

Effect of Protein Binding on Renal Extraction of ^{131}I -OIH and $^{99\text{m}}\text{Tc}$ -Labeled Tubular Agents

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The clearance of $^{99\text{m}}\text{Tc}$ -mercaptoacetyltriglycine (MAG3) is less than the clearances of o - ^{131}I -iodohippurate (OIH) and $^{99\text{m}}\text{Tc}$ -labeled DD- and LL-ethylenedicycysteine (EC). This difference could be associated with the lower affinity of MAG3 for the tubular transport receptor, but MAG3 is more highly protein bound than OIH and the EC isomers; protein binding could also be an important factor governing tubular extraction. To separate the effects of protein binding from tubular receptor affinity, the extraction fractions (EFs) of MAG3, OIH, and the DD, LL, and DL isomers of $^{99\text{m}}\text{Tc}$ -EC were measured in an isolated perfused rat kidney model using a protein-free perfusate and perfusates containing bovine serum albumin. **Methods:** The right kidney was removed from the rat and perfused with modified Krebs-Henseleit buffers containing 7.5 or 2.5 g/dL bovine serum albumin or a protein-free perfusate. OIH was coinjected into the renal artery with each of the $^{99\text{m}}\text{Tc}$ -tracers. Protein binding was measured in each of the perfusates, and the venous outflow was collected to determine the EF. **Results:** The protein binding of MAG3 in the albumin perfusates ranged from 87% to 95%, significantly higher than the 20%–34% range of protein binding observed with the three EC complexes ($P < 0.05$). In the 2.5 g/dL albumin perfusate, the EF of MAG3 was 44%, significantly less than the 57%–77% EF of the three EC complexes; in the 7.5 g/dL perfusate, the MAG3 EF fell to 18% versus 39%–45% for the EC complexes ($P < 0.05$). However, in the protein-free perfusate, the EF of MAG3 was 64%, equal to or higher than the 46%–62% EF of the three EC complexes. **Conclusion:** Protein binding modulates the tubular extraction of renal tracers. Protein binding and receptor affinity must be considered in the design of future renal radiopharmaceuticals as well as radiopharmaceuticals targeting other receptors.

Key Words: $^{99\text{m}}\text{Tc}$ -MAG3; protein binding; extraction fraction; kidney

J Nucl Med 2000; 41:2077–2082

Two renal tubular agents that are actively transported across the membrane of proximal tubular cells are $^{99\text{m}}\text{Tc}$ -mercaptoacetyltriglycine (MAG3) and o - ^{131}I -iodohippurate (OIH); however, the clearance of $^{99\text{m}}\text{Tc}$ -MAG3 is only 50%–60% of the OIH clearance (1). A potential alternative to $^{99\text{m}}\text{Tc}$ -MAG3 is L,L- $^{99\text{m}}\text{Tc}$ -ethylenedicycysteine (LL-EC),

which has been reported to have a slightly better clearance than $^{99\text{m}}\text{Tc}$ -MAG3 in healthy volunteers, averaging 70% of OIH (2–5). However, several stereochemical forms of the $^{99\text{m}}\text{Tc}$ -EC complex exist because of the two chiral carbons in the ligand (6). Furthermore, recent studies have shown that the clearance of DD- $^{99\text{m}}\text{Tc}$ -EC in humans may be even higher than that of LL- $^{99\text{m}}\text{Tc}$ -EC, averaging ~80% of OIH (7). The differences in the clearances of these tubular function agents could be associated with differences in the affinity for the tubular transport protein, differences in plasma protein binding, or a combination of both factors. Because development of improved tubular transport agents requires an understanding of the factors associated with efficient tubular transport, an isolated perfused rat kidney model was used to distinguish the effects of protein binding from those of transport affinity.

MATERIALS AND METHODS

Radiopharmaceutical Preparation

$^{99\text{m}}\text{Tc}$ -MAG3 was prepared by heating an aqueous solution of the MAG3 ligand at 100°C in the presence of $^{99\text{m}}\text{Tc}$ -pertechnetate and SnCl_2 (8). The DD, LL, and DL ligands were synthesized and radiolabeled according to procedures reported in the literature (7,9,10). All $^{99\text{m}}\text{Tc}$ -labeled complexes were purified by high-performance liquid chromatography before use. ^{131}I -OIH was obtained commercially (Mallinckrodt, Inc., St. Louis, MO).

