VIA MEDICA

Nuclear Medicine Review 2004
Vol. 7, No. 1, pp. 1–5

ISSN 1506–9680

Original

Chemical and biological evaluation of technetium (I) Tricarbonyl complexes with EHIDA and DPD

^{99m}Tc (I) complexes of EHIDA and DPD

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[Received 17 XI 2003; Accepted 22 II 2004]

Abstract

BACKGROUND: ^{99m}Tc-phosphate and ^{99m}Tc-IDA complexes, made by the addition of $^{99m}TcO_4^-$ to the kits, have been applied to bone and gallbladder imaging respectively, for many years. In this paper, an effort to label DPD and EHIDA with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was carried out.

MATERIAL AND METHODS: DPD and EHIDA were synthesised and prepared in kit form in INS "Vinča". A carbonyl labelling agent Isolink™ (Mallinckrodt Medical B.V.) and a carbonyl precursor (NCRS Demokritos) were applied. The samples of each compound were added to a vial containing ^{99m}Tc-carbonyl precursor, in which original pH (10÷11) was neutralised to a pH of around 5.5 or 7.5, the same one as the pH of the investigated compounds. After heating, the reaction products were analysed by HPLC equipped with UV and g-detector, with TEAP 0.05 M, methanol and water as solvent. The biological evaluation of ^{99m}Tc(I)-coordinated compounds, as well as ^{99m}Tc-DPD and ^{99m}Tc-EHIDA complexes, involved a bio distribution examination on Wistar rats.

RESULTS: The results have shown that hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor facilitates the formation of Tc (I) complexes with these ligands, based on the tricarbonyltechnetium (I) core. The changes in structure of DPD and EHIDA labelled molecules influenced biological behaviour: $^{99}Tc(CO)_3$-DPD did not accumulate in bone (< 1% of the complex was found in the femur), while $^{99}Tc(CO)_3$- EHIDA has shown slower billiary excretion and faster filtration through the kidneys.

DISCUSSION: The results of the labelling of DPD and EHIDA with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and their chemical and biological behaviour, in comparison with the same one for ^{99m}Tc-DPD and ^{99m}Tc-EHIDA, confirmed that different oxidation states of technetium make the formation of a variety of complexes with quite different behaviour possible.

Key words: Technetium-99m, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, labelling, EHIDA, DPD

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Introduction

The first results of a synthesis of $[^{99m}Tc(CO)_3(H_2O)_3]^+$, the air and water stable organometallic aqua complex obtained directly from $^{98}Mo/^{99m}Tc$ generator, were presented by Albeto et al. in 1998 [1–4]. This precursor allowed the complex-formation ^{99m}Tc (I) aqua ion with different mono, bi- and tridentate ligands based on the

tricarbonyl technetium (I) core, by changing of three labile coordinated H_2O molecules with other ligands [5].

A number of ^{99m}Tc-phosphate compounds and ^{99m}Tc-IDA complexes, made by adding $^{99m}TcO_4^-$ to a penicillin vial with a lyophilised form of phosphate compounds or IDA derivatives (kit form), were applied for bone and gallbladder imaging respectively. They formed mixed-metal complexes containing Tc (+3, +4 or +5) and Sn (II) in variable concentration [6].

The results of the labelling of EHIDA and DPD with $[^{99m}Tc(CO)_3(H_2O)_3]^+$, and the examination of their chemical, pharmacological and biological behaviour in comparison with same one for ^{99m}Tc-EHIDA and ^{99m}Tc-DPD complexes, are presented in this paper.

Material and methods

EHIDA and DPD were synthesised and prepared in kit form for ^{99m}Tc-labelling by the direct tin (II) reduction method in INS "Vinca" (Vinca kits).

Each vial of the inactive freeze-dried EHIDA kit contained 40 mg N-(2, 6 diethylacetanilid) iminodiacetic acid, 1.2 mg $SnCl_2 \times \times 2H_2O$ and 9 mg NaCl in inert atmosphere. The pH of preparation was 5.5. ^{99m}Tc-EHIDA was prepared by adding 4 ml of 99m-technetiumpertechnetate ($^{99m}TcO_4^-$) in saline (Tc-generator, Vinča).

