99mTc(CO)₃-Nitrilotriacetic Acid: A New Renal Radiopharmaceutical Showing Pharmacokinetic Properties in Rats Comparable to Those of ¹³¹I-OIH

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To develop a 99mTc renal tracer with a capacity to measure effective renal plasma flow comparable to that of the clinical gold standard ¹³¹I-o-iodohippurate (¹³¹I-OIH) and superior to that of 99mTc-mercaptoacetyltriglycine, which has a clearance only 50%–60% that of ¹³¹I-OIH, we investigated 99mTc-tricarbonyl nitrilotriacetic acid (Na₂[99mTc(CO)₃(NTA)]). This radiopharmaceutical, which is based on an aminopolycarboxylate ligand, is formed as a single species and has a dangling carboxylate group favoring tubular transport. Methods: Na₂[99mTc(CO)₃(NTA)] was prepared by using commercially available NTA and an IsoLink kit and isolated by high-performance liquid chromatography. The stability of Na₂[99mTc(CO)₃(NTA)] in isotonic saline was assessed for 24 h and was further evaluated by incubation in 0.1 M cysteine and histidine for 4 h at 37°C. The biodistribution of Na₂[99mTc(CO)₃(NTA)], coinjected with ¹³¹I-OIH as an internal control, was evaluated in 5 normal Sprague-Dawley rats at 10 min, 5 normal Sprague-Dawley rats at 60 min (group A) and 6 rats with renal pedicle ligation at 60 min (group B) after injection. Clearance and extraction fraction studies were conducted in 2 normal Sprague-Dawley rats, and urine and plasma from 2 additional normal rats each were analyzed for metabolites by high-performance liquid chromatography. Results: The radiochemical purity of Na₂[99mTc(CO)₃(NTA)] was greater than 99%, the complex was stable for 24 h at physiologic pH, and the challenge experiments showed no degradation. In normal rats, the percentage dose in the urine at 10 and 60 min was 108% ± 5% and 101% ± 5%, respectively, that of ¹³¹I-OIH; minimal hepatic or gastrointestinal activity was demonstrated. In group B rats, Na₂[99mTc(CO)₃(NTA)] was better retained in the blood and had less excretion into the bowel than did ¹³¹I-OIH (P < 0.01). The plasma clearances of Na₂[99mTc(CO)₃(NTA)] and ¹³¹I-OIH were comparable, but the extraction fraction of Na₂[99mTc(CO)₃(NTA)] was 93.5% ± 3.8%, compared with 67.9% ± 6.1% for ¹³¹I-OIH. Plasma protein binding of Na₂[99mTc(CO)₃(NTA)] averaged 67% ± 7%, and red cell uptake was 7% ± 2%. Conclusion: Na₂[99mTc(CO)₃(NTA)] is stable, exists as a single species, and has pharmacodynamic properties in rats comparable to those of ¹³¹I-OIH.

Key Words: ⁹⁹mTc-tricarbonyl; renal radiopharmaceuticals; ⁹⁹mTc(CO)₃(NTA); ¹³¹I-ortho-iodohippurate (¹³¹I-OIH); ⁹⁹mTc-mercaptoacetyltriglycine (⁹⁹mTcO-MAG3)

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Our goal has been the development of a ⁹⁹mTc renal tracer with a capacity to measure effective renal plasma flow (ERPF) comparable to that of the gold standard, p-aminohippurate. ¹³¹I-o-iodohippurate (¹³¹I-OIH) has biologic properties similar to p-aminohippurate and has been used as an imaging agent and as a tracer to measure ERPF. However, ¹³¹I-OIH has suboptimal imaging characteristics because of its 363-keV photon, delivers relatively high radiation doses to the kidneys and thyroid in patients with impaired renal function as a result of the β-emission of ¹³¹I (I), and is no longer clinically available in the United States.

