THE IMPACT OF TECHNETIUM-99m-RADIOPHARMACEUTICALS' DESIGN ON THEIR BIOLOGICAL BEHAVOIR

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ABSTRACT

The coordination has a great and not always predictable impact on the in-vivo behaviour of the small molecule into which the technetium-bearing chelate units is integrated. The different valence state of technetium in the complexes with some ligands changes the properties of these complexes, such as physico-chemical parameters and biological behaviour. The change of their biological behaviour has a great impact on quality of imaging study and on radiation dose to the patient. The results of the labelling of DPD and EHIDA with ^{99m}Tc(I) and their biological behaviour, in comparison with the same one for ^{99m}Tc(III)-DPD and ^{99m}Tc(III)-EHIDA complexes, confirmed that different oxidation state of ^{99m}Tc make possible forming variety of complexes with quite a different and unexpected biological behaviour.

Key words*:* Technetium-99m, oxidation state, labelling, EHIDA, DPD

Introduction

Radiopharmacies are used for the preparation of a wide range of products in a variety of dosage forms. The majority of radiopharmaceuticals are administered parentally and hence need to be prepared in conditions which satisfy both pharmaceutical and radiation safety requirements [1].

An ideal radiopharaceuticals should be organ specific, should not alter in vivo after administration to the patient and the target to nontarget activity ratio should be large, with a minimum radiation dose to the patient [2].

As technetium-99m has ideal physical properties (the 6-h physical half-life, the absence of β radiation, and the monochromatic 140-keV photons) for many applications in nuclear medicine, it is still the radionuclide of choice. For radiopharmaceuticals preparation it was often used like technetium pertechnetate (TcO_4^-), which had to be reduced in lower oxidation state using stannous chloride as reducing agent. Several factors will influence the resultant oxidation state following reduction of pertechnetate and complexation, including the nature of reductant and ligand, pH and temperature.

Besides the direct labelling approach, the use of bifunctional chelating agent (BFCA) has been more fruitful [3]. Nowadays, hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor allows the forming of Tc (I) radiopharmaceuticals based on the tricarbonyltechnetium (I) core [4, 5, 6].

A number of ^{99m}Tc-compounds, made by adding $^{99m}TcO_4^-$ to a penicillin vial with lyophilised form of those compounds (kits form), have been applied for diagnostic imaging. They formed mixed-metal complexes containing Tc (+3, +4 or +5) and Sn (II) in variable concentration. Numerous studies of structure-biodistribution relationships helped to make the design approaches more rational. But it is obvious that the complexity of determinants of desired bioactivity is not yet understood.

The coordination chemistry of technetium is complicated by the ease with which it can move from one oxidation state to another. Given any new ligand and/ or new set of reduction/coordination conditions, it is often difficult to predict which oxidation state(s) of technetium will result [7].

The results of the labelling of EHIDA and DPD with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $^{99m}TcO_4^-$ examination of their pharmacological behaviour in comparison with same one for ^{99m}Tc - EHIDA and ^{99m}Tc - DPD complexes, were presented in this paper.

Material and methods

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

^{99m}Tc(III)-EHIDA and ^{99m}Tc(III) - DPD were prepared from lyophilised kit by adding of 4 and 10 ml technetiumpertechnetate ($^{99m}TcO_4^-$) in saline (Tc-generator, Vinča) respectively.

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ ion was prepared by addition of 1 ml of ^{99m}Tc-pertechnetate (20 – 100 mCi $^{99m}TcO_4^-$ eluted in saline from ^{99m}Tc-generator, Vinča Institute) 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^{TM} ,Mallinckrodt Medical B.V., The Netherlands). After heating for 30 min in boiling water bath and cooling, pH of solutions were adjusted to around 5.5 and 7.5 (as pH of preparations) with 1 M HCl.

The samples of EHIDA or DPD were prepared by dissolving in water 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 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 boiling water bath.

Animal biodistribution - Organ biodistribution studies of ^{99m}Tc(III) – and ^{99m}Tc (I) - labelled compounds were carried out on healthy white Wistar rats (four weeks old). The animals were sacrificed in corresponding time (3.5 and 60 min for labelled EHIDA and 60 min for DPD) after application of 0.1 ml of ^{99m}Tc-labelled compound (~74kBq). 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 administered dose was determined.

