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[^{161}Tb]Tb-Thz-Phe-D-Trp-Lys-Thr-DOTA: a potential radiopharmaceutical for the treatment of neuroendocrine tumors

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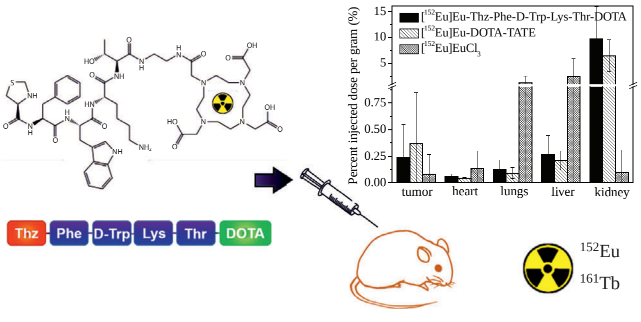
Thz-Phe-D-Trp-Lys-Thr-DOTA, a conjugate of the DOTA chelator and the Thz-Phe-D-Trp-Lys-Thr pentapeptide, was labeled with ^{152}Eu and ^{161}Tb radionuclides, where ^{161}Tb has decay characteristics suitable for its use in cancer therapy. For the [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA complex, the biodistribution in nude mice bearing IMR-32 tumors was evaluated for the first time. It was shown that the complexes of the conjugate demonstrate accumulation in the tumor at the level of DOTA-TATE, another peptide conjugate widely used in nuclear medicine for the diagnosis and therapy of neuroendocrine tumors, which allows Thz-Phe-D-Trp-Lys-Thr-DOTA to be considered as a potential biological vector for radiopharmaceuticals.



Keywords: peptide receptor radionuclide therapy, somatostatin analogs, neuroendocrine tumors, short peptides, ^{161}Tb.

Neuroendocrine tumors are rare and difficult to diagnose diseases characterized by overexpression of somatostatin hormone (SST) receptors on the surface of tumor cells.[1,2] Various somatostatin analogs are used for therapy of such diseases, among which cyclic octapeptides and their conjugates are already widely put in clinical practice worldwide.[3–9] In this work, we consider the Thz-Phe-D-Trp-Lys-Thr-DOTA conjugate, which includes the DOTA [2,2′,2′′,2′′′-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid] chelator and the Thz-Phe-D-Trp-Lys-Thr pentapeptide (see Figure S1 in Online Supplementary Materials), where Phe-D-Trp-Lys-Thr is the key amino acid sequence, *i.e.* it is responsible for recognition by somatostatin receptors (SSTR). Previously, [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA [$T_{1/2}$(^{152}Eu) = 13.537 y, with a decay mode of β^-/EC (electron capture)], has demonstrated affinity for SSTR types 2 and 5 on the surface of human neuroblastoma cells IMR-32 *in vitro* [$K_d(1) = 0.1$ nM and $K_d(2) = 2.25$ nM for two types of binding sites], as well as the presence of beta turn conformation, the required type of secondary structure, shown by circular dichroism spectroscopy.[10] On the one hand, expanding the range of different somatostatin analogs with demonstrated affinity for various types of SST receptors may contribute to the development of personalized medicine methods, since different types of tumors are characterized by different SSTR expression.[11–13] On the other hand, the transition to shorter biological vectors in the composition of radiopharmaceuticals will lead to cheaper production and, accordingly, an increase in the availability of radiopharmaceuticals to the population for timely diagnosis and treatment.

Terbium attracts great attention among the elements with potential medical radionuclides, since it has four isotopes with 149, 152, 155 and 161 mass numbers, covering the entire range of possible medical applications. Among them, ^{161}Tb has decay characteristics suitable for its use both in therapy ($T_{1/2}$ = 6.88 d; with the β^- decay mode, the average energy of beta particles is 150 keV) and in theranostics as the presence of low-energy gamma lines allows visualization in a gamma camera using single-photon emission computed tomography (SPECT) or scintigraphy. According to some estimates, ^{161}Tb in terms of its decay properties even surpasses the widely used therapeutic ^{177}Lu, since ^{161}Tb emits a significant amount of conversion and Auger electrons, which provide a much higher local dose density due to their shorter range in the tissue (0.5–30 µm). Previously ^{161}Tb has been used for labeling somatostatin analogs and other molecules, and has also been used in therapeutic practice.[14–17]

In our previous work, Thz-Phe-D-Trp-Lys-Thr-DOTA was successfully labeled with ^{44}Sc, ^{90}Y, ^{152}Eu, and ^{207}Bi radionuclides, while ^{152}Eu was used as a long-lived surrogate of medical rare earth element (REE) radionuclides, in particular, ^{161}Tb due to similar chemical and coordination properties of lanthanide cations.[10,18] The Tb^{3+} and Eu^{3+} cations indeed have similar characteristics: close ionic radii[19] and hydrolytic properties[20]

Table 1 Comparative characteristics of the considered cations.