To accomplish our goal, we initially explored renal agents with the [⁹⁹mTc(V)O]⁺³ core, such as ⁹⁹mTc-mercaptoacetyltriglycine (⁹⁹mTcO-MAG3) (2–5), ⁹⁹mTcO-ethylenedicysteine (⁹⁹mTcO-EC) (6), and ⁹⁹mTcO-mercaptoacetamide-ethylenecysteine (⁹⁹mTcO-MAEC) (7,8). All of those agents produced excellent scintigraphic images, but their plasma clearances were still significantly less than that of ¹³¹I-OIH. In a continuing effort to develop renal imaging agents with higher clearances, we shifted our focus to the fac-[⁹⁹mTc(I)(CO)₃]⁺ core: This core provides a straightforward reaction with a multitude of ligands with a variety of ligating groups (9); the small size of this core relative to ⁹⁹mTcO complexes such as ⁹⁹mTcO-MAG3 might facilitate more efficient tubular transport; the high chemical and kinetic stability resulting from the ⁹⁹mTc-tricarbonyl core provides ideal parameters for the efficient labeling of low-molecular-weight biomolecules; an IsoLink kit (Covidien) is available for the convenient preparation of the water- and air-stable [⁹⁹mTc(I)(CO)₃(H₂O)₃]⁺ precursor (10,11); and the first renal radiopharmaceutical with a ⁹⁹mTc-tricarbonyl core...
evaluated in humans, Na$_2$[99mTc(CO)$_3$(lanthionine), proved to be an excellent renal imaging agent (12), although its plasma clearance and the rate of renal excretion were still lower than those of 131I-OIH.

We chose the aminopolycarboxylate ligand nitrilotri-acetic acid (NTA) for further investigation because it has a suitable chelating moiety, with the amine and carboxyl donor groups allowing tridentate coordination to the 99mTc(CO)$_3$ core to form a stable, single product with a dangling carboxyl group that is highly hydrophilic, favoring tubular transport rather than hepatobiliary excretion. Finally, we expected that Na$_2$[99mTc(CO)$_3$(NTA)] would be dianionic at physiologic pH, with one negative charge associated with the metal inner coordination sphere and the second negative charge associated with the dangling CO$_2^-$: This charge distribution is shared by 99mTcO-MAG3 and is associated with a rapid plasma clearance, efficient tubular extraction, and a rapid rate of renal excretion.

**MATERIALS AND METHODS**

**General**

Nitrilotriacetic acid, as a trisodium salt monohydrate (NTA), was purchased from Aldrich. [Re(CO)$_3$(H$_2$O)$_3$]trifluoromethanesulfonate (or triflate) ([Re(CO)$_3$(H$_2$O)$_3$]OTf) was prepared as previously reported (13) and was stored and used as a 0.1 M stock aqueous solution. 1H nuclear magnetic resonance (NMR) spectra were recorded on a 600-MHz spectrometer (Varian) in D$_2$O. Electrospray mass spectrometry (MS) was performed on a Finnigan LTQ-FIT instrument (Thermo Electron). 99mTc-pertechnetate ([99mTcO$_4^-$]) was eluted from a 99Mo/99mTc generator (Amersham Health), with 0.9% saline. IsoLink vials were obtained as a gift from Covidien. 99mTc(CO)$_3$(H$_2$O)$_3$ was prepared as previously reported (13) and was stored and used as a 0.1 M stock aqueous solution. 1H NMR [99mTc(CO)$_3$(H$_2$O)$_3$] OTf (3 mL); the pH was adjusted to 7 with 1 M sodium hydroxide, and the reaction mixture was stirred at room temperature for 2 h. The volume of the reaction mixture was reduced under vacuum, and the white residue was dried to yield Na$_2$[99mTc(CO)$_3$(NTA)]. The product fractions were collected, the solvent was removed from a column (Sephadex G-15; Aldrich) eluted with deionized water.