Results and discussion

^{99m}Tc(III)-EHIDA radiopharmaceutical has been applied for gallbladder and biliary tree imaging.

The biodistribution investigation results for ^{99m}Tc(I)-EHIDA and ^{99m}Tc(III)–EHIDA are presented in Table 1.

These resultes have shown that the biological behaviour of labelled EHIDA were different: the elimination through the intestine of ^{99m}Tc(III) - EHIDA was faster, but slower elimination through the kidneys than ^{99m}Tc(I) - EHIDA. The radioactivity in liver 3.5 min after administration of ^{99m}Tc(I) - EHIDA was high and after 60 min it was still to high, twofold lower than after 3.5 min, which means that the billiary excretion was slow. In comparison with ^{99m}Tc(I) - EHIDA, ^{99m}Tc(III) – labelled EHIDA had lower values of radioactivity in liver, but faster billiary excretion: 60 min after application of radiopharmaceutical, negligible values of radioactivity were in liver, but over 90 % of it was in intestine.

^{99m}Tc(III)-DPD radiopharmaceutical has been applied as bone scanning agent, because it bind strongly to the surface of hydroxyapatite crystal by chemical adsorption.

The biodistribution investigation results for ^{99m}Tc(I) - DPD are presented in Table 2., in comparison with the same one for ^{99m}Tc(III) – DPD.

Table 1.Organ distribution data of ^{99m}Tc(III) - EHIDA and ^{99m}Tc(I) - 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	Intestine	Blood*
^{99m}Tc(III) - EHIDA	3.5	0.6±0.2	30.2±4.4	0.2±0.1	3.1±1.6	50.4±11.9	1.1±0.4
	60	0.1±0.1	1.5±0.1	0.1±0.1	1.6±0.3	93.0±2.7	-
^{99m}Tc(I) - EHIDA	3.5	1.1±0.2	70.7±3.5	0.3±0.1	13.8±2.3	11.1±0.3	1.4±0.2
	60	0.3±0.1	37.3±4.1	0.2±0.1	0.9±0.2	37.5±5.0	0.7±0.1

** % ID / g*

Six animals for each of radiopharmaceuticals and time intervals

Table 2. Organ distribution data of ^{99m}Tc(III)-DPD and ^{99m}Tc(I) 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(III) – DPD[1]	0.2±0.1	0.7±0.3	1.7±0.7	0.2±0.1	0.04±0.02	8.8±1.9
^{99m}Tc(I) – DPD[2]	0.5±0.1	3.6±0.6	1.0±0.2	0.9±0.1	0.12±0.01	0.4±0.1

** % ID / g*

[1] Mean values ± SD for 20 series of comercial available ^{99m}Tc(III)-DPD

[2] Mean values ± SD for six animals

The changes in structure of DPD labelled complexes influence the biological behaviour, too. ^{99m} Tc (I) complexes of DPD did not accumulate in bone (<1 % / g of complex were found out in femur). The accumulation of labelled compound in liver was higher, as well as in blood and muscle, not much higher in lungs, but lower in kidneys.

Conclusion

From recent investigations it can be concluded that oxidation state in technetium coordination complexes has a great impact on in vivo behaviour of the these complexes. The different structures of technetium complexes have shown difference in pharmacological behaviour. The complexes EHIDA and DPD with ^{99m}Tc (I) showed difference in the biological behaviour from that of the conventional $SnCl_2$-EHIDA and –DPD preparation. Accumulation ^{99m}Tc (I)-DPD in target tissues was to lower than the conventional ^{99m}Tc(III)-DPD and the target-to-nontarget activity ratio was very small. The higher accumulation in nontarget tissues increase radiation dose in those tissues while the efficiency in the diagnosis of diseases was a minimal. The longer retain of ^{99m}Tc(I)-EHIDA in liver than conventional ^{99m}Tc(III)-EHIDA increase radiation dose in that organ without possibilities of evaluation the functional status of the hepatocytes (rapid hepatic clearance) and patency of the billiary duct. Because

of that, they must consider the physicochemical conditions during the labelling suitable compounds with ^{99m}Tc.

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