also arise from a fault in the animal model, which is largest for the di-isopropyl/p-butyl pair. However, subsequent crossover studies in man (*25*) with the compound predicted by these data to have the best in vivo characteristics (XXIII) show that the discrepancies in the animal compared with human data do not affect the ability of these data to predict an efficacious agent for human use.

The remaining parameter deemed necessary for a good hepatobiliary agent was that it should exhibit a high degree of resistance to the competitive effects of bilirubinaemia (*26*). This characteristic was tested in isolated hepatocytes, since animal models of bilirubinaemia are unreliable (*11*). The data in Table 2 show that Tc-mebrofenin has a high degree of resistance to bilirubin and BSP challenge and should be efficacious in the jaundiced patient.

CONCLUSION

We have shown that lipophilicity can be used to predict the protein binding and in vivo distribution of Tc-HIDAs. Substituents in the ortho position contribute less to the measured lipophilicity than theory predicts. This leads to less protein binding and hence higher renal extraction. HIDAs with small alkyl substituents in the ortho position have faster hepatocellular transit times than their unsubstituted counterparts.

We have been able to develop a new compound that has excellent properties as a cholescintigraphic agent. This agent, Tc-mebrofenin, has low renal extraction,

high hepatobiliary specificity and rapid hepatocellular transit time. It is now undergoing clinical trials in man and showing similar characteristics to those obtained using animal models (*25*).

Mebrofenin disproves the previously held opinion exemplified by the data of Molter et al (*3*) and stated by Kato-Azuma (*27*) that for the HIDAs low renal extraction and rapid hepatocellular transit are mutually exclusive.

FOOTNOTES

* Alltech Associates.
† Schoeffel SF 770.
‡ Altex 420.
§ Gelman.

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