**Radiosynthesis of Na$_2$[99mTc(CO)$_3$(NTA)] and 131I-OIH**

The NTA ligand was labeled as previously described (15). Briefly, 0.5 mL of a freshly prepared solution of the [99mTc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor (pH $\sim$ 7–8) was added to a vial containing approximately 1.0 mg of the NTA ligand in 0.2 mL of water. The pH of the solution was adjusted to approximately 7 with 1 M sodium hydroxide, heated at 70°C for 15 min, and cooled to room temperature. Na$_2$[99mTc(CO)$_3$(NTA)] was separated from unlabeled ligand by HPLC; the radiochemical purity was greater than 99%. Methanol was partially removed by nitrogen gas, and the aqueous solution of Na$_2$[99mTc(CO)$_3$(NTA)] was buffered in a physiologic phosphate buffer at pH 7.4.

131I-OIH was prepared by the isotope exchange reaction between nonradioactive hippuran (OIH) and radioactive sodium iodide (Na$_2$[131I]) according to the method reported by Anghileri (16) and modified as follows for simplicity and to improve yield and purity. Cold OIH (10–20 mg) and ammonium sulfate (5–10 mg) were placed in a sterile vial, which was closed with a rubber stopper and sealed with aluminum. Na$_2$[131I] (1 mL of a 185–370 MBq/mL solution) was transferred to the vial, and a syringe filled with activated carbon was connected to the vial via a needle placed through the rubber stopper. The solution was heated at 140°C for 30 min. During the heating, all the solvent evaporated, leaving solid residue at the bottom of the vial. Sterile water (1 mL) was added to the dry residue, and the process was repeated. After the residue had been cooled to room temperature, the solid was dissolved in 5 mL of saline. The solution was then transferred to a vial containing microporous carbon chips, which had been impregnated with freshly precipitated silver chloride (17), and the vial was shaken for 10 min. Next, the solution was passed through a sterile 0.22-$\mu$m filter unit (Millipore; Millipore) into a sterile, pyrogen-free empty vial to ensure sterility. The final concentration was approximately 111–296 MBq/5 mL. The radiochemical purity of 131I-OIH was determined by thin-layer chromatography using silica gel plates (60F-254; Merck) as the solid phase and ethanol:ethyl acetate:ammonium hydroxide (20:20:1) as a mobile phase. In this system, the 131I-OIH had a retention factor of 0.3, and radioiodide had a retention factor of 0.9. 131I-OIH was obtained with a 98%–99% labeling yield.

**In Vitro Stability**

The buffered solution of Na$_2$[99mTc(CO)$_3$(NTA)] was evaluated by HPLC at 24 h to assess stability. In addition, HPLC-purified samples of Na$_2$[99mTc(CO)$_3$(NTA)] (0.1 mL) were mixed with 0.1 M solutions of histidine and cysteine (0.9 mL) and incubated at 37°C; aliquots were analyzed by HPLC at 1, 2, and 4 h to evaluate decomposition.

**Biodistribution Studies**

All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Care and Use Committee of Emory University. Na$_2$[99mTc(CO)$_3$(NTA)] was evaluated in 2 experimental groups of Sprague-Dawley rats. Rats in both groups were anesthetized with ketamine and xylazine (2 mg/kg of body weight) injected intramuscularly, with additional supplemental anesthetic as needed. In the first group of 10 normal rats (group A), the bladder was catheterized by use of heat-flared polyethylene (PE-50) tubing (Becton, Dickinson and Co.). In the second group of 6 rats (group B), the abdomen was opened by a
midline incision, and both renal pedicles were ligated to produce a model of renal failure; thus, no urine was collected.

A solution containing Na₂[²⁹⁹Tc(CO)₃(NTA)] (3.7 MBq/mL [100 μCi/mL]) and ¹³¹I-OIH (925 kBq/mL [25 μCi/mL]) was prepared (pH ~ 7.4), and 0.2-mL doses were injected via a tail vein. One additional aliquot (0.2 mL) for each time point was diluted to 100 mL, and three 1-mL portions of the resulting solution were used as standards.

