99mTc(CO)3(NTA): A 99mTc Renal Tracer with Pharmacokinetic Properties Comparable to Those of 131I-OIH in Healthy Volunteers

Andrew T Taylor Jr., Emory University
Malgorzata Lipowska, Emory University
Luigi G. Marzilli, Louisiana State University

Journal Title: Journal of Nuclear Medicine and Radiation Therapy
Volume: Volume 51, Number 3
Publisher: Society of Nuclear Medicine | 2010-03, Pages 391-396
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.2967/jnumed.109.070813
Permanent URL: http://pid.emory.edu/ark:/25593/fhg26

Final published version: http://jnm.snmjournals.org/content/51/3/391

Copyright information:
© 2010 by the Society of Nuclear Medicine, Inc.

Accessed February 5, 2020 5:23 AM EST
$^{99m}$Tc(CO)$_3$-Nitrilotriacetic Acid: A New Renal Radiopharmaceutical Showing Pharmacokinetic Properties in Rats Comparable to Those of $^{131}$I-OIH

Malgorzata Lipowska$^1$, Luigi G. Marzilli$^2$, and Andrew T. Taylor$^1$

$^1$ Department of Radiology, Emory University, Atlanta, GA 30322, USA
$^2$ Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA

Abstract

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 $^{99m}$Tc-mercaptoacetyltriglycine ($^{99m}$TcO-MAG3), 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 physiological pH, and the challenge experiments showed no degradation. In normal rats, the percent dose in the urine at 10 and 60 min was 108 ± 9 % and 101 ± 5 %, respectively, that of $^{131}$I-OIH; minimal hepatic and 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 to 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 %.

Conclusions—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.
INTRODUCTION

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, $^{131}$I-o-iodohippurate ($^{131}$I-OIH). $^{99m}$Tc-ortho-iodohippurate ($^{131}$I-OIH) has similar biological properties 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 kidney and thyroid in patients with impaired renal function as a result of the beta emission of $^{131}$I (1), and it 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 gave 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)$_3$]$^{3+}$ 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)$_3$]$^{3+}$ 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$[^{99m}$Tc(CO)$_3$(NTA)] would be dianionic at physiological 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)$_3$]$^{3+}$ 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 light 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 gamma 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$_2$Re 505.94684 and found 505.94691. Both $^1$H NMR and MS confirmed the identity of the purified product.

**Radiosynthesis of Na$_2$[99mTc(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 1.0 mg of the NTA ligand in 0.2 mL of water. The pH of the solution was adjusted to ~ 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 physiological phosphate buffer at pH 7.4.

$^{131}$I-OIH was prepared by the isotope exchange reaction between non-radioactive 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 (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 (TLC) 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 open 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 intravenously 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. Following tracheostomy, the left jugular vein was cannulated with 2 pieces of PE-50 tubing (1 for infusion of radiopharmaceuticals and 1 to infuse 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 continuously 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 percent 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 cells 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 Na₃[¹⁹⁹mTc(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 if the complex was metabolized in the plasma or by the kidney.

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)] 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 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 Na$_2$[${}^{99m}$Tc(CO)$_3$(NTA)] was examined in vitro in a physiological 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 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 HPLC 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 one 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 ${}^{131}$I-OIH, with only 4.2 ± 0.9% of the injected dose remaining in the blood 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 heart, lungs, spleen, blood and liver at 60 min, compared to 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% versus 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 to 44 ± 10% and 35 ± 1%, respectively, for ${}^{131}$I-OIH. The plasma clearance of Na$_2$[${}^{99m}$Tc(CO)$_3$(NTA)] was comparable to that of ${}^{131}$I-OIH (3.08 mL/min/100g vs. 2.96 mL/min/100g, 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 very 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, a number of 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 dangling 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 analytical characterization of its rhenium analogue. 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[^99mTc] complexes have a diaionic overall charge at physiological pH similar to Na$_2$[^99mTcO(MAG3)], Na$_2$[^99mTcO(EC)], Na$_2$[^99mTcO(MAEC)], and Na$_2$[^99mTc(CO)$_3$(carboxymethylmercaptosuccinic acid)] (14).

Na$_2$[^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, Na$_2$[^99mTc(CO)$_3$(NTA)] was highly promising because it had significantly less renal activity than ^131I-OIH. In addition, the renal clearance of Na$_2$[^99mTc(CO)$_3$(NTA)] in rats was comparable to that of ^131I-OIH, which was equivalent to that reported in the literature (3). Although Na$_2$[^99mTc(CO)$_3$(NTA)] and ^131I-OIH had similar clearances, the extraction fraction of Na$_2$[^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 to 7% for Na$_2$[^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 Na$_2$[^99mTc(CO)$_3$(NTA)]. The minimal red cell binding is another potential advantage of Na$_2$[^99mTc(CO)$_3$(NTA)], compared to ^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 (24,25,26). 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$_3$NTA-H$_2$O in rodents is about 2000 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 two-year 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 prior to injection, and even the administrated dose of the Na$_2$[^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 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.