Each vial of the inactive freeze-dried DPD kit contained 11.0 mg 2, 3-dicarboxypropane-1,1-diphosphonic acid; 2.0 mg methylendiphosphonic acid; 0.5 mg $SnCl_2 \times 2H_2O$; 2.0 mg (4-aminobenzoyl)-glutamic acid and 40 mg NaCl in inert atmosphere. The pH of the preparation was 7.5. ^{99m}Tc-DPD was prepared by adding 10 ml of $^{99m}TcO_4^-$ in saline.

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ ion was prepared by the addition of 1 ml of ^{99m}Tc-pertechnetate (20–100 mCi $^{99m}TcO_4^-$) to a penicillin vial with lyophilised form of 7.15 mg sodium carbonate, 4.5 mg sodium boranocarbonate, 2.85 mg sodium tetraborate and 8.5 mg sodium tartrate (IsoLink™, Mallinckrodt Medical B.V., The Netherlands). After heating for 30 min in a boiling water bath and cooling, the basic solution (pH = 10 ± 11) was neutralised to pH 5.5 or 7.5 with 1 M HCl.

The samples of EHIDA or DPD were prepared by dissolving in water an appropriate amount of substances for obtaining 10^{-3} mol dm^{-3} solutions. The pH of solutions was around 5.5 and 7.5 respectively. ^{99m}Tc-carbonyl EHIDA and DPD complexes were prepared by the addition of 0.1 ml of DPD or EHIDA solutions to 0.9 ml of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with appropriate pH values. The vials were heated for 30 min in a boiling water bath.

HPLC analysis

A quality control inspection of the obtained $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (pH = 10 ± 11) was performed according Mallinckrodt Medical instruction, using gradient HPLC (Liquid Chromatograph, Hewlett Packard 1050, S/N with UV and Raytest gamma flow detector) on RP C18 column (250 × 4.6 mm). The solutions of 0.05 M triethylammonium phosphate (TEAP) of pH = 2.25 and methanol were used as mobile phases. The labelling efficiency for ^{99m}Tc-carbonyl targeted EHIDA or DPD was determined in isocratic HPLC with 80 % H_2O: 20 % TEAP, pH = 3.0 as a mobile phase (flow rate 0.7 ml/min).

Protein binding and lipophilicity measurements

The trichloracetic acid (TCA) precipitation method for determining the percentage of ^{99m}Tc- and ^{99m}Tc (I)-labelled EHIDA or DPD bound to 12% human albumin (12% HA, National Blood Transfusion Institute, Belgrade) during incubation at 37°C for different time intervals, was employed [7]. All lipophilicity measurements for ^{99m}Tc-labelled compounds were made by the solvent extraction method with n-octanol equilibrated with 0.15 M phosphate buffers (pH = 3.5 ÷ 7.5) [7]. The measurements were performed at room temperature.

Animal biodistribution

Organ biodistribution studies of ^{99m}Tc- and ^{99m}Tc (I)-labelled compounds were carried out on healthy white Wistar rats (four weeks old). The animals were sacrificed at corresponding times (3.5 and 60 min for labelled EHIDA and 60 min for DPD) after the application of 0.1 ml of ^{99m}Tc-labelled compound (~74 kBq). The radioactivity per whole organ of interest (or per gram) was measured in a NaI (Tl) well type detector and the percentage of radioactivity related to the administered dose was determined.

Results and discussion

The HPLC radiochromatogram of obtained $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (pH = 10 ± 11), is presented in Figure 1. For flow rate of 0.7 ml/min, retention times were R_t = 4.510 min for $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and R_t = 11.258 min for free $^{99m}TcO_4^-$. The peaks at R_t higher than 11.258 min were attributed to unknown radiochemical impurities.

