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Characterisation of $^{67}Ga^{3+}$ Complexes of Triaza Macrocyclic Ligands: Biodistribution and Clearance Studies

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ABSTRACT. The $^{67}Ga^{3+}$ complexes of three triazamacrocycles, 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (NOTA), its phosphonate analog 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonic) acid (NOTP), and the monoethyl ester of NOTP, 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris (methylenephosphonate-monoethylester) (NOTPME) were studied for possible use as radiopharmaceuticals. Biodistribution studies and gamma imaging were performed in Wistar rats. The present results demonstrated that all the macrocyclic complexes studied display renal clearance and are almost completely eliminated within 24 h. The $[^{67}Ga](NOTP)^{3-}$ chelate, with a large negative charge, has a considerably slower uptake and elimination by the kidneys than the neutral $[^{67}Ga]$(NOTA) and $[^{67}Ga]$(NOTPME) chelates. We have thus demonstrated a charge-clearance relationship for a series of stable and well characterized complexes. The high stability and rapid renal excretion properties displayed by the NOTA and NOTPME chelates support their possible application as imaging agents for kidney structural and functional studies. NUCL MED BIOL 26;6:707–710, 1999. **© 1999 Elsevier Science Inc. All rights reserved.**

KEY WORDS. Radiopharmaceutical, Gamma imaging, Biodistribution, $^{67}Ga^{3+}$ complexes, Triazamacrocycles

INTRODUCTION

The importance of complexes of gallium (III) in diagnostic nuclear medicine has led to an increasing interest in its coordination chemistry. The positron emitting radioisotope ^{68}Ga (β^+, $t_{1/2}$ = 68 min) may be applicable in positron emission tomography (PET) (3, 14, 19), whereas ^{67}Ga (β^-,γ, $t_{1/2}$ = 3.35 days) is a useful tracer in conventional nuclear medicine scintigraphy for tumor and inflammation detection (14, 20–23).

When $^{67}Ga^{3+}$ is injected into the blood stream in the commonly used form of gallium citrate (a weak chelate), the Ga^{3+} ion is transchelated to transferrin (13, 24, 25) and is then found in areas of high iron uptake (bone marrow, liver, spleen, gastrointestinal tract, salivary glands, and in the breast tissue of young adult or lactating females [8]). The radioisotope is cleared slowly from the body. A clinically useful chelating ligand should form inert complexes with the appropriate metal ions, while at the same time conferring to these complexes desirable chemical, physical, and biological properties. Recently, there has been considerable interest in polyaza macrocyclic ligands that form highly stable chelates with trivalent metal ions, with slow rates of metal dissociation (1, 5–7, 10, 11, 13). The ligand 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (NOTA) has been found to fulfill the criteria of high thermodynamic and kinetic stability for binding to Ga^{3+}. In fact, the Ga(NOTA) chelate remains intact in nitric acid over a period of 6 months (4).

In the present study, we investigated the *in vivo* behavior of $^{67}Ga^{3+}$ chelates of NOTA, its phosphonate analog 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonicacid) (NOTP), and the monoethyl ester of NOTP, 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris (methylenephosphonate monoethylester) (NOTPME) (see Fig. 1 for their chemical structures). The ligands form neutral (NOTA and NOTPME) or negatively charged (NOTP) complexes with $^{67}Ga^{3+}$, which display varying molecular properties, which are known to influence the biodistribution and excretion of substances injected into the blood stream (5, 10, 18). These properties include molecular size, molecular weight, charge, and hydrophilicity of the complexes. Thus, the present study may be useful in assessing the correlation between such chelate molecular properties and their *in vivo* pharmacokinetics, helping in the design of new, more specific radiopharmaceuticals.