In group A, 5 animals were sacrificed at 10 min, and 5 at 60 min, after injection. A blood sample was obtained, and the kidneys, heart, lungs, spleen, whole stomach, and sections of the duodenum and ascending colon were removed and placed in counting vials. The whole liver was weighed, and random sections were obtained for counting. Samples of blood and urine were also placed in counting vials and weighed. Each sample and the standards were placed in a γ-counter; counts were corrected for background radiation, physical decay, and spillover of ¹³¹I counts into the ⁹⁹mTc window. The percentage of the dose in each tissue or organ was calculated by dividing the counts in each tissue or organ by the total injected counts. The percentage injected dose (%ID) reported for the bowel was based on the combined counts of the duodenum and colon samples. The %ID in whole blood was estimated by assuming a blood volume of 6.5% of total body weight.

The 6 group B rats were sacrificed 60 min after injection. Selected organs, blood, and all of the small and large intestines were collected and counted as described above. The %ID reported for the bowel was based on the combined counts in the small and large intestines.

**Renal Clearance, Extraction Fraction, Plasma Protein Binding (PPB), and Erythrocyte Uptake**

Two male rats were anesthetized as described above and placed on a heated surgical table. After the tracheostomy, the left jugular vein was cannulated with 2 pieces of PE-50 tubing (1 for infusion of radiopharmaceuticals and 1 for infusion of normal saline [5.8 mL/h] to maintain hydration and additional anesthetic [5 mg/h], as necessary). The right carotid artery was cannulated for blood sampling, and the bladder was catheterized with PE-50 tubing. The core temperature of each animal was continually monitored throughout the study using a rectal temperature probe. Na₂[²⁹⁹Tc(CO)₃(NTA)] (3.7 MBq/mL [100 μCi/mL]) and ¹³¹I-OIH (1.85 MBq/mL [50 μCi/mL]) were coinjected at a flow rate of 1.7 mL/h for 60 min to establish steady-state blood levels. Urine was then collected for three 10-min clearance periods, and midpoint blood samples (0.5 mL) were obtained. The blood samples were centrifuged for 15 min, and plasma samples were obtained. Plasma clearance (mL/min) was determined as UV/P, where U is the urine radiotracer concentration, V is the urine volume excreted per minute, and P is the plasma radioactivity concentration. The average of the three 10-min clearance measurements was used as the clearance value.

The extraction fraction was measured at the conclusion of the clearance measurements by obtaining a left renal venous blood sample (0.5 mL), followed immediately by a carotid artery sample (3 mL). Both blood samples were centrifuged immediately after collection to obtain plasma samples. Extraction fraction was calculated by measuring the difference between the arterial and venous plasma sample: (arterial concentration – venous concentration)/arterial concentration.

PPB was determined by ultrafiltration (Centrifree micropartition system; Amican Inc.) of 1 mL of plasma obtained from the carotid artery sample: (1 – [ultrafiltrate concentration/plasma concentration]) × 100. Arterial blood samples were placed in capillary tubes and centrifuged to determine the hematocrit. Samples of the whole blood and packed cells (~0.3 mL each) were pipetted into counting tubes, weighed, and counted. The percentage uptake in the erythrocytes was calculated from the whole blood (counts/g) and packed cells (counts/g). Percentage erythrocyte uptake was calculated as (counts/g in erythrocytes × hematocrit)/counts/g in whole blood. No correction was made for plasma trapped in the red blood cell sample. PPB and erythrocyte uptake were calculated in duplicate and the mean values reported.

**In Vivo Stability**

To assess in vivo stability, 4 rats were anesthetized and injected with Na₂[²⁹⁹Tc(CO)₃(NTA)] (18.5 MBq [0.5 mCi]) via a tail vein. Two rats were prepared for a 10-min urine collection as described above, and arterial blood was collected by cardiac puncture from the remaining 2 rats at 2–3 min after injection. Urine and plasma samples were analyzed by HPLC to determine whether the complex was metabolized in the plasma or by the kidneys.

**Statistical Analysis**

All results are expressed as the mean ± SD. To determine the statistical significance of differences between the 2 groups, comparisons were made with the 2-tailed Student t test for paired data; a P value of less than 0.05 was considered to be statistically significant.