At Figure 2 and Figure 3 the HPLC radiochromatograms for $[^{99m}Tc(CO)_3]^+$ targeted EHIDA and DPD respectively are presented. The retention times and labelling yield for EHIDA and DPD coordinated to $[^{99m}Tc(CO)_3]^+$, as well as radiochemical purity of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor are presented in Table 1. The differences in R_t values for $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $^{99m}TcO_4^-$ at HPLC radiochromatograms of precursor and Tc (I)-EHIDA are the consequence of differences in applied HPLC analyses (gradient or isocratic), as well as pH of mobile phases and preparation.

The results confirmed that the hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor facilitates the formation of Tc (I) complexes with both EHIDA and DPD, based on the tricarbonyl-technetium (I) core: radiochemical purity of $^{99m}Tc(CO)_3$-EHIDA was ~70 %, with 9.67 % of free $^{99m}TcO_4^-$ and 20.69 % of free, not bound $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as radiochemical impurities. The radiochemical purity of $^{99m}Tc(CO)_3$-DPD was much higher and exceeded 99 %.

The results of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, $^{99m}TcO_4^-$, ^{99m}Tc-carbonyl targeted EHIDA and DPD, as well as ^{99m}Tc labelled EHIDA and DPD, bound to 12% HA, determined by the TCA precipitation method, are presented in Table 3. As can be seen from the presented results, the binding to HA was species-dependent process. The values obtained for pertechnetate-99m in saline solution were much lower (~ 3.5%) than the values obtained for the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (~ 85%). While the stable no-reactive $^{99m}TcO_4^-$ showed only a weak binding to human albumin, the most reactive radiolabelled tricarbonyl precursor bound itself to that protein more effectively. This confirmed that the use

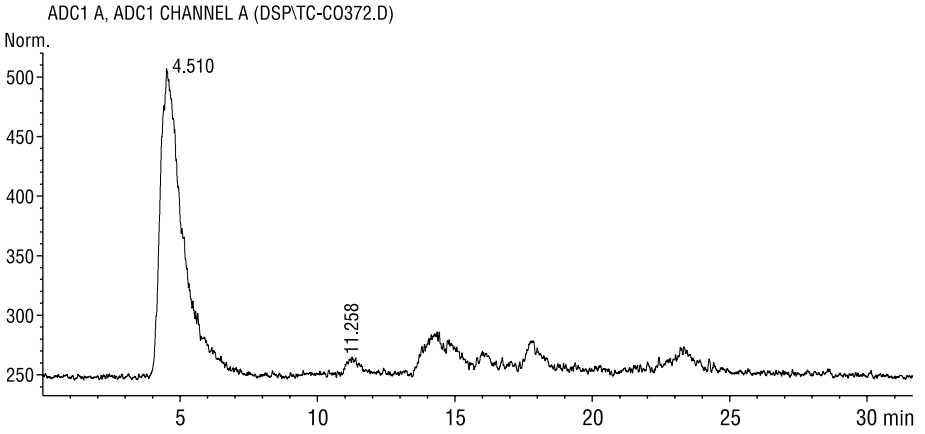

Figure 1. HPLC radiochromatogram of $[Tc(CO)_3(H_2O)_3]^+$ precursor, pH = 10 ÷ 11, flow rate 0.7 ml/min, R_t = 4.510 min for $[Tc(CO)_3(H_2O)_3]^+$, R_t = 11.258 min for free $^{99m}TcO_4^-$ and $R_t \geq 11.258$ min for unknown radiochemical impurities.

