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Comparison of 'classic' ^{99m}Tc–DTPA, ^{99m}Tc(CO)$_3$–DTPA and ^{99m}Tc(CO)$_2$(NO)–DTPA

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Abstract—Diethylenetriamine pentaacetic acid (DTPA) was labeled with ^{99m}Tc in three different ways, resulting in 'classic' ^{99m}Tc–DTPA, ^{99m}Tc(CO)$_3$–DTPA and ^{99m}Tc(CO)$_2$(NO)–DTPA. The biodistribution of the formed DTPA-complexes was studied in mice with a special emphasis on the behavior of the novel tricarbonyl and dicarbonyl-nitrosyl complexes, which was clearly differing from that of 'classic' ^{99m}Tc–DTPA. The conversion of a Tc-tricarbonyl complex to a Tc-dicarbonyl-nitrosyl complex using NO^+ reagents offers a synthetic tool for preparing a novel class of ^{99m}Tc labeled compounds.

1. Introduction

A few years ago, Alberto et al. developed a convenient high-yield synthesis for ^{99m}Tc-tricarbonyl complexes.[1] A large number of such complexes has been reported since then and the access to this kind of technetium chemistry became even easier with the introduction of the IsoLink™ kit (Tyco-Mallinckrodt, Petten, The Netherlands). ^{99m}Tc(I)-complexes with a Tc(CO)$_3$-moiety show particular characteristics as compared to 'classic' Tc-compounds.[2–6] First clinical studies using Tc-tricarbonyl labeled biomolecules also revealed the potential clinical usefulness of such ^{99m}Tc-complexes.[7] However, for only a limited number of ^{99m}Tc-tricarbonyl complexes with simple chelators (not attached to a biomolecule) biological properties have been reported.[8–11]

Recently, this kind of bioorganometallic complex chemistry has been extended to dicarbonyl-nitrosyl complexes with a M(CO)$_2$(NO)-moiety (M=Re, Tc), which can be prepared starting from Re- or Tc-tricarbonyl complexes.[12] The introduction of a charged NO^+ group instead of a neutral CO group changes the charge of the formed Tc-dicarbonyl-nitrosyl complexes by +1 as compared to the original corresponding Tc-tricarbonyl compounds. Also the electronic properties of the Tc-nitrosyl complex differ, as NO^+ is a stronger π-acceptor, but a weaker σ-donor as compared to the isoelectronic CO.[13] To our knowledge, no reports about the biological properties of this new class of ^{99m}Tc(CO)$_2$(NO)-complexes have been published to date.

The chelating agent diethylenetriamine pentaacetic acid (DTPA) is a ligand known to form Tc-tricarbonyl and Tc-dicarbonyl-nitrosyl complexes.[14,15] The distance between the available oxo- and nitrogen heteroatoms is ideal, resulting in two five-membered rings when the radiometal is attached to the ligand.[16] Moreover, also the 'classic' ^{99m}Tc–DTPA-complex is well known, made by reduction of $^{99m}TcO_4^-$ with stannous ions in the presence of DTPA and used as a renal imaging radiopharmaceutical.[17] This made DTPA the ligand of choice to study the different Tc-cores mentioned above.

In the present study, we have compared the labeling and biodistribution characteristics in mice of the three different ^{99m}Tc–DTPA complexes: the 'classic' nuclear imaging agent ^{99m}Tc–DTPA (**1**), ^{99m}Tc(CO)$_3$–DTPA (**2**) and ^{99m}Tc(CO)$_2$(NO)–DTPA (**3**). Biodistribution experiments with complexes containing the same chelating ligand, but different Tc-cores, might provide the best insight in the influence of changes at the Tc-center on biological properties. A special emphasis was put on the novel tricarbonyl and dicarbonyl-nitrosyl complexes to investigate the potential of these ^{99m}Tc-complexes as clinically useful radiopharmaceuticals.

