^{99m}Tc(CO)$_3$-Nitrilotriacetic Acid: A New Renal Radiopharmaceutical Showing Pharmacokinetic Properties in Rats Comparable to Those of ^{131}I-OIH

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To develop a ^{99m}Tc renal tracer with a capacity to measure effective renal plasma flow comparable to that of the clinical gold standard ^{131}I-*o*-iodohippurate (^{131}I-OIH) and superior to that of ^{99m}TcO-mercaptoacetyltriglycine, which has a clearance only 50%−60% that of ^{131}I-OIH, we investigated ^{99m}Tc-tricarbonyl nitrilotriacetic acid (Na$_2$[^{99m}Tc(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:** Na$_2$[^{99m}Tc(CO)$_3$(NTA)] was prepared by using commercially available NTA and an IsoLink kit and isolated by high-performance liquid chromatography. The stability of Na$_2$[^{99m}Tc(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 Na$_2$[^{99m}Tc(CO)$_3$(NTA)], coinjected with ^{131}I-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 Na$_2$[^{99m}Tc(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% ± 9% and 101% ± 5%, respectively, that of ^{131}I-OIH; minimal hepatic or gastrointestinal activity was demonstrated. In group B rats, Na$_2$[^{99m}Tc(CO)$_3$(NTA)] was better retained in the blood and had less excretion into the bowel than did ^{131}I-OIH ($P < 0.01$). The plasma clearances of Na$_2$[^{99m}Tc(CO)$_3$(NTA)] and ^{131}I-OIH were comparable, but the extraction fraction of Na$_2$[^{99m}Tc(CO)$_3$(NTA)] was 93.5% ± 3.8%, compared with 67.9% ± 6.1% for ^{131}I-OIH. Plasma protein binding of Na$_2$[^{99m}Tc(CO)$_3$(NTA)] averaged 67% ± 7%, and red cell uptake was 7% ± 2%. **Conclusion:** Na$_2$[^{99m}Tc(CO)$_3$(NTA)] is stable, exists as a single species, and has pharmacodynamic properties in rats comparable to those of ^{131}I-OIH.

Our goal has been the development of a ^{99m}Tc renal tracer with a capacity to measure effective renal plasma flow (ERPF) comparable to that of the gold standard, *p*-aminohippurate. ^{131}I-*o*-iodohippurate (^{131}I-OIH) has biologic properties similar to *p*-aminohippurate and has been used as an imaging agent and as a tracer to measure ERPF. However, ^{131}I-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 ^{131}I (*1*), and is no longer clinically available in the United States.

To accomplish our goal, we initially explored renal agents with the [^{99m}Tc(V)O]$^{3+}$ core, such as ^{99m}TcO-mercaptoacetyltriglycine (^{99m}TcO-MAG3) (*2–5*), ^{99m}TcO-ethylenedicysteine (^{99m}TcO-EC) (*6*), and ^{99m}TcO-mercaptoacetamide-ethylenecysteine (^{99m}TcO-MAEC) (*7,8*). All of those agents produced excellent scintigraphic images, but their plasma clearances were still significantly less than that of ^{131}I-OIH. In a continuing effort to develop renal imaging agents with higher clearances, we shifted our focus to the *fac*-[^{99m}Tc(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 ^{99m}TcO complexes such as ^{99m}TcO-MAG3 might facilitate more efficient tubular transport; the high chemical and kinetic stability resulting from the ^{99m}Tc-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 [^{99m}Tc(I)(CO)$_3$(H$_2$O)$_3$]$^+$ precursor (*10,11*); and the first renal radiopharmaceutical with a ^{99m}Tc-tricarbonyl core

evaluated in humans, Na[^{99m}Tc(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 ^{131}I-OIH.

We chose the aminopolycarboxylate ligand nitrilotriacetic acid (NTA) for further investigation because it has a suitable chelating moiety, with the amine and carboxyl donor groups allowing tridentate coordination to the ^{99m}Tc(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$_2$[^{99m}Tc(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 ^{99m}TcO-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. ^{1}H 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-FT instrument (Thermo Electron). ^{99m}Tc-pertechnetate (^{99m}TcO$_4^-$) was eluted from a ^{99}Mo/^{99m}Tc generator (Amersham Health), with 0.9% saline. IsoLink vials were obtained as a gift from Covidien. [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was prepared according to the manufacturer's insert. The radiolabeled and nonradiolabeled compounds were analyzed on a high-performance liquid chromatography (HPLC) instrument (System Gold Nouveau; Beckman Coulter) equipped with a model 170 radiometric detector and a model 166 ultraviolet light–visible detector, 32 Karat chromatography software (Beckman Coulter), and an octyldecyl silane column (C18 RP Ultrasphere; Beckman Coulter) (5-μm, 4.6 × 250 mm). The flow rate and mobile phase were the same as reported previously (*14*). Tissue and organ radioactivity was measured with a γ-counter (Packard Cobra II γ-Counter; Perkin Elmer).