*J Nucl Med. Author manuscript; available in PMC 2009 August 7.*
CONCLUSION

Initial results in normal rats showed that Na$_2^{[99mTc(CO)_3(NTA)]}$ is excreted in the urine as rapidly as $^{131I}$-OIH, has a high specificity for renal excretion, has minimal activity associated with red cells, and has lower activity than dose $^{131I}$-OIH in the liver at 60 min ($P < 0.05$). In the renal failure model (renal pedicle ligation), Na$_2^{[99mTc(CO)_3(NTA)]}$ 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 superior to $^{131I}$-OIH in humans. Moreover, Na$_2^{[99mTc(CO)_3(NTA)]}$ 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$_2^{[99mTc(CO)_3(NTA)]}$ may be a superior $^{99mTc}$ renal tubular imaging agent for imaging and for the measurement of effective renal plasma flow in man.

Acknowledgments

This research was supported by NIH R01 DK38842

We thank Dr. Patricia A. Marzilli for her valuable comments during the preparation of the article and Eugene Malveaux and Mel Camp for their technical assistance. Covidien is gratefully acknowledged for providing the IsoLink kits. This research was supported by a grant from National Institute of Health (NIH R01 DK38842).

References


27. National Cancer Institute. Bioassays of Nitrilotriacetic Acid (NTA) and Nitrilotriacetic Acid, Trisodium Salt, Monohydrate (Na\textsubscript{3}NTA, H\textsubscript{2}O) for Possible Carcinogenicity. Bethesda, MD: Natinal Cancer Institute; 1977. p. 77-806.NCI-CG-TR-6; DHEW Publication No. [NIH]

FIGURE 1.
Synthesis of $[\text{M(CO)}_3(\text{NTA})]^\text{2−}$; $\text{M} = \text{rhenium (2 h, room temperature) and } ^{99m}\text{Tc (15 min, 70 °C)}$. 

Nitrilotriacetic acid (NTA) $\xrightarrow{\text{[M(CO)_3(H_2O)_3]^+}}$ Coordinating Sites $\xrightarrow{\text{pH 7}} [\text{M(CO)}_3(\text{NTA})]^\text{2−}; \text{M} = \text{Re, } ^{99m}\text{Tc}$
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).
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.
<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Kidney</th>
<th>Urine</th>
<th>Liver</th>
<th>Bowel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%99mTc-NTA</td>
<td>%99mTc-NTA</td>
<td>%99mTc-NTA</td>
<td>%131I-OIH</td>
<td>%99mTc-NTA</td>
</tr>
<tr>
<td></td>
<td>131I-OIH</td>
<td>131I-OIH</td>
<td>131I-OIH</td>
<td>%99mTc-131I-OIH</td>
<td>131I-OIH</td>
</tr>
<tr>
<td>Group A (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>4.2 ± 0.9</td>
<td>5.6 ± 10</td>
<td>61 ± 0.7</td>
<td>57.1 ± 8.4</td>
<td>108 ± 9</td>
</tr>
<tr>
<td>60 min</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 00</td>
<td>0.4 ± 0.2</td>
<td>93.0 ± 40</td>
<td>91.4 ± 3.7</td>
</tr>
<tr>
<td>Group B (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>199 ± 1.2</td>
<td>15.1 ± 09</td>
<td>0.9 ± 0.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
<table>
<thead>
<tr>
<th>Urine (99mTc/131I) ratio at</th>
<th>[99mTc(CO)₃(NTA)]²⁻</th>
<th>[99mTc(CO)₃(CMSA)]²⁻</th>
<th>[99mTc(CO)₃(TDSA)]³⁻</th>
<th>[99mTc(CO)₃(LAN)]⁻</th>
<th>[99mTc(CO)₃(ENDAC)]⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>108 ± 9</td>
<td>82 ± 4</td>
<td>41 ± 5</td>
<td>69 ± 6</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>60 min</td>
<td>101 ± 5</td>
<td>98 ± 1</td>
<td>68 ± 8</td>
<td>89 ± 6</td>
<td>90 ± 4</td>
</tr>
</tbody>
</table>

Biodistribution data for [99mTcO(MAG3)]²⁻ in rats at 10 and 60 min after injection are not available for comparison. CMSA = carboxymethylmercaptosuccinic acid (14); TDSA = thiodisuccinic acid (14); LAN = lanthionine (12); ENDAC = ethylenediamine-N,N'-diacetic acid (23).