**RESULTS**

**Chemistry and Radiochemistry**

The synthesis of the [Re(CO)₃(NTA)]²⁻ complex has been previously reported (18). However, that method used (NEt₄)₂[Re(CO)₃Br₃] as a rhenium-tricarbonyl precursor and required heating at 80°C for 2.5 h to form the Na/NEt₄[Re(CO)₃(NTA)] complex in good yield but as a mixture of Na/NEt₄ salts. To avoid byproducts containing the [NEt₄]⁺ counterion and to obviate an extensive purification process, we started with our [Re(CO)₃(H₂O)₃]OTf precursor and were able to obtain the Na₂[Re(CO)₃(NTA)] complex exclusively as a Na⁺ salt, as confirmed by MS, in 89% yield after only 2 h of stirring at room temperature. The only signals present in the ¹H NMR spectrum were those from the coordinated NTA ligand and were consistent with data reported in the literature (18).

The NTA ligand was successfully labeled with the ⁹⁹mTc-tricarbonyl precursor (Fig. 1), and Na₂[⁹⁹mTc(CO)₃(NTA)] [Fig. 1] was isolated with the high radiocurium purity of more than 99%. Because technetium and rhenium complexes with identical ligands have essentially identical coordination parameters, we confirmed the identity of Na₂[⁹⁹mTc(CO)₃(NTA)] by coinjecting it with Na₂[Re(CO)₃(NTA)] and comparing their HPLC profiles. The rhenium and ⁹⁹mTc-tricarbonyl complexes had the same retention time (17 min).

**In Vitro and In Vivo Stability**

The stability of Na₂[⁹⁹mTc(CO)₃(NTA)] was examined in vitro in a physiologic phosphate buffer at pH 7.4. HPLC analysis of an aliquot of the incubated sample revealed only intact ⁹⁹mTc complex for 24 h. When challenged with an
excess of cysteine and histidine at 37°C for 4 h, Na₂[^99mTc(CO)₃(NTA)] was completely inert and showed no sign of transchelation or decomposition.

We evaluated the stability of Na₂[^99mTc(CO)₃(NTA)] in vivo by comparing the high-performance liquid chromatograms of the injected[^99mTc] complex with the[^99mTc] complex recovered in the urine collected during the first 10 min after injection and in the plasma sample obtained 2–3 min after injection. As shown in Figure 2, there was only 1 peak in urine (Fig. 2B) and plasma (Fig. 2C), and each had an elution time identical to that of the injected complex (Fig. 2A), indicating in vivo stability.

**Biodistribution Studies**

The biodistribution of Na₂[^99mTc(CO)₃(NTA)] in normal rats (group A) and in rats with simulated renal failure (group B) is shown in Table 1 and Figure 3. In the normal group A rats, the blood clearance of Na₂[^99mTc(CO)₃(NTA)] was rapid and comparable to that of ^131I-OIH, with only 4.2% ± 0.9% of the injected dose remaining in the blood at 10 min and 0.4% ± 0.2% at 60 min after injection (Table 1). The activity of Na₂[^99mTc(CO)₃(NTA)] in urine as a percentage of ^131I-OIH was 108% ± 9% at 10 min and 101% ± 5% at 60 min; there was no difference in the %ID in the urine for Na₂[^99mTc(CO)₃(NTA)] and ^131I-OIH at 10 and 60 min (P = 0.14 and 0.5, respectively). Na₂[^99mTc(CO)₃(NTA)] demonstrated a high specificity of renal excretion, with less than a mean of 0.8% of the total injected activity present in the heart, lungs, spleen, blood, and liver at 60 min, compared with 1.1% for ^131I-OIH (P = 0.01).