Preparation of Modified Krebs-Henseleit Buffer Containing Bovine Serum Albumin, Fraction V

A modified Krebs-Henseleit buffer was prepared as the perfusion medium (11). One liter of this stock buffer contained the following: 118.4 mmol/L NaCl, 1.2 mmol/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 24.8 mmol/L NaHCO_3 , 10 mmol/L dextrose, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4 , and 2.5 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Bovine serum albumin, fraction V (Sigma; St. Louis, MO), at a concentration of 10 g/dL, was added to the stock buffer and dialyzed through a Spectra/Por 1 dialysis membrane (Spectrum Medical Industries, Inc., VWR Scientific Products, Suwanee, GA) against an 11-fold excess of the stock buffer with frequent changes over 48 h (12,13). Aliquots of the dialyzed buffer were stored at -20°C until the day of the experiment. The dialyzed buffer was then diluted with the modified Krebs-Henseleit buffer to a final concentration of either 2.5 or 7.5 g/dL bovine albumin. The diluted buffer was filtered through a 0.45- μm Gelman filter (Gelman Science, Ann Arbor, MI), and the pH was adjusted to 7.0.

Received Dec. 1, 1999; revision accepted May 5, 2000.

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Preparation of Modified Krebs-Henseleit Buffer Containing G-70 Dextran

The modified Krebs-Henseleit buffer was identical to that reported above; however, G-70 dextran (Sigma; average molecular weight, 77,000) was dissolved in the stock buffer, producing a final concentration of 2.5 or 7.5 g/dL. The perfusate was filtered through a 0.45- μ m Gelman filter, and the pH was adjusted to 7.0 with 1N hydrochloric acid.

Isolated Perfused Kidney Apparatus

The perfusion apparatus was similar to that described by De Mello and Maack (14). The modified Krebs-Henseleit buffer was added to the perfusion reservoir and allowed to circulate throughout the closed system using two perfusion pumps. The dialyzed buffer was filtered continuously through two 8- μ m Millipore filters (Millipore, Bedford, MA) and oxygenated with 95% O₂ and 5% CO₂ for at least 30 min before the attachment of the isolated kidney; after oxygenation, the pH rose to \sim 7.4 in the perfusates. The oxygenated buffer then traveled through a flow meter that was connected to a mercury manometer for pressure measurements. The connecting tubing was made of Tygon (Nalgene; Fisher Scientific, Norcross, GA) except at the attachment site to the kidney, where an intravenous admixture set was attached to the renal artery cannula to serve as the injection site. The temperature of the perfusates and kidney were maintained at 37°C throughout the experiment.

Protein Binding Measurements

¹³¹I-OIH and each of the ^{99m}Tc-labeled agents were incubated for 60 min in 10 mL modified Krebs-Henseleit buffer containing 2.5 g/dL albumin, 7.5 g/dL albumin, and 2.5 g/dL dextran. ^{99m}Tc-MAG3 and OIH were also incubated in 7.5 g/dL dextran for 60 min. At 10 and 60 min after incubation, 1-mL samples were removed for protein binding determination using a Centrifree micropartition system (Amicon, Beverly, MA). Protein binding measurements were performed in duplicate during each perfusion experiment; there were six protein binding measurements per datum point.

Preparation of Isolated Perfused Kidney

Male Sprague-Dawley rats (Charles Rivers, Raleigh, NC) weighing 220–465 g were anesthetized with a 50:50 mixture of ketamine and xylazine, which was supplemented as needed. An abdominal incision was made and the right kidney was isolated. The right ureter was cannulated with PE-10 tubing (Fisher Scientific). Heparin (1000 U/kg of body weight) was then injected into the left femoral vein. The vena cava below the right renal vein was cannulated with PE-205 tubing (Fisher Scientific). The right renal artery was cannulated through the superior mesenteric artery as reported by Nishiitsutsuji-Uwo et al. (15) and Ross et al. (16) with a blunt 20-gauge needle. The needle was attached to a syringe containing the modified Krebs-Henseleit buffer and the buffer was flushed through the right kidney. The kidney was immediately removed from the rat, positioned in the perfusion apparatus, and perfused with the modified Krebs-Henseleit buffer at a flow rate of 11–13 mL/min for 15 min before the injection of the protein-bound OIH and ^{99m}Tc-labeled agents. The perfusion pressure averaged 81.5 \pm 17.3 mm Hg.