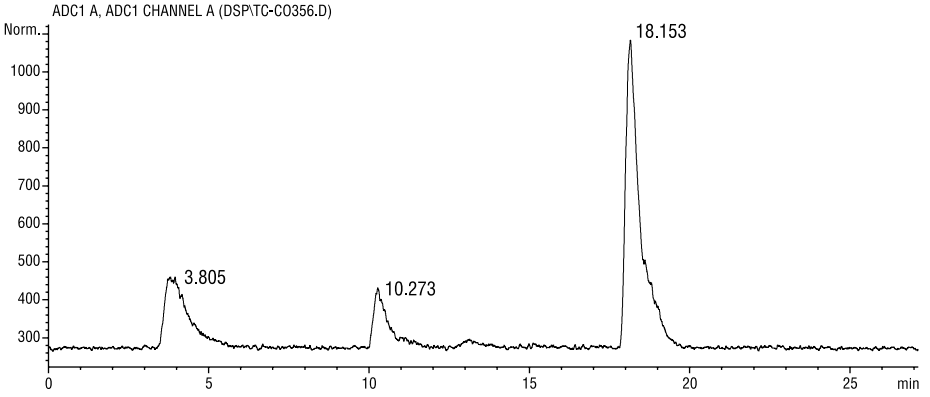

Figure 2. HPLC radiochromatograms of $^{99m}Tc(CO)_3$-EHIDA, pH ≅ 5.5, mobile phase: 20% TEAP: 80% H_2O (pH = 3), flow rate 0.7 ml/min, R_t = 18.153 min for $^{99m}Tc(CO)_3$-EHIDA; R_t = 3.805 min for $[Tc(CO)_3(H_2O)_3]^+$ and R_t = 10.273 min for free $^{99m}TcO_4^-$.

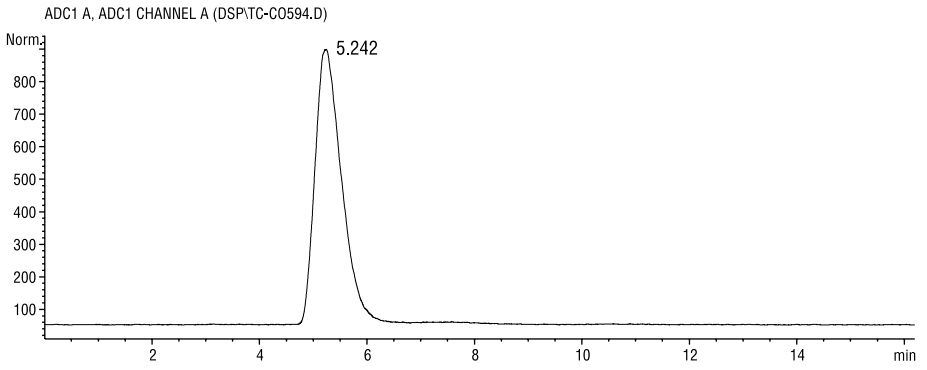

Figure 3. HPLC radiochromatograms of $^{99m}Tc(CO)_3$-DPD, pH ≅ 7.5, mobile phase: 20% TEAP: 80% H_2O (pH = 3), flow rate 0.7 ml/min, R_t = 5.242 min for $^{99m}Tc(CO)_3$-DPD.

of reduction agents like Sn (II) were necessary for higher ^{99m}Tc-labelling efficiency.

The percentage of labelled compounds bound to protein was higher for ^{99m}Tc-EHIDA than $^{99m}Tc(CO)_3$-EHIDA. The similarity was found for DPD: the values of protein binding percentage for ^{99m}Tc-DPD were higher than for $^{99m}Tc(CO)_3$-DPD.

The results of lipophilicity measurements for $^{99m}Tc(CO)_3$-EHIDA and ^{99m}Tc-EHIDA together with the results for $^{99m}Tc(CO)_3$-DPD and ^{99m}Tc-DPD, are presented in Figure 4. as a distribution coefficient in function of the pH for given preparation. The obtained l-octanol-buffer distribution pH profiles have shown that the distribution coefficient for EHIDA labelled with $[^{99m}Tc(CO)_3]^+$ and

Table 1. Retention times and labelling yield for EHIDA and DPD coordinated to $[Tc(CO)_3(H_2O)_3]^+$ (30 min heating in boiling water bath)

Samples	R_t [min]	Labelling yield (%)
$[Tc(CO)_3(H_2O)_3]^+$	4.510	97.51
Free $^{99m}TcO_4^-$	11.258	0.65
Unknown radiochemical impurities	>11.258	1.84
Free $[Tc(CO)_3(H_2O)_3]^+$	3.805	20.69
Free $^{99m}TcO_4^-$	10.273	9.67
$^{99m}Tc(CO)_3$-EHIDA	18.153	69.64
$^{99m}Tc(CO)_3$-DPD	5.242	> 99.00