The group B rats had ligation of both renal pedicles to simulate renal failure. Na₂[^99mTc(CO)₃(NTA)] was better retained in the blood at 60 min (19.9%) than was ^131I-OIH (15.1%; P < 0.001). Bowel activity was substantially less for Na₂[^99mTc(CO)₃(NTA)] than for ^131I-OIH (5.6% vs. 14.6%, respectively [P < 0.001]), indicating that renal failure results in less hepatobiliary excretion or intestinal secretion of Na₂[^99mTc(CO)₃(NTA)] than of ^131I-OIH (Table 1). The minimal renal activity noted with both tracers was probably secondary to capsular blood flow (Table 1; Fig. 3).

The PPB of Na₂[^99mTc(CO)₃(NTA)] averaged 67% ± 7% and erythrocyte uptake was low (7% ± 2%), compared with 44% ± 10% and 35% ± 1%, respectively, for ^131I-OIH. The
plasma clearance of Na$_2$[99mTc(CO)$_3$(NTA)] was comparable to that of 131I-OIH (3.08 mL/min/100 g vs. 2.96 mL/min/100 g, respectively). The extraction fraction of Na$_2$[99mTc(CO)$_3$(NTA)] was 93.5% versus an extraction fraction of 67.9% for 131I-OIH.

**DISCUSSION**

[99mTc(CO)$_3$(NTA)]$^{2-}$ has been synthesized previously (15,19), but we could not find any biodistribution data reported in the literature. 99mTc complexes of NTA and NTA derivatives have been prepared by stannous reduction of pertechnetate and investigated in rats (20,21) and dogs (22). These results (20–22) have been disappointing in regard to the rate and specificity for renal excretion, but the stannous reduction–labeling procedure likely produced a mixture of products, including dimers, trimers, and tetramers, all with different rates and specificities for renal excretion. Consequently, the suboptimal renal characteristics of 99mTc-NTA prepared by stannous reduction might not be applicable to a well-characterized tracer with a tricarbonyl core such as Na$_2$[99mTc(CO)$_3$(NTA)].

In fact, several factors led us to believe that Na$_2$[99mTc(CO)$_3$(NTA)] would be an excellent renal agent. Na$_2$[99mTc(CO)$_3$(NTA)] is highly hydrophilic, with a dan-

![FIGURE 3. Biodistribution of [99mTc(CO)$_3$(NTA)]$^{2-}$ and 131I-OIH in normal rats (n = 5) at 10 min after injection (A) and 60 min after injection (B) and in rats with renal pedicle ligation (n = 6) at 60 min after injection (C), expressed as %ID per organ, blood, and urine.](image-url)
gling carboxylate group favoring tubular transport rather than hepatobiliary excretion, and it is formed as a single species with a well-established structure based on the analytic characterization of its rhenium analog. The rhenium(I) center and the anionic part of the [NTA]^{2−} ligand, the tertiary amine and both carboxylate groups from the coordinatedIDA chelating moiety, have a net negative charge. At pH 7.4, the pendant carboxyl group is deprotonated (the pK_a of the carboxylic acid is ~3); thus, both the rhenium and the 99mTc complexes have a dianionic overall charge at physiologic pH similar to Na2[99mTcO(EC)], Na2[99mTcO(MAEC)], and Na2[99mTcO(MAG3)].