M^{3+}	R_i (CN 8)†/Å	lg $\beta_{M\text{-}OH}$‡	lg $\beta_{M\text{-}DOTA}$§,21,22
Eu^{3+}	1.07	-7.9	26.2; 28.2
Tb^{3+}	1.04	-7.86	28.6

(Table 1), which allows one to expect similar results both in optimal labeling conditions and in the stability of the formed complexes in various media.

To compare labeling results, Thz-Phe-D-Trp-Lys-Thr-DOTA was labeled with ^{161}Tb produced by irradiating a natural gadolinium oxide target with 13–15 MeV deuterons on the U-120 cyclotron at the D. V. Skobeltsyn Institute of Nuclear Physics (M. V. Lomonosov Moscow State University) by the ^{160}Gd(d,n) reaction. Figure 1 shows the combined results of Thz-Phe-D-Trp-Lys-Thr-DOTA labeling with both cations. It can be seen that the terbium complex formation proceeds even faster than the formation of the europium complex: the labeling yield of 98% is achieved already in 5 min at pH 4 and T = 90 °C. This increase in the reaction rate may be due to a larger value of the stability constant of the Tb–DOTA complex compared to the Eu analog: for the latter, the published data are somewhat different, but both values are less than the value for the constant with terbium (Table 1). An increase in the pH value up to 6.5 leads to a decrease in the rate of the labeling reaction, possibly due to hydrolysis of the cation.

For the most effective delivery of a therapeutic dose of ionizing radiation, the radiopharmaceutical should not undergo transchelation by proteins in the blood plasma. In order to evaluate the stability of the complexes under similar conditions, the M-Thz-Phe-D-Trp-Lys-Thr-DOTA (M = Eu^{3+}, Tb^{3+}) complexes with radiochemical purity from 95 to 98% were used for *in vitro* stability evaluation. The radioactive fraction bound by serum proteins was determined by separating the serum proteins by ethanol precipitation. The obtained results demonstrate that with a 100-fold excess of fetal bovine serum (peptide diluted to 1 μmol dm^{-3}), there is a release of 0 to 10% of the cation from the M-Thz-Phe-D-Trp-Lys-Thr-DOTA complexes within 30–60 min (Figure 2). After 24 h of incubation, the radiochemical purity of M-Thz-Phe-D-Trp-Lys-Thr-DOTA did not change.

In general, the stability of [^{161}Tb]Tb-Thz-Phe-D-Trp-Lys-Thr-DOTA is consistent both with data for other complexes with DOTA–peptide conjugates, which are characterized by high stability in serum for at least 24 h, and with high stability of [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA in particular. The obtained labeling and stability results confirm the high similarity of the labeled complexes behavior and make it possible to use $^{152}Eu^{3+}$ as a long-lived surrogate of ^{161}Tb under *in vivo* conditions.

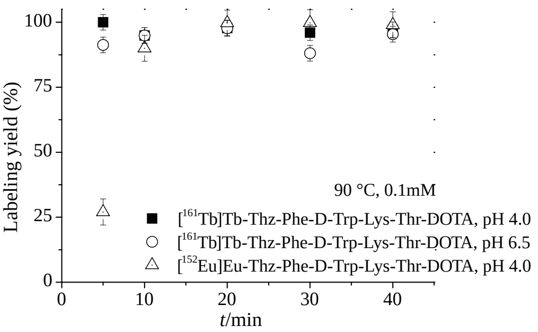


Figure 1 Time dependence of the labeling yield of Thz-Phe-D-Trp-Lys-Thr-DOTA with $^{152}Eu^{3+}$ and $^{161}Tb^{3+}$ at 90 °C.

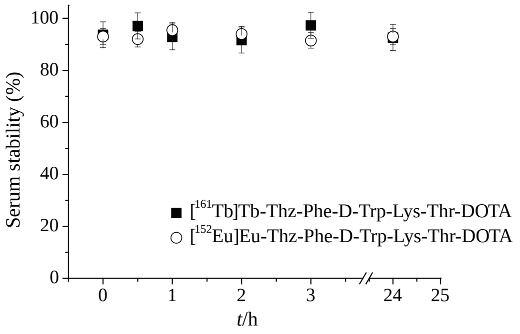


Figure 2 Stability of M-Thz-Phe-D-Trp-Lys-Thr-DOTA in bovine serum.

To analyze the biodistribution of Thz-Phe-D-Trp-Lys-Thr-DOTA complexes in nude mice, we used the IMR-32 (human neuroblastoma) cell line expressing SSTR2 and SSTR5, which was previously used to evaluate the affinity to receptors *in vitro*. At this stage, we assumed both the confirmation of targeting and accumulation of the conjugate in a tumor and the analysis of the stability of the labeled complexes *in vivo*. For this purpose, the biodistribution of the labeled conjugate is usually compared with the distribution of the free radionuclide cation. Another option would be to 'block' the target receptors on the surface of the tumor and some other organs by pre-injecting a drug that reliably binds to the same receptors. In this work, an octapeptide octreotide that specifically binds to SSTR2 and SSTR5 was used as a 'blocking' substance.