Extraction Fraction Measurements

Duplicate extraction fraction (EF) measurements were performed in three kidneys, making a total of six EF measurements for

each agent and buffer. To determine the EF, the radiopharmaceuticals were incubated in each test perfusate for 60 min. A 0.5-mL aliquot of the test perfusate containing \sim 1.85 MBq (50 μ Ci) ¹³¹I-OIH and 7.8 MBq (200 μ Ci) ^{99m}Tc-labeled complex was then injected through the injection port of the intravenous admixture set followed by continuous perfusion of tracer-free perfusate until \sim 40 mL of the venous output were collected. A second extraction was then performed \sim 2 min later by injecting a second aliquot of the test perfusate into the port with a second collection of 40 mL. To determine the EF of OIH and each ^{99m}Tc-labeled agent, duplicate 1-mL venous aliquots were obtained and assayed in a Beckman automated well counter (Beckman, Fullerton, CA). All samples were corrected for background, decay, and cross talk. The extraction efficiency was then determined by subtracting the percentage dose in the venous output perfusate from 100%.

Statistical Analysis

The data are expressed as the mean \pm SD. *P* values were determined using the Tukey method for multiple comparisons. Significance was defined as *P* < 0.05.

RESULTS

The results of protein binding measurements are presented in Table 1. There was no significant difference between the percentage protein binding measurements at 10 and 60 min for any of the tracers in the albumin buffers, and there was no significant tracer binding to dextran. In both the 2.5 and 7.5 g/dL albumin perfusates, the protein binding of ^{99m}Tc-MAG3 (87% and 95%, respectively) was significantly higher than the 20%–34% protein binding of DD-, DL-, and LL-^{99m}Tc-EC (*P* < 0.05) (Table 1).

The results of the EF measurements are presented in Table 2. In the isolated perfused rat kidney studies, the EF of each of the ^{99m}Tc-labeled agents was divided by the simultaneously measured OIH EF. This EF ratio was calculated to correct for any differences in the isolated rat kidney preparations and to allow a better comparison between studies. In the 2.5 g/dL albumin perfusate, the EF of ^{99m}Tc-MAG3 and the ^{99m}Tc-MAG3/OIH EF ratio were significantly less than the corresponding EFs of DD-, DL-, and LL-EC and significantly less than the EF ratios of DD-^{99m}Tc-EC/OIH, DL-^{99m}Tc-EC/OIH, and LL-^{99m}Tc-EC/OIH (*P* < 0.05) (Table 2).

TABLE 1
Protein Binding (%) of ¹³¹I-OIH, ^{99m}Tc-MAG3, and LL-, DD-, and DL-EC in Isolated Rat Kidney Perfusates Containing Albumin and Dextran

Agent	Albumin		Dextran (2.5 g/dL)
	2.5 g/dL	7.5 g/dL	
MAG3	86.8 \pm 1.0	95.1 \pm 0.1	0
OIH	51.9 \pm 2.7	80.9 \pm 0.1	0
DD-EC	25.8 \pm 1.0	30.3 \pm 0.8	1.9 \pm 0.9
OIH	63.0 \pm 2.1	76.7 \pm 0.2	1.7 \pm 1.2
DL-EC	27.8 \pm 1.0	34.2 \pm 0.8	0
OIH	61.9 \pm 1.1	74.2 \pm 0.5	0
LL-EC	20.2 \pm 3.0	30.5 \pm 1.9	0.2 \pm 0.3
OIH	55.6 \pm 1.2	69.0 \pm 0.8	0.6 \pm 0.09

TABLE 2
Isolated Rat Kidney EFs (%) of ^{131}I -OIH, $^{99\text{m}}\text{Tc}$ -MAG3, and
LL-, DD-, and DL- $^{99\text{m}}\text{Tc}$ -EC from Perfusates Containing
Albumin and Dextran