Synthesis of Na$_2$[Re(CO)$_3$(NTA)]

The Na$_2$[Re(CO)$_3$(NTA)] complex was synthesized as a nonradioactive reference compound. A solution of NTA (0.082 g, 0.3 mmol in 3 mL of water) was added to a 0.1 M aqueous solution of [Re(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 to 2 mL by rotary evaporation, and this solution was passed through a column (Sephadex G-15; Aldrich) eluted with deionized water. The product fractions were collected, the solvent was removed under vacuum, and the white residue was dried to yield Na$_2$[Re(CO)$_3$(NTA)] (0.134 g, 89%). ^{1}H NMR [δ (ppm)]: 4.07 (s, 2H), 4.04 (d, 2H, J = 16.8 Hz), 3.9 (d, 2H, J = 16.8 Hz)]. MS (ESI): m/z 506 (100%, M + Na$_2$)$^+$; high-resolution mass spectroscopy calculated C$_9$H$_7$O$_9$NNa$_2$^{187}Re 505.94684 and found 505.94691.

Both ^{1}H NMR and MS confirmed the identity of the purified product.

Radiosynthesis of Na$_2$[^{99m}Tc(CO)$_3$(NTA)] and ^{131}I-OIH

The NTA ligand was labeled as previously described (*15*). Briefly, 0.5 mL of a freshly prepared solution of the [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor (pH ~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$_2$[^{99m}Tc(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$_2$[^{99m}Tc(CO)$_3$(NTA)] was buffered in a physiologic phosphate buffer at pH 7.4.

^{131}I-OIH was prepared by the isotope exchange reaction between nonradioactive hippuran (OIH) and radioactive sodium iodide (Na^{131}I) 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^{131}I (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-μm filter unit (Millex-GS; 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 ^{131}I-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 ^{131}I-OIH had a retention factor of 0.3, and radioiodide had a retention factor of 0.9. ^{131}I-OIH was obtained with a 98%–99% labeling yield.

In Vitro Stability

The buffered solution of Na$_2$[^{99m}Tc(CO)$_3$(NTA)] was evaluated by HPLC at 24 h to assess stability. In addition, HPLC-purified samples of Na$_2$[^{99m}Tc(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$_2$[^{99m}Tc(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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ (3.7 MBq/mL [100 µCi/mL]) and ^{131}I-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 ^{131}I counts into the ^{99m}Tc 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. $Na_2[^{99m}Tc(CO)_3(NTA)]$ (3.7 MBq/mL [100 µCi/mL]) and ^{131}I-OIH (1.85 MBq/mL [50 µCi/mL]) were coinfused 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 radioactivity 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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ (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 $(NEt_4)_2[Re(CO)_3Br_3]$ as a rhenium-tricarbonyl precursor and required heating at 80°C for 2.5 h to form the $Na/NEt_4[Re(CO)_3(NTA)]$ complex in good yield but as a mixture of Na/NEt_4 salts. To avoid byproducts containing the $[NEt_4]^+$ counterion and to obviate an extensive purification process, we started with our $[Re(CO)_3(H_2O)_3]OTf$ precursor and were able to obtain the $Na_2[Re(CO)_3(NTA)]$ 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 ^{99m}Tc-tricarbonyl precursor (Fig. 1), and $Na_2[^{99m}Tc(CO)_3(NTA)]$ was isolated with the high radiochemical purity of more than 99%. Because technetium and rhenium complexes with identical ligands have essentially identical coordination parameters, we confirmed the identity of $Na_2[^{99m}Tc(CO)_3(NTA)]$ by coinjecting it with $Na_2[Re(CO)_3(NTA)]$ and comparing their HPLC profiles. The rhenium and ^{99m}Tc-tricarbonyl complexes had the same retention time (17 min).