MATERIALS AND METHODS

Materials, Reagents, and General Methods

$[^{67}Ga]$ citrate was obtained from CIS-Biointernational. The triazamacrocyclic ligands NOTA, NOTP, and NOTPME were synthesized and characterized by nuclear magnetic resonance (NMR) spectroscopy as described elsewhere (11, 15, 16). Solutions of the Ga^{3+} chelates for NMR analysis were obtained by mixing stoichiometric amounts of $Ga(NO_3)_3$ and each of the ligands in D_2O (at 20-mM concentrations) and adjusting the pH to 7 with diluted DCl and NaOD. All the reagents and solvents were obtained from either Aldrich or Sigma and used as received. ^1H and ^{71}Ga-NMR spectra were recorded on a Varian Unity-500 Fourier Transform spectrometer (at an external field of 11.8 T) operating at 499.8 and 152.4 MHz, respectively. The resonance shifts were measured relative to tetrametylsilane (TMS) and the $[Ga(H_2O)_6]^{3+}$ species present in 0.1 M $Ga(NO_3)_3$ in D_2O, respectively. Assignments of

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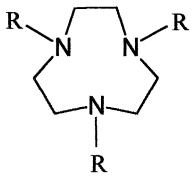

R= CH_2COOH NOTA

R = $CH_2PO_4H_2$ NOTP

R = $CH_2PO_2H(OC_2H_5)$ NOTPME

FIG. 1. Chemical structures of the ligands used in this study.

the proton NMR spectra were based on literature data on similar systems and the results of two-dimensional homonuclear correlation spectra (COSY) (2).

A gamma camera-computer system (GE 400 AC\STARPORT) was used for acquisition and pre-processing. Data processing and display were performed with a CityDesk IBM AT compatible computer using software developed specifically for this type of experiments. A well counter (DPC-Gamma C12) with a Compaq DeskPro compatible computer was used for activity counting in the biodistribution studies.

Gamma Imaging

Stock solutions of the ligands were prepared in isotonic HEPES pH 7 buffer and mixed (in a mole ratio of 1:1, with a small ligand excess) with [^{67}Ga] citrate. Gamma images and the biological distribution for the three $^{67}Ga^{3+}$ complexes were determined using Wistar rats weighing 300 g. All animal studies were carried out in compliance with procedures approved by the appropriate institutional review committees. Conscious rats were allowed free access of food and water *ad libitum*. Three groups of four animals (one group for each complex) were anaesthetized with Ketamine (50 mg/mL)/chloropromazine (2.5%) (10:3) and injected in the tail vein with ca. 150 μCi of the respective $^{67}Ga^{3+}$ chelate. The animals were then positioned in ventral *decubitus* over the detector. Image acquisition was initiated immediately before radiotracer injection. Sequences of 180 images (10 s each), were acquired to 64 × 64 matrices. Blood samples were taken during the dynamic acquisition and subsequently counted in a γ well counter.

To analyze the transport of radiotracer over time, three regions of interest (ROI) were drawn on the image files, corresponding to the thorax, liver, and left kidney. From these ROI, time–activity curves were obtained using homemade software. In addition, static data were acquired at 24 and 48 h after the radiotracer injection.

Biodistribution Experiments

Four groups of four animals were injected with ca. 100 μCi of the three $^{67}Ga^{3+}$ complexes and the [^{67}Ga]citrate complex (for comparative purposes). All animals were sacrificed 2 h later. The majors organs were removed, weighted, and counted in a γ well counter. Similar biodistribution studies were also performed with the rats referred in the previous section sacrificed at 48 h.

RESULTS AND DISCUSSION

A series of structurally related $^{67}Ga^{3+}$ chelates of triazamacrocyclic ligands with different types of pendant arms (Fig. 1), having different molecular properties such as molecular weight and net charge, were prepared aiming at the elucidation of structure–activity relationships governing biodistribution and clearance of $^{67}Ga^{3+}$ complexes.

Figure 2 shows the averaged time–activity curves, obtained from dynamic acquisitions for each ROI. The thorax activity/pixel was considered as the background activity. The values of mean activity/pixel for each ROI, after background deduction, were used to obtain regional time–activity curves. The curves were normalized relative to the maximum activity obtained for each complex. The complexes studied seem to undergo different kidney clearance processes. The liver–spleen curve is similar to the thorax curve, corresponding only to blood activity. [^{67}Ga](NOTA) and [^{67}Ga](NOTPME), show a much higher depuration efficiency and faster transit time through the kidneys than [^{67}Ga](NOTP)$^{3-}$. The kidney curve for [^{67}Ga](NOTP)$^{3-}$ indicates slow uptake and long retention of the tracer by the organ.