Agent	Albumin		Dextran (2.5 g/dL)
	2.5 g/dL	7.5 g/dL	
MAG3	43.9 \pm 2.8	18.2 \pm 3.1	63.6 \pm 6.2
OIH	62.0 \pm 4.7	21.4 \pm 8.7	65.0 \pm 4.2
Ratio	0.71 \pm 0.06	0.98 \pm 0.36	0.98 \pm 0.06
DD-EC	77.1 \pm 4.6	39.3 \pm 10.0	61.6 \pm 4.1
OIH	78.0 \pm 5.4	29.8 \pm 6.6	78.2 \pm 4.2
Ratio	0.99 \pm 0.07	1.31 \pm 0.11	0.79 \pm 0.05
DL-EC	59.7 \pm 8.2	41.7 \pm 13.7	52.0 \pm 10.4
OIH	67.4 \pm 7.2	28.6 \pm 12.8	73.9 \pm 6.4
Ratio	0.88 \pm 0.04	1.54 \pm 0.21	0.70 \pm 0.10
LL-EC	56.7 \pm 6.5	45.3 \pm 4.7	46.2 \pm 6.6
OIH	61.5 \pm 7.5	29.4 \pm 8.9	73.2 \pm 9.1
Ratio	0.93 \pm 0.08	1.67 \pm 0.5	0.63 \pm 0.04

Similarly, in the 7.5 g/dL albumin perfusate, the EF of $^{99\text{m}}\text{Tc}$ -MAG3 and the $^{99\text{m}}\text{Tc}$ -MAG3/OIH EF ratio were also significantly less than the corresponding values for DD- $^{99\text{m}}\text{Tc}$ -EC, DL- $^{99\text{m}}\text{Tc}$ -EC, and LL- $^{99\text{m}}\text{Tc}$ -EC ($P < 0.05$) (Table 2). However, in the nonprotein perfusate containing 2.5 g/dL dextran, the $^{99\text{m}}\text{Tc}$ -MAG3/OIH EF ratio was 0.98, significantly higher than the 0.63–0.79 EF ratios of the three $^{99\text{m}}\text{Tc}$ -EC complexes ($P < 0.05$) (Table 2). Furthermore, the absolute EF of $^{99\text{m}}\text{Tc}$ -MAG3 in the nonprotein protein perfusate was higher than the EFs of DL- $^{99\text{m}}\text{Tc}$ -EC and LL- $^{99\text{m}}\text{Tc}$ -EC ($P < 0.05$) and was equal to the EF of DD- $^{99\text{m}}\text{Tc}$ -EC.

The EF of $^{99\text{m}}\text{Tc}$ -MAG3 in the 2.5g/dL albumin perfusate was 44% (Table 2). When the albumin concentration in the perfusate was increased to 7.5g/dL, the EF of $^{99\text{m}}\text{Tc}$ -MAG3 fell to 18%. To determine if this effect could be caused by an increase in osmolality, the $^{99\text{m}}\text{Tc}$ -MAG3 and OIH EFs in 2.5 g/dL dextran were compared with an additional set of studies using 7.5 g/dL dextran. When the osmolality of the dextran perfusate was increased by a factor of 3, there was no significant difference in the $^{99\text{m}}\text{Tc}$ -MAG3 and OIH EFs, 65% versus 74% for OIH and 64% versus 65% for $^{99\text{m}}\text{Tc}$ -MAG3 in 2.5 and 7.5 g/dL dextran, respectively.

DISCUSSION

Differences in the renal EFs of the various tubular agents are often attributed to differences in the affinity for the tubular receptor. The effect of plasma protein binding has been largely disregarded. However, plasma protein binding may compete with the transport receptor for the tracer and consequently reduce the EF, particularly if the plasma protein has a high affinity for the tracer. We used the isolated perfused rat kidney to separate the effects of plasma protein binding from the radiopharmaceutical affinity for the tubular transport receptor.