Nude mice were inoculated with tumor cells and the animals were divided into three groups depending on the administered substance: 1) [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA; 2) [^{152}Eu]Eu-DOTA-TATE as a positive control substance, since DOTA-TATE (DOTA-D-Phe^1-Cys^2-Tyr^3-D-Trp^4-Lys^5-Thr^6-Cys^7-Thr^8-ol, disulfide bridge: 2–7) should accumulate in the SSTR2- and SSTR5-expressing tumors; and 3) [^{152}Eu]$EuCl_3$ as a negative control substance. The number of animals was 4 for each group. The results are presented in Figure 3 and in Table S1.¶

It can be seen from the obtained results that free europium cations mainly accumulate in the lungs, liver, and spleen, without demonstrating clearance, while for [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA and [^{152}Eu]Eu-DOTA-TATE the greatest accumulation is observed in kidneys. It can be assumed that [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA undergoes excretion *via* kidneys, which was also confirmed by the results of TLC analysis of urine samples (Figure S2) and collected urine measurements over 6 h (Figure S3), where the highest counting rate is observed after 2 h after injection. Interestingly, the [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA accumulation in kidneys exceeds the same parameter for [^{152}Eu]Eu-DOTA-TATE, which may be due to the shortness of the amino acid sequence in the peptide and its greater hydrophilicity. In general, the biodistribution profile of the complex correlates with the distribution of [^{152}Eu]Eu-DOTA-TATE and differs significantly from [^{152}Eu]$EuCl_3$, so we can talk about both the stability of the complex *in vivo* and its ability to accumulate in the target organ, namely tumor. That is, the affinity of the labeled non-cyclic short pentapeptide conjugate is maintained under *in vivo* conditions.

[†] R_i (CN 8) are the ionic radii of the eight-coordinated M^{3+} ions.

[‡] $\beta_{M\text{-}OH}$ are the hydrolysis constants for the reactions: $M^{3+} + H_2O = H^+ + M\text{-}OH^{2+}$.

[§] $\beta_{M\text{-}DOTA}$ are the stability constants of M–DOTA complexes.

[¶] All *in vivo* experiments were performed in accordance with the EU Directive 2010/63/EU for animal experiments and were approved by the Bioethics Commission of M. V. Lomonosov Moscow State University, meeting no. 128-a, 31.05.2021, protocol no. 131-d.

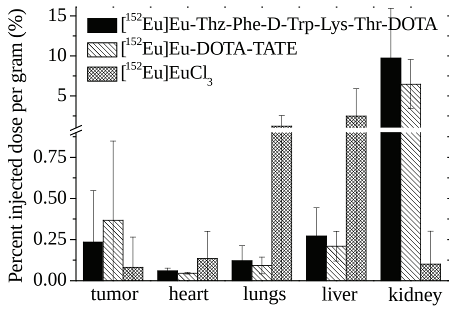


Figure 3 Distribution of ^{152}Eu-labeled compounds in nude mice at 6 h after administration, $P = 95\%$, $n = 4$ for each group.

In addition to the tumor, SSTR expression is also characteristic of several organs, primarily kidneys, adrenals, pancreas, gastrointestinal tract and lungs. According to the results of the experiment with blocked receptors, the accumulation of [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA in these organs is significantly reduced compared to animals that were not previously injected with octreotide (Figure 4).

Noteworthy that the ability of such peptides (short analogs of somatostatin) to selectively accumulate in the SSTR-expressing tumors was demonstrated for the first time. Earlier, besides our *in vitro* experiments[10] only indirect action inhibiting some hormone and expression has been shown *in vivo*.[23] In the future it seems reasonable to analyze the biodistribution of the labeled complex over a longer period.

From the point of view of delivery of a therapeutic dose of radiation, small molecule compounds can be an attractive alternative for antibodies, since they have a comparable affinity to the target receptors, as well as improved pharmacokinetic characteristics. In turn, ultrashort peptides, which include only the key amino acid sequence for receptor recognition, remain little studied objects, although the effectiveness of such compounds has been demonstrated using small prostate-specific membrane antigen (PSMA) ligands as an example. Thz-Phe-D-Trp-Lys-Thr-DOTA is the first ultrashort linear peptide conjugate with demonstrated affinity for somatostatin receptors type 2 and 5 *in vivo* and the ability to accumulate in the tumor, which allows it to be considered as a potential biological vector in the composition of radiopharmaceuticals.

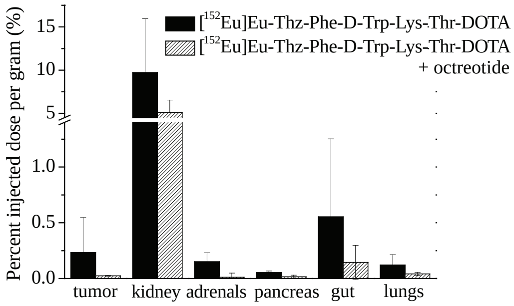


Figure 4 Distribution of [^{152}Eu]Eu-Thz-Phe-D-Trp-Lys-Thr-DOTA in mice at 6 h after administration in the presence and absence of an excess of the blocking substance (octreotide), $P = 95\%$, $n = 4$ for each group.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.06.009.

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