Table 2. The percentage of protein binding of $^{99m}TcO_4^-$ $[^{99m}Tc(CO)_3(H_2O)_3]^+$, ^{99m}Tc-EHIDA and $^{99m}Tc(CO)_3$-EHIDA, as well as of ^{99m}Tc-DPD and $^{99m}Tc(CO)_3$- DPD, determined by TCA precipitation method

Labelled compound	Incubation time [min] 20	60
$^{99m}TcO_4^-$	3.47 ± 0.34	3.14 ± 0.40
$[^{99m}Tc(CO)_3(H_2O)_3]^+$	84.97 ± 1.18	83.01 ± 0.81
^{99m}Tc-EHIDA	91.38 ± 0.61	90.56 ± 0.10
$^{99m}Tc(CO)_3$-EHIDA	66.22 ± 0.07	64.59 ± 0.71
^{99m}Tc-DPD	78.16 ± 0.11	69.65 ± 0.19
$^{99m}Tc(CO)_3$- DPD	47.06 ± 1.72	45.21 ± 0.86

The percentage of protein binding is the mean values ± SD from five measurements

$^{99m}TcO_4^-$ are dependent on pH: at pH = 3.5 distribution coefficients are almost equal to 0.7 and with increased pH the distribution coefficient for ^{99m}Tc-EHIDA falls quickly to almost zero, whereas for $^{99m}Tc(CO)_3$-EHIDA it remains at about 0.3. The distribution coefficients for DPD labelled with $[^{99m}Tc(CO)_3]^+$ and $^{99m}TcO_4^-$ were almost equal, close to zero and independent from pH: this means that both compounds had a hydrophylic character for all examined pH values.

The biodistribution investigation results for $^{99m}Tc(CO)_3$-EHIDA and ^{99m}Tc-EHIDA are presented in Table 3. These results have shown that the biological behaviour of labelled EHIDA was different: the elimination through the intestine of ^{99m}Tc - EHIDA was faster, but slower elimination through the kidneys than $^{99m}Tc(CO)_3$-EHIDA.

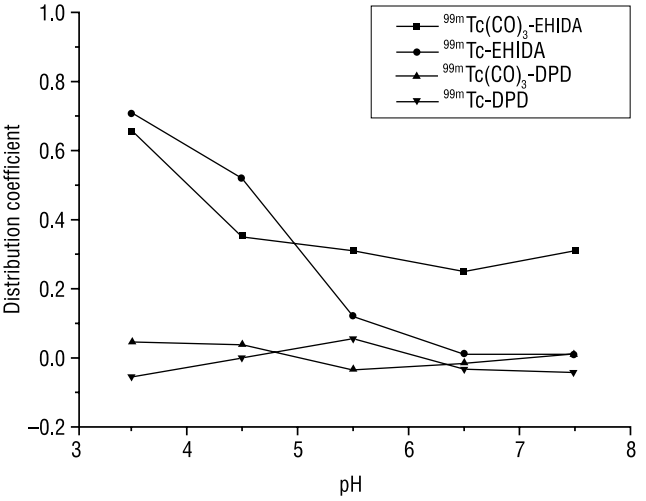

Figure 4. l-octanole buffer distribution pH profiles for $^{99m}Tc(CO)_3^-$ and ^{99m}Tc-labelled EHIDA and DPD.

The radioactivity in the liver of 3.5 min after the administration of $^{99m}Tc(CO)_3$-EHIDA was high and after 60 min it was still too high, twice as low than after 3.5 min, which means that the billiary excretion was slow. In comparison with $^{99m}Tc(CO)_3$-EHIDA, ^{99m}Tc-labelled EHIDA had lower values of radioactivity in the liver, but faster billiary excretion: 60 min after the application of radiopharmaceutical, negligible values of radioactivity were in the liver, but over 90% of it was in the intestine.