The isolated perfused rat kidney requires specialized equipment and is a technically demanding model. Differ-

ences in surgical technique, such as difficulty in cannulating a ureter, cannulating a vein, or a delay in isolating the kidney and connecting it to the perfusion apparatus, could produce different degrees of renal injury and result in differences in tubular extraction. For example, the EF was measured twice in each isolated perfused kidney; for all tracers, the mean change in EF from the first to second measurement ranged from 4.6% to -17.9% with a combined mean of -5.6% . This slight decrease in EF was statistically significant and contributed the variability of the results. Another potential source of variability was pH. The pH of the perfusate was adjusted to 7.0, but the perfusate was filtered continuously and oxygenated with 95% O_2 and 5% CO_2 for at least 30 min before the attachment of the isolated kidney; after oxygenation, the pH rises to ~ 7.4 . Small differences in pH may affect protein binding and possibly affect tubular transport (24). The variability within the model is illustrated by the OIH data. OIH was used as a control in the four $^{99\text{m}}\text{Tc}$ experiments at 2.5 and 7.5 g/dL albumin (Tables 1 and 2). When the albumin concentration was increased from 2.5 to 7.5 g/dL, the protein binding of OIH increased by 12.3%–29% and was associated with a decrease in EF of 32.1%–48.2%. In theory, there should not have been a spread in the data because the coinfused tracer quantities of the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals would not be expected to affect the protein binding of OIH or the tubular transport. The important point is that, in every experiment, an increase in protein binding was associated with a decrease in EF of OIH and of each $^{99\text{m}}\text{Tc}$ tracer.

For each perfusate, any changes in experimental conditions that would affect the EFs of the $^{99\text{m}}\text{Tc}$ -labeled complexes and OIH were assumed to affect the EFs proportionally. Consequently, the ratios of the EF of each $^{99\text{m}}\text{Tc}$ -labeled complex to the OIH EF were calculated to normalize for any differences that might have occurred in the experimental model. The ratios provide an additional method of comparing the EFs of the $^{99\text{m}}\text{Tc}$ -labeled complexes within a single perfusate. This normalization cannot be used to compare results at different albumin concentrations because the protein binding of the $^{99\text{m}}\text{Tc}$ -labeled complexes and OIH changes as the albumin concentrations change, and these changes are not proportional (Table 1).

Another potential source of variability was the use of 2.5 g/dL dextran. The osmolality of 2.5 g/dL dextran is not the same as that of 2.5 or 7.5 g/dL albumin, and there may have been some movement of water into the tubular cells that could have affected tubular cell function. This was probably not a major factor in our short-term experiments because the $^{99\text{m}}\text{Tc}$ -MAG3 and OIH EFs in 2.5 and 7.5 g/dL dextran were essentially the same.

All three $^{99\text{m}}\text{Tc}$ -EC isomers had higher EC/OIH EF ratios (0.88–0.99) than that of $^{99\text{m}}\text{Tc}$ -MAG3 (0.71) at an albumin concentration of 2.5 g/dL; these results could be interpreted as showing that the $^{99\text{m}}\text{Tc}$ -EC complexes have a higher affinity for the tubular transport receptor (Table 2). When the albumin concentration was increased to 7.5 g/dL, the EF

ratios for the three ^{99m}Tc -EC isomers (1.31–1.67) were still higher than the 0.98 EF ratio for ^{99m}Tc -MAG3, which supports this interpretation. However, when protein was removed and the kidneys were perfused with 2.5 g/dL dextran, the EF of ^{99m}Tc -MAG3 was 0.98, higher than EF ratios of all three ^{99m}Tc -EC complexes (0.63–0.79), indicating that the binding of ^{99m}Tc -MAG3 to albumin preferentially reduced the availability of ^{99m}Tc -MAG3 for tubular transport.

Sudlow et al. (17,18) have shown that acidic drugs bind predominantly to albumin and have proposed the existence of two distinct binding sites for anionic drugs on human serum albumin. Continuing these observations, Noctor et al. (19) have reported that site I binding seems to be nonstereoselective, whereas site II binding tends to be highly stereoselective. However, they found it difficult to identify the precise structural features that disposed an isolate to bind to a particular site. Because ^{99m}Tc -MAG3 (and the EC complexes) is anionic at physiologic pH, Kawai et al. (20) conducted competitive displacement studies to determine the ^{99m}Tc -MAG3 binding site on human and rat albumin. MAG3 was displaced in vitro by bucolome, a type I site inhibitor. Some albumin binding sites mirror biologically active receptors in terms of selectivity and specificity, and the binding of small ligands to albumin may induce small conformational changes of the environment of the binding sites (21). Furthermore, binding of the ligand may change the conformation of its own binding site and alter the binding affinity (21).