2. Results

Radiochemistry. ^{99m}Tc(CO)$_3$–DTPA (**2**) was obtained in similarly high yields (>98%) as 'classic' ^{99m}Tc–DTPA (**1**). Reversed phase high pressure liquid chromatography (RP-HPLC) showed the peak of (**1**) at a retention time (t_R) of

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3.5 min, while the more lipophilic (**2**) showed up at a t_R of 11.5 min (Fig. 1). In the same HPLC-system, $[^{99m}TcO_4]^-$ and the ^{99m}Tc-tricarbonyl precursor (**4**) had a t_R of 4.5 and 6.5 min, respectively. As shown in the HPLC-chromatograms of the unpurified reaction mixtures, none of these starting or intermediary ^{99m}Tc-compounds was present in any of the ^{99m}Tc–DTPA preparations.

The formation of $^{99m}Tc(CO)_2(NO)$–DTPA (**3**) in water using $NOHSO_4$ afforded only moderate to poor yields and not a single, well-defined product. The conversion with $NOBF_4$ in CH_2Cl_2 or acetonitrile gave better results, but disadvantages were the time consuming procedure (including a complete change from saline to the organic solvent) and the overall lower yield as compared to the model complexes of rhenium with picolinic acid and iminodiacetic acid.[12,14]

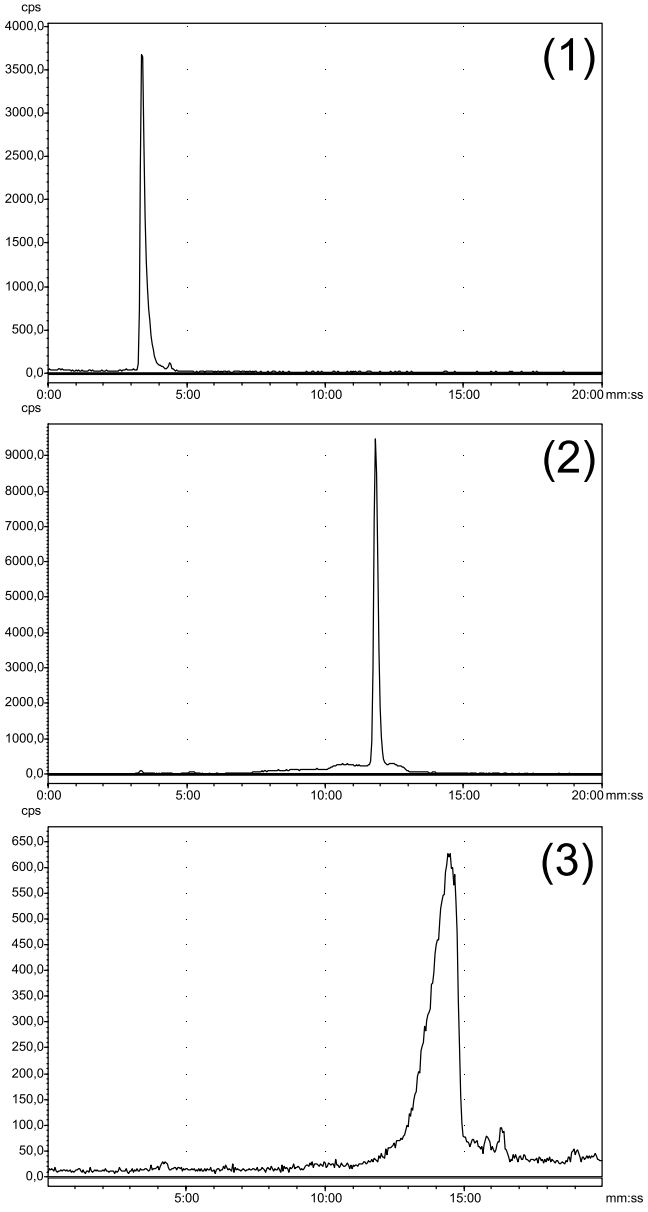

Figure 1. Reversed phase HPLC-analysis of the unpurified reaction mixtures of 'classic' ^{99m}Tc–DTPA (**1**), $^{99m}Tc(CO)_3$–DTPA (**2**), and $^{99m}Tc(CO)_2(NO)$–DTPA (**3**) (X-Terra RP-18 column, gradient elution from 0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in acetonitrile in 20 min; flow rate 1 ml/min).