In Vitro and In Vivo Stability

The stability of $Na_2[^{99m}Tc(CO)_3(NTA)]$ 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 ^{99m}Tc complex for 24 h. When challenged with an
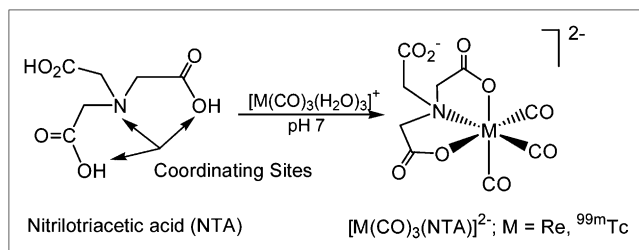
FIGURE 1. Synthesis of $[M(CO)_3(NTA)]^{2-}$. M = rhenium (2 h, room temperature) and ^{99m}Tc (15 min, 70°C).

excess of cysteine and histidine at 37°C for 4 h, $Na_2[^{99m}Tc(CO)_3(NTA)]$ was completely inert and showed no sign of transchelation or decomposition.

We evaluated the stability of $Na_2[^{99m}Tc(CO)_3(NTA)]$ in vivo by comparing the high-performance liquid chromatograms of the injected ^{99m}Tc complex with the ^{99m}Tc 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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ 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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ was rapid and comparable to that of ^{131}I-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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ in urine as a percentage of ^{131}I-OIH was 108% ± 9% at 10 min and 101% ± 5% at 60 min; there was no difference in the %ID in the urine for $Na_2[^{99m}Tc(CO)_3(NTA)]$ and ^{131}I-OIH at 10 and 60 min ($P = 0.14$ and 0.5, respectively). $Na_2[^{99m}Tc(CO)_3(NTA)]$ 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 ^{131}I-OIH ($P = 0.01$).

The group B rats had ligation of both renal pedicles to simulate renal failure. $Na_2[^{99m}Tc(CO)_3(NTA)]$ was better retained in the blood at 60 min (19.9%) than was ^{131}I-OIH (15.1%; $P < 0.001$). Bowel activity was substantially less for $Na_2[^{99m}Tc(CO)_3(NTA)]$ than for ^{131}I-OIH (5.6% vs. 14.6%, respectively [$P < 0.001$]), indicating that renal failure results in less hepatobiliary excretion or intestinal secretion of $Na_2[^{99m}Tc(CO)_3(NTA)]$ than of ^{131}I-OIH (Table 1). The minimal renal activity noted with both tracers was probably secondary to capsular blood flow (Table 1; Fig. 3).
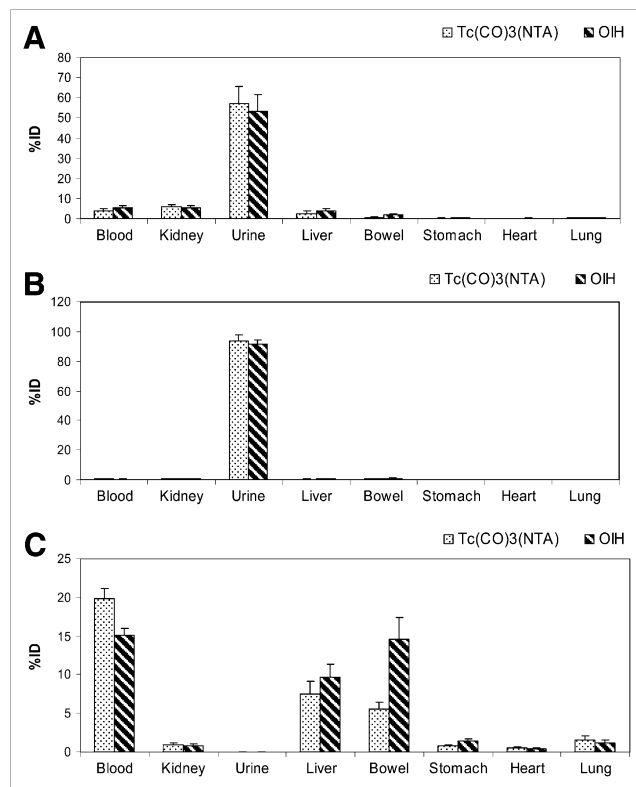
The PPB of $Na_2[^{99m}Tc(CO)_3(NTA)]$ averaged 67% ± 7% and erythrocyte uptake was low (7% ± 2%), compared with 44% ± 10% and 35% ± 1%, respectively, for ^{131}I-OIH. The
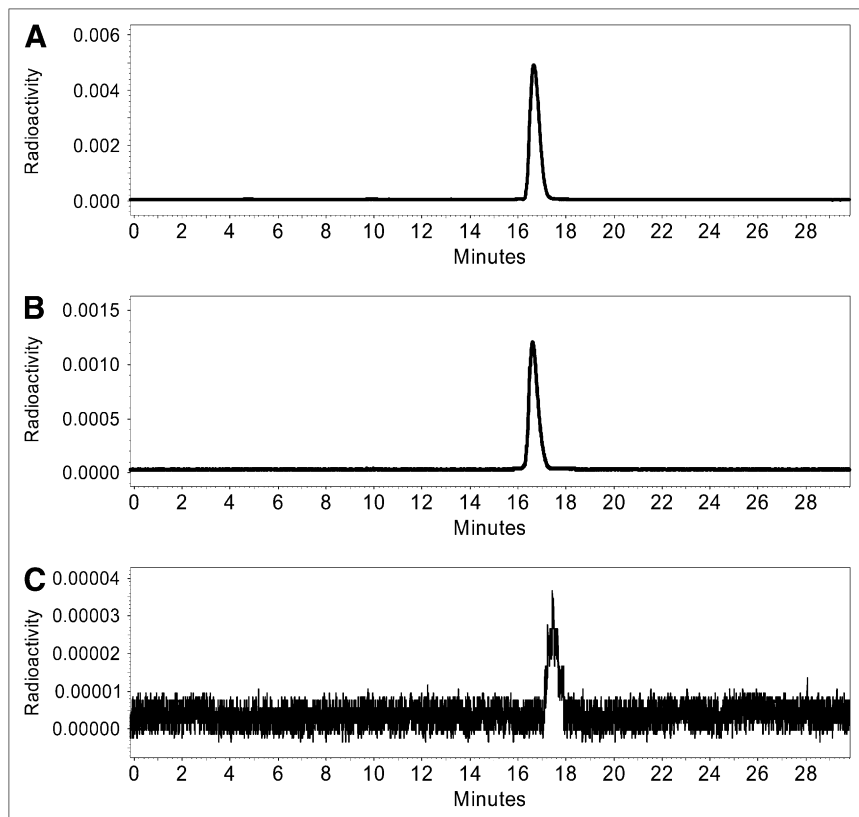
FIGURE 2. High-performance liquid chromatograms of $[^{99m}Tc(CO)_3(NTA)]^{2-}$ before injection (A), in urine at 10 min after injection (B), and in plasma at 2–3 min after injection (C).