Tubular secretion of a drug has not been thought to be influenced by protein binding if binding is reversible (22). However, using the isolated perfused rat kidney, Bowman (23) has shown that secretion of furosemide is related to the albumin concentration and, hence, the concentration of the unbound drug. As the perfusate albumin concentration was raised from 2.5 to 7.5 g/dL, the unbound fraction of furosemide concentration decreased from 9.4% to 2.2% and the secretory rate fell by $\sim 80\%$. The decrease in the secretory rate seems disproportionally large compared with the small change in protein binding; yet, comparable results were obtained in this study. As the perfusate concentration of albumin was increased from 2.5 to 7.5 mg/dL, the unbound portion of ^{99m}Tc -MAG3 decreased from 13.2% to 4.9% and the EF fell from 43.9% to 18.2%. In support of this concept, Kawai et al. (20) have reported that the excretion of ^{99m}Tc -MAG3 could be accelerated by administering bucolome to increase the unbound fraction of ^{99m}Tc -MAG3 in the plasma.

The unbound portion of ^{99m}Tc -MAG3 as well as the unbound portion of OIH and the ^{99m}Tc -EC complexes are available for glomerular filtration. Glomerular filtration contributes to the overall EF; however, the magnitude of the decrease in the EF far exceeds any effect contributed by glomerular filtration, and our data represent an effect on tubular extraction. The filtration fraction in humans is $\sim 20\%$, whereas the filtration fraction in Sprague-Dawley

rats is 30% (24). Thus, in vivo, glomerular filtration can contribute substantially to the total renal excretion (filtration fraction \times the unbound fraction). If a tracer were 70% unbound, 21% of the unbound drug in the plasma would be cleared by glomerular filtration with each pass through the kidney of a Sprague-Dawley rat (70% unbound \times 30% EF). The glomerular filtration rate can be reduced in the isolated perfused rat kidney model because of ureteral back pressure, but this effect is low at albumin concentrations >4 g/100 mL (12). Furthermore, and most important, the filtration fraction in the isolated perfused rat kidney is $\sim 3\%$; consequently, glomerular filtration could have little effect on the EFs at different albumin concentrations (12).

It is an accepted pharmacologic principle that only the nonprotein bound or free fraction of a drug in blood is available for distribution to tissue (25–27). Albumin accounts for much of the drug binding observed in plasma, and as a drug carrier the binding capacity of albumin is generally considered to be nonsaturable (26–29). Because a drug bound to albumin is practically nondiffusible (30), strong albumin binding of a radiopharmaceutical will directly impair its rate of renal extraction. The ultrafiltration technique used in these studies separates the aqueous phase and the unbound drug from the protein and protein-bound drug at the ultrafiltration membrane (31), providing results that reflect the magnitude of the equilibrium constant, K .

Mathias et al. (32) have reported that the albumin binding of ^{67}Cu -labeled pyruvaldehyde bis(N^4 -methylthiosemicarbazonato)-copper (II) adversely affects the myocardial extraction of the tracer at high flow rates. The off rate, k_2 , is reported to be too slow and becomes the rate-limiting step for tissue extraction of the tracer. It is clear from our perfused rat kidney studies that the off rate of ^{99m}Tc -MAG3 and OIH is fast because the percentage extracted exceeds the initial percentage of unbound drug. For example, in 2.5 g/dL albumin, only 14% of the ^{99m}Tc -MAG3 is not bound to protein but 44% of the dose is extracted. Consequently, even in this unfavorable case of highly protein-bound ^{99m}Tc -MAG3, the off rate is reasonably fast compared with the time of passage through the tubules. This result indicates a high extraction rate constant for ^{99m}Tc -MAG3 that is confirmed by the 64% EF in the absence of protein. In comparison with ^{99m}Tc -MAG3, only 20%–30% of the dose of the ^{99m}Tc -EC complexes is protein bound in 2.5 g/dL albumin, so the percentage unbound is $\sim 70\%$ – 80% . Because dissociation is very fast compared with the time of passage through the tubules, the percentage extracted of the LL- and DL- ^{99m}Tc -EC complexes was essentially unaffected by protein binding.