A novel strategy to solve this problem was the application of a two-layer system with water and CH_2Cl_2 for the conversion. $NOHSO_4$ was covered with CH_2Cl_2 and an aqueous solution containing $^{99m}Tc(CO)_3$–DTPA (**2**) was directly applied on top of it as second layer. Complex (**2**) was able to switch over to the organic layer and reacted with $NOHSO_4$, while $^{99m}TcO_4^-$ and inorganic salts remained in the aqueous layer. In this system, $NOHSO_4$ acts as suitable source of NO^+, whereas in direct contact with water it spontaneously releases brown gases (NO_2) in a vigorous reaction. $^{99m}Tc(CO)_2(NO)$–DTPA (**3**) was obtained almost pure from the organic layer, but the yields were quite low. Mass spectral analysis of the isolated compound by LC-MS supported the formation of a $Tc(CO)_2(NO)$–DTPA complex. The detected mass of 576.90 Da corresponds with the theoretical value of 577.02 Da. Kryptofix 2.2.2 (377.26 Da) was added to the mobile phase and served as lock mass for accurate mass determination (Fig. 2). The conversion to (**3**) was completed after 4–5 h. For practical reasons the reaction was allowed to proceed overnight.

A second new strategy to form $^{99m}Tc(CO)_2(NO)$–DTPA (**3**) was the isolation of the $^{99m}Tc(CO)_3$-precursor (**4**) in acetonitrile by RP-HPLC and adding this solution to a mixture of DTPA and $NOHSO_4$ (solid). This is the fastest of the three methods, but the yields were varying from 60 to 89%. RP-HPLC analysis showed the main peak of the intended complex at a retention time of 14.2 min and smaller peaks of yet unidentified ^{99m}Tc-compounds in the region 16 min (Fig. 1). For biodistribution experiments the main peak was isolated by RP-HPLC.

Stability tests. Stability tests of (**3**) have been performed in acetonitrile, CH_2Cl_2, water, phosphate buffer (0.025 M, pH=7.4) and blood plasma. Complex (**3**) was almost unchanged in the organic solvents CH_2Cl_2 and acetonitrile after 24 h. In water and phosphate buffer only a limited degradation of $^{99m}Tc(CO)_2(NO)$–DTPA was detected after 24 h After incubation in blood plasma analysis using size exclusion HPLC (SEC-HPLC, Superdex™ column, phosphate buffer as mobile phase) showed a peak at a retention time of 12–15 min in case of $^{99m}Tc(CO)_2(NO)$–DTPA, which was not observed for any of the two other ^{99m}Tc–DTPA-complexes. In aqueous solution, complexes (**2**) and (**3**) eluted at 31–32 min as well-defined peaks, showing almost identical SEC-HPLC chromatograms. In a control experiment, ^{99m}Tc-labeled albumin eluted also at a comparable retention time of 12–15 min, strongly suggesting a rapid and pronounced binding of the radionuclide or the intact complex to plasma proteins in case of $^{99m}Tc(CO)_2(NO)$–DTPA.

As serum proteins contain essentially cysteine and histidine as competing coordination sites,[8] the stability of $^{99m}Tc(CO)_2(NO)$–DTPA against an excess of histidine and cysteine was tested ('histidine and cysteine challenge'). Complex (**3**) remained unchanged under these conditions after 30 min, 1, 2, and 20 h and formation of a $^{99m}Tc(CO)_2(NO)$-species with histidine or cysteine was not observed. The histidine complex $[^{99m}Tc(CO)_2(NO)(HIS)]^+$ was