TABLE 1. %ID of $[^{99m}Tc(CO)_3(NTA)]^{2-}$ and ^{131}I-OIH at 10 and 60 Minutes in Group A and Group B Rats

Time (min)	Blood		Kidney		Urine			Liver		Bowel	
	^{99m}Tc-NTA	^{131}I-OIH	^{99m}Tc-NTA	^{131}I-OIH	^{99m}Tc-NTA	^{131}I-OIH	% ^{99m}Tc/^{131}I-OIH	^{99m}Tc-NTA	^{131}I-OIH	^{99m}Tc-NTA	^{131}I-OIH
Group A (n = 5)											
10	4.2 ± 0.9	5.6 ± 1.0	6.1 ± 0.7	5.6 ± 0.6	57.1 ± 8.4	53.2 ± 7.2	108 ± 9	2.7 ± 1.2	4.0 ± 0.7	0.7 ± 0.3	2.2 ± 0.4
60	0.4 ± 0.2	0.5 ± 0.0	0.4 ± 0.2	0.5 ± 0.0	93.0 ± 4.0	91.4 ± 3.7	101 ± 5	0.3 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	1.0 ± 0.5
Group B (n = 6)											
60	19.9 ± 1.2	15.1 ± 0.9	0.9 ± 0.2	0.8 ± 0.2	—	—	—	7.5 ± 1.6	9.7 ± 1.7	5.6 ± 0.9	14.6 ± 2.8

Data are presented as mean ± SD.

FIGURE 3. Biodistribution of $[^{99m}Tc(CO)_3(NTA)]^{2-}$ and ^{131}I-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.

plasma clearance of $Na_2[^{99m}Tc(CO)_3(NTA)]$ was comparable to that of ^{131}I-OIH (3.08 mL/min/100 g vs. 2.96 mL/min/100 g, respectively). The extraction fraction of $Na_2[^{99m}Tc(CO)_3(NTA)]$ was 93.5% ± 3.8%, versus an extraction fraction of 67.9% ± 6.1% for ^{131}I-OIH.