Interpretation of the DD- ^{99m}Tc -EC data is more problematic. There was a minimum increase in protein binding of DD- ^{99m}Tc -EC from 26% in 2.5 g/dL albumin to 30% in 7.5 g/dL albumin. The increase in protein binding was less than would be expected from theoretic considerations when the albumin concentration was increased from 2.5 to 7.5 g/dL, and the small change observed in protein binding cannot

explain why the EF fell from 77% to 39%. This result is unlikely to reflect a problem with the isolated perfused rat kidney in the DD- ^{99m}Tc -EC set of experiments because the EF of the internal control, OIH, was comparable in all four sets of experiments at each protein concentration. The protein binding results were very consistent within each set of experiments; however, there were minor differences in the protein binding of OIH in the different studies. For example, the protein binding of OIH in 2.5 g/dL albumin was $51.9\% \pm 2.7\%$ in the ^{99m}Tc -MAG3 set of experiments compared with $63\% \pm 2.1\%$ in the DD- ^{99m}Tc -EC studies. A new perfusate was prepared for each set of experiments and there may have been minor differences between perfusates. Each stock albumin solution was dialyzed against an 11-fold excess of stock buffer with frequent changes over 48 h. Furthermore, protein binding was measured after incubation in each perfusate for 30 min at pH 7.0; however, the tracers were injected into the oxygenated perfusate, which has a pH of ~ 7.4 . Variations in pH can affect protein binding (24).

The rate of tubular extraction depends on the product of the free or unbound concentration of the drug (C_u) \times the rate constant for tubular extraction (k_{ext}) integrated over the time of passage past the tubules. C_u is the concentration at any given time of the unbound drug (i.e., drug not bound to protein) in the plasma as it passes the tubules. High protein binding makes C_u small. In an equilibrating system such as the extraction of a tracer by the renal tubules, the value of C_u also depends on the off rate from the protein. For the product (C_u)(k_{ext}) or the rate of extraction to be high, both C_u and k_{ext} need to be high.

These considerations suggest two approaches for developing a drug with an EF approaching 100%. The first consists of the traditional approach of developing tracers with a greater affinity for the tubular transport receptors; however, the extraction efficiency for ^{99m}Tc -MAG3, OIH, and the ^{99m}Tc -EC complexes is already high. Given the 50%–60% EFs of the better technetium agents in the absence of protein (Table 2), increasing k_{ext} cannot increase EFs by a factor of >2 . A potentially greater effect on the rate of extraction may be derived from the second approach, which is to develop tracers with a low protein binding affinity. C_u and, hence, the extraction rate can be increased by developing a drug with an unbound fraction that is significantly higher than the 10%–15% unbound fraction associated with ^{99m}Tc -MAG3 (33,34). This is the case with DD- and LL- ^{99m}Tc -EC, both of which have a higher clearance in humans than ^{99m}Tc -MAG3.

C_u depends on both the binding constant and the off rate. Whereas the value of an equilibrium binding constant does not necessarily give an indication of the magnitude of a component's rate constants, for closely related equilibria, including those involving protein drug adducts with structurally related drugs, it is well accepted that rates and equilibria are related. However, only at equilibrium does $K = k_1/k_2$; under nonequilibrium conditions, the forward rate, ($k_1[\text{tracer}][\text{albumin}]$), and the backward rate, $k_2[\text{tracer-albumin complex}]$, depend on the concentration of the

reacting species. Our data indeed show that the off rate from albumin is appreciable. As the drug is extracted while passing through the tubules, more protein-bound drug dissociates. This sequence of events continues as the solution passes through the perfused kidney, but C_u never exceeds its equilibrium value. Where the binding is very weak, the off rate is irrelevant. Thus, an improved renal tracer requires a species with high k_{ext} , but an optimized renal tubular tracer is likely to have a lower protein binding than MAG3 and, hence, a higher unbound concentration in the plasma.

CONCLUSION

Protein binding modulates the tubular extraction of renal tracers. Complexes with the highest affinity for the tubular transport receptors may be inferior renal agents once protein binding is considered. Both protein binding and receptor affinity must be considered in the design of future renal radiopharmaceuticals as well as radiopharmaceuticals targeting other receptors.

ACKNOWLEDGMENTS

The authors thank William Mitch, MD, for the loan of the isolated kidney perfusion apparatus and for his generous assistance in setting up this model. This work was supported by National Institutes of Health grant DK38842-07.

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J Nucl Med. 2000;41:2077-2082.

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The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

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