Figure 3 illustrates the scintigraphic images obtained 30 min after injection of the $^{67}Ga^{3+}$ chelates. For [^{67}Ga](NOTA) and [^{67}Ga](NOTPME), only a slight activity in kidneys above the tissue background was seen, but there was high activity in the bladder. In contrast, a smaller background activity and no elimination of the tracer after kidney retention was noticed for [^{67}Ga](NOTP)$^{3-}$. Almost all the radioactivity was cleared from tissues and organs within 24 h (data not shown) and no deposition of the complexes or any $^{67}Ga^{3+}$ particles in the liver–spleen region were observed.

All three Ga^{3+} complexes were cleared rapidly from the blood stream. In fact, 30 min after administration less than 0.03% of the injected dose was found in the blood. This behavior suggests that the chelates remained intact for the time interval they remained in the blood. The results of animal biodistribution studies at 2 and 48 h (in percent of injected dose per gram of organ) for [^{67}Ga](NOTA), [^{67}Ga](NOTP)$^{3-}$, and [^{67}Ga](NOTPME) are summarized in Figure 4, along with data for [^{67}Ga] citrate for comparative purposes. These results agree with the gamma-imaging data. They show clearly that, as opposed to [^{67}Ga] citrate, all the chelates studied were very specific and underwent only renal clearance, similar to other blood pool agents, *e.g.*, [^{111}In] DTPA (12). [^{67}Ga]citrate has low tissue specificity due both to transchelation of Ga^{3+} to transferrin and

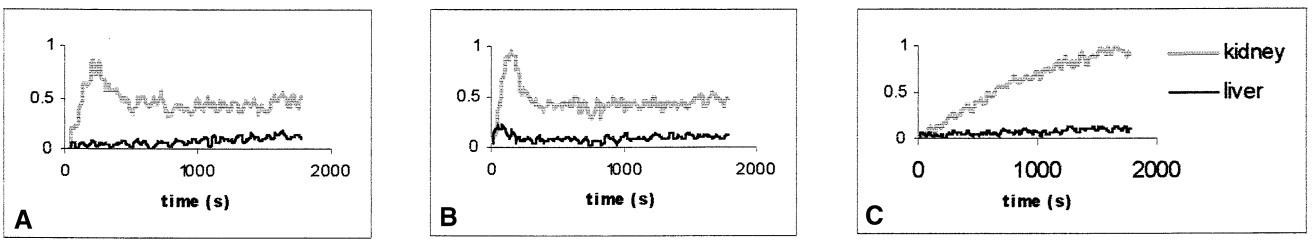

FIG. 2. Time–activity curves at the various regions of interest for (A) [^{67}Ga](NOTA), (B) [^{67}Ga](NOTPME), and (C) [^{67}Ga](NOTP)$^{3-}$.

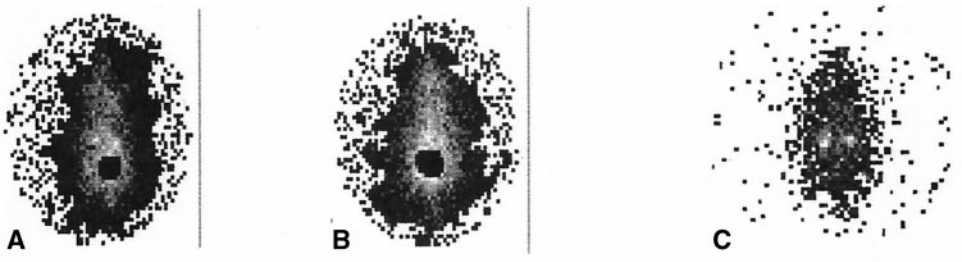

FIG. 3. Scintigraphic images at 30 min after injection with (from left to right) (A) [^{67}Ga](NOTA), (B) [^{67}Ga](NOTPME), and (C) [^{67}Ga](NOTP)$^{3-}$. Both (A) and (B) have a mask over the bladder due to its high activity.

formation of $Ga(OH)_4^-$ colloids that were trapped by the reticulo endothelial system (13, 24, 25).