DISCUSSION

$[^{99m}Tc(CO)_3(NTA)]^{2-}$ has been synthesized previously (15,19), but we could not find any biodistribution data reported in the literature. ^{99m}Tc 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 ^{99m}Tc-NTA prepared by stannous reduction might not be applicable to a well-characterized tracer with a tricarbonyl core such as $Na_2[^{99m}Tc(CO)_3(NTA)]$.

In fact, several factors led us to believe that $Na_2[^{99m}Tc(CO)_3(NTA)]$ would be an excellent renal agent. $Na_2[^{99m}Tc(CO)_3(NTA)]$ is highly hydrophilic, with a dan-

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 coordinated IDA 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 ^{99m}Tc complexes have a dianionic overall charge at physiologic pH similar to $Na_2[^{99m}TcO(MAG3)]$, $Na_2[^{99m}TcO(EC)]$, $Na_2[^{99m}TcO(MAEC)]$, and $Na_2[^{99m}Tc(CO)_3(carboxymethylmercaptosuccinic acid)]$ (14).

$Na_2[^{99m}Tc(CO)_3(NTA)]$ proved to be a stable complex, and its pharmacokinetic characteristics in normal rats were superior to those of the other ^{99m}Tc-tricarbonyl renal tubular tracers we previously tested (12,14,23) and comparable to those of ^{131}I-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, $Na_2[^{99m}Tc(CO)_3(NTA)]$ was highly promising because it had significantly less intestinal activity than did ^{131}I-OIH. In addition, the renal clearance of $Na_2[^{99m}Tc(CO)_3(NTA)]$ in rats was comparable to that of ^{131}I-OIH, which was equivalent to that reported in the literature (3). Although $Na_2[^{99m}Tc(CO)_3(NTA)]$ and ^{131}I-OIH had similar clearances, the extraction fraction of $Na_2[^{99m}Tc(CO)_3(NTA)]$ appeared to be higher than that of ^{131}I-OIH (93.5% vs. 67.9%). This observation probably reflects an underestimation of the ^{131}I-OIH extraction fraction due to dissociation or leakage of ^{131}I-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 ^{131}I-OIH in rats was 35%, compared with 7% for $Na_2[^{99m}Tc(CO)_3(NTA)]$; consequently, leakage of the tracer from the red cells back into the renal vein plasma would be more problematic for ^{131}I-OIH than for $Na_2[^{99m}Tc(CO)_3(NTA)]$. The minimal red cell binding is another potential advantage of $Na_2[^{99m}Tc(CO)_3(NTA)]$, compared with ^{131}I-OIH, because under equilibrium conditions 15%−20% of the activity is from ^{131}I-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 $Na_3NTA \cdot H_2O$ 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 administered dose of the $Na_2[^{99m}Tc(CO)_3(NTA)]$ 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 ^{99m}Tc 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 $Na_2[^{99m}Tc(CO)_3(NTA)]$ is excreted in the urine as rapidly as is ^{131}I-OIH, has a high specificity for renal excretion, has minimal activity associated with red cells, and has lower activity than does ^{131}I-OIH in the liver at 60 min ($P < 0.05$). In the renal failure model (renal pedicle ligation), $Na_2[^{99m}Tc(CO)_3(NTA)]$ showed higher retention than did ^{131}I-OIH in the blood at 60 min and less activity in the bowel, suggesting that in these respects it may be

TABLE 2. Injected Dose of $[^{99m}Tc(CO)_3(NTA)]^{2-}$, and Other ^{99m}Tc-Tricarbonyl Tracers, as a Percentage of ^{131}I-OIH in Urine of Rats at 10 and 60 Minutes After Injection

Urine ($^{99m}Tc/^{131}I$ ratio) at…	$[^{99m}Tc(CO)_3(NTA)]^{2-}$	$[^{99m}Tc(CO)_3(CMSA)]^{2-}$	$[^{99m}Tc(CO)_3(TDSA)]^{3-}$	$[^{99m}Tc(CO)_3(LAN)]^{-}$	$[^{99m}Tc(CO)_3(ENDAC)]^{-}$
10 min	108 ± 9	82 ± 4	41 ± 5	69 ± 6	56 ± 5
60 min	101 ± 5	98 ± 1	68 ± 8	89 ± 6	90 ± 4

Biodistribution data for $[^{99m}TcO(MAG3)]^{2-}$ in rats at 10 and 60 min after injection are not available for comparison. CMSA = carboxymethylmercaptosuccinic acid; TDSA = thiodisuccinic acid (14); LAN = lanthionine (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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