The biodistribution investigation results for $^{99m}Tc(CO)_3$-DPD are presented in Table 4, in comparison with the same one for ^{99m}Tc-DPD. Changes in the structure of DPD labelled complexes also influence biological behaviour. ^{99m}Tc (I) complexes of DPD did not accumulate in bone (< 1%/g of complex were found out in the femur). The accumulation of labelled compounds in liver was higher, as well as in the blood and muscle, not much higher in the lungs, but lower in the kidneys.

Conclusion

The presented results confirmed that oxidation state of technetium had a great influence on *in vitro* and *in vivo* behaviour of the investigated technetium coordination complexes, e.g. on their physicochemical parameters, pharmacological and biological behaviour. ^{99m}Tc (I) complexes of EHIDA showed lower percentages of protein binding and higher distribution coefficient values

Table 3. Organ distribution data of ^{99m}Tc-EHIDA and $^{99m}Tc(CO)_3$-EHIDA in Wistar rats, 3.5 and 60 min after administration (mean values ± SD, % ID/organ)

^{99m}Tc-labelled EHIDA	Time [min]	Lungs	Liver	Spleen	Kidneys	Stomach	Intestine	Blood*
^{99m}Tc-EHIDA[1]	3.5	0.57 ± 0.16	30.15 ± 8.36	0.23 ± 0.20	3.13 ± 1.59	0.62 ± 0.03	50.41 ± 11.91	1.10 ± 0.42
	60	0.11 ± 0.03	1.54 ± 0.13	0.05 ± 0.01	1.61 ± 0.29	–	93.01 ± 2.67	–
$^{99m}Tc(CO)_3$-EHIDA[2]	3.5	1.09 ± 0.24	70.67 ± 7.46	0.25 ± 0.13	13.73 ± 2.31	0.83 ± 0.18	11.12 ± 0.31	1.41 ± 0.17
	60	0.27 ± 0.05	37.30 ± 4.03	0.17 ± 0.07	0.92 ± 0.16	1.69 ± 0.40	37.51 ± 4.98	0.66 ± 0.03

* % ID/g; [1] Mean values ± SD for 20 series of commercial available ^{99m}Tc-EHIDA; [2] Mean values ± SD for 6 animals

Table 4. Organ distribution data of ^{99m}Tc-DPD and $^{99m}Tc(CO)_3$-DPD in Wistar rats, 60 min after administration (mean values ± SD, % ID/organ)

^{99m}Tc-labelled DPD	Lungs	Liver	Kidneys	Blood*	Muscle*	Bones*
^{99m}Tc-DPD[1]	0.20 ± 0.02	0.65 ± 0.29	1.71 ± 0.68	0.18 ± 0.02	0.04 ± 0.02	8.8 ± 1.9
$^{99m}Tc(CO)_3$- DPD[2]	0.47 ± 0.11	3.63 ± 0.61	1.00 ± 0.20	0.87 ± 0.11	0.12 ± 0.01	0.37 ± 0.03

* % ID / g; [1] Mean values ± SD for 20 series of commercially available ^{99m}Tc-DPD; [2] Mean values ± SD for 6 animals

than the conventional ^{99m}Tc-Sn (II)-EHIDA. The similar results from DPD complexes pointed out the differences in protein binding results between ^{99m}Tc (I)-DPD and ^{99m}Tc-Sn (II)-DPD complexes, but there was no significant difference in lipophilicity between them. Therefore, it could be for these reasons that ^{99m}Tc(I)-EHIDA had a slower billiary excretion than ^{99m}Tc-labelled EHIDA and ^{99m} Tc (I) complexes of DPD did not accumulate in bone as did ^{99m}Tc-DPD. In this way we obtained labelled compounds with quite different characteristics.

This paper has been financially supported by the Ministry of Science and Technology and the Development of Serbia, under Project OI 1980.

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (IsoLink™) was obtained free of charge owing to the generosity of Tyco Healthcare, Mallinckrodt Medical B.V., The Netherlands.

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