This study demonstrates the high *in vivo* stability of the chelates. We found no evidence of bone marrow accumulation, which is observed when the gallium(III)-transferrin complex is formed (25). The thermodynamic stability constant of Ga(NOTA) has been reported elsewhere (log K_{st} = 30.98 [6]) and is considerably higher than that determined for the complex of Ga^{3+} with transferrin (log K_{st} = 20.3 [13]), which is the main competitor for Ga^{3+} in serum

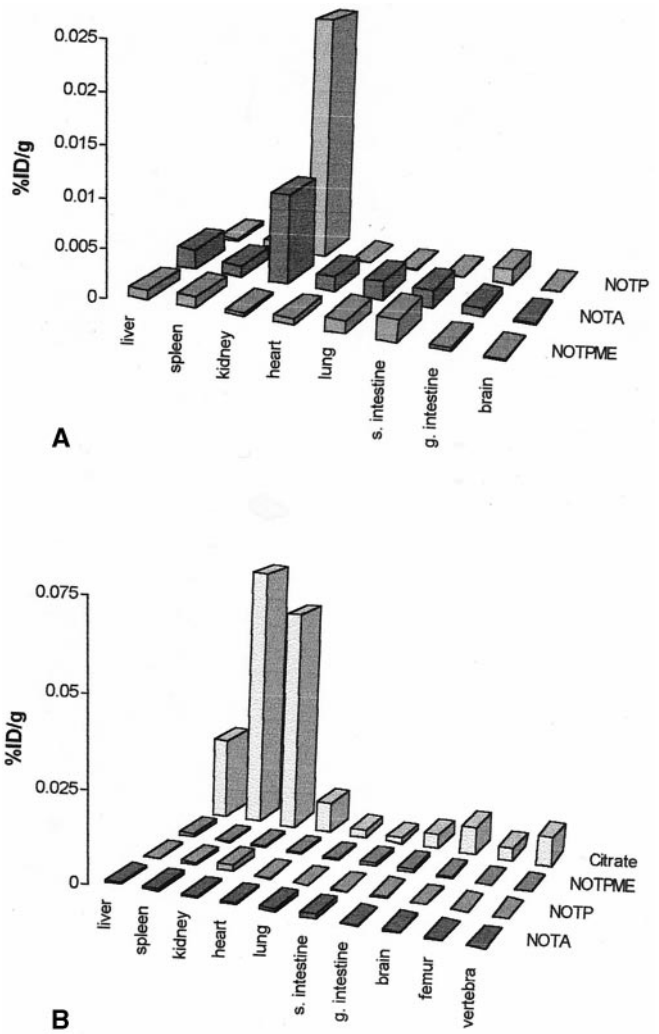

FIG. 4. Biodistribution of the $^{67}Ga^{3+}$ complexes in rat tissues at (A) 2 and (B) 48 h after injection of the chelates.

(13, 24, 25). The thermodynamic stability constant for $Ga(NOTP)^{3-}$ has not been determined, but our solution studies by ^{71}Ga NMR spectroscopy demonstrate that this complex remains intact in aqueous solution, in the pH range of 2–11 (M.I.M. Prata and C.F.G.C. Geraldes, manuscript in preparation). Other Ga^{3+} complexes with triazamacrocyclic ligands, such as 1,4,7-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane and 1,4,7-tris(2 mercaptoethyl)-1,4,7-triazacyclononane are formed with very high *in vitro* and *in vivo* stability (7, 22). The triazamacrocycles display an high conformational and size selectivity toward cations and the high stability of these Ga^{3+} complexes may arise from the good fit of the metal ion (ionic radii = 0.76 Å) in the triazacyclononane macrocycle cavity. All these ligands seem to encapsulate the metal ion, insulating it efficiently from competing ligands.

None of the complexes passed through the blood–brain barrier, as expected for nonlipophilic complexes (9, 17).

In conclusion, we found that the neutral chelates [^{67}Ga]NOTA and [^{67}Ga]NOTPME have similar *in vivo* behavior, with high stability and rapid renal excretion. The replacement of the carboxylate pendant arms of NOTA by the methylenephosphonate monoethylester groups of NOTPME seemed not to affect the biodistribution and clearance of these complexes. However, the high negatively charged chelate of the NOTP ligand, [^{67}Ga](NOTP)$^{3-}$, had a considerably slower uptake and elimination by the kidneys. The main reason for this different *in vivo* behavior may be the neutral *versus* negative charge of the complexes. The high stability and rapid renal excretion of the NOTA and NOTPME chelates are favorable properties for their possible application as kidneys imaging agents, for both structural and functional studies.

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