Na2[99mTc(CO)3(NTA)] proved to be a stable complex, and its pharmacokinetic characteristics in normal rats were superior to those of the other 99mTc-tricarbonyl renal tubular tracers we previously tested (12,14,23) and comparable to those of 131I-OIH (Table 2). In humans, a renal tracer must be highly specific for renal excretion so that its plasma clearance provides an accurate measurement of renal function; consequently, it is particularly important that the tracer not be cleared via the hepatobiliary tract or secreted across the intestinal mucosa in patients with impaired renal function. In our animal model of renal failure, Na2[99mTc(CO)3(NTA)] was highly promising because it had significantly less intestinal activity than did 131I-OIH. In addition, the renal clearance of Na2[99mTc(CO)3(NTA)] in rats was comparable to that of 131I-OIH, which was equivalent to that reported in the literature (3). Although Na2[99mTc(CO)3(NTA)] and 131I-OIH had similar clearances, the extraction fraction of Na2[99mTc(CO)3(NTA)] appeared to be higher than that of 131I-OIH (93.5% vs. 67.9%). This observation probably reflects an underestimation of the 131I-OIH extraction fraction due to dissociation or leakage of 131I-OIH from the red blood cells back into the plasma in the renal vein sample before or during centrifugation. Our results showed that the red cell uptake of 131I-OIH in rats was 35%, compared with 7% for Na2[99mTc(CO)3(NTA)]; consequently, leakage of the tracer from the red cells back into the renal vein plasma would be more problematic for 131I-OIH than for Na2[99mTc(CO)3(NTA)]. The minimal red cell binding is another potential advantage of Na2[99mTc(CO)3(NTA)], compared with 131I-OIH, because under equilibrium conditions 15%–20% of the activity is from 131I-OIH in human blood bound to or inside of the red cells (4).

The kinetics and metabolism of the NTA ligand itself have been investigated in several species, including humans (24–26). NTA has not been found to be teratogenic or genotoxic but has induced urinary tract tumors in rats and mice at extremely high doses (840 mg/kg of body weight per day for 2 y (27)). The oral median lethal dose of Na3NTA H2O in rodents is about 2,000 mg/kg (28). Limited information exists regarding the toxicity of NTA in humans. Eight human volunteers did ingest a single dose of NTA (10 mg), and physical examination, blood chemistry analysis, and urinalysis showed no evidence of adverse effects (24). Because NTA induces tumors only at doses higher than those that are nephrotoxic, NTA is classified in group IIIB (possibly carcinogenic to man). On the basis of 2-y studies in rats to determine the lowest no-observed-adverse-effect level for a nephrotoxic effect, Health Canada has determined the acceptable daily intake in drinking water to be 10 μg/kg per day (25). In our studies, no free NTA ligand was injected because the ligand was separated from the complex by HPLC before injection, and even the administrated dose of the Na2[99mTc(CO)3(NTA)] complex was extremely small, at less than 0.2 μg/kg, which is lower than the acceptable daily intake of NTA. Consequently, even if all the 99mTc dissociated from the complex, the remaining NTA would still be at a safe level. In a kit formulation, free NTA would be injected, but assuming that it would be on the same order as MAG3 (1 mg/vial) then the dose of NTA injected (μg/kg) would likely still be below the toxicity level established by Health Canada for acceptable daily intake.

CONCLUSION

Initial results in normal rats showed that Na2[99mTc(CO)3(NTA)] is excreted in the urine as rapidly as is 131I-OIH, has a high specificity for renal excretion, has minimal activity associated with red cells, and has lower activity than does 131I-OIH in the liver at 60 min (P < 0.05). In the renal failure model (renal pedicle ligation), Na2[99mTc(CO)3(NTA)] showed higher retention than did 131I-OIH in the blood at 60 min and less activity in the bowel, suggesting that in these respects it may be

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<th>TABLE 2. Injected Dose of [99mTc(CO)3(NTA)]2−, and Other 99mTc-Tricarbonyl Tracers, as a Percentage of 131I-OIH in Urine of Rats at 10 and 60 Minutes After Injection</th>
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Biodistribution data for [99mTcO(MAG3)]2− in rats at 10 and 60 min after injection are not available for comparison. CMSA = carboxymethylmercaptopusuccinic acid; TDSA = thiodisuccinic acid (14); LAN = lantionine (12); ENDAC = ethylenediamine-N,N’-diacetic acid (23).
superior to $^{131}$I-OIH in humans. Moreover, Na$_2$[$^{99m}$Tc(CO)$_3$(NTA)] is formed as a single species and is amenable to kit formulation, and the unreacted NTA is below accepted toxicity levels in humans. These combined results suggest that Na$_2$[$^{99m}$Tc(CO)$_3$(NTA)] may be a superior $^{99m}$Tc renal tubular imaging agent for imaging and for the measurement of ERPF in humans.

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