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^{99m}Tc-COMPLEX OF NOVEL DIAMINE-DIOXIME LIGAND

M. Mirković[1], S. Vranješ-Đurić[1], D. Stanković[1], Đ. Petrović[1], D. Mijin[2], N. Nikolić[1]

[1]*Laboratory for Radioisotopes, Vinča Institute of Nuclear Sciences, University of Belgrade, P.O.Box 522, 11001 Belgrade, Serbia*
[2]*Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, P.O. Box 3503, 11120 Belgrade, Serbia*

Abstract
Novel diamine-dioxime ligand, 4,7-diaza-3,8-diethyldecane-2,9-dione bis oxime (LH_2), derivative of hexamethyl-propylene amine oxime (HMPAO), was synthesized in order to develop new brain perfusion imaging agent, based on ^{99m}Tc(V) complexes. The ^{99m}Tc(V)-d,l-HMPAO complex is well-known radiopharmaceutical for brain imaging. The structures of the synthesized compound were characterized by UV-Vis, IR, ^{1}H NMR and ^{13}C NMR. The procedure for radiolabeling of diamine-dioxime with ^{99m}Tc was developed and radiolabeling yield of the ^{99m}Tc-complex was followed by paper and thin-layer chromatography. The maximum radiolabeling yield was obtained when the reactions were carried out at pH~9 within 10 min at room temperature (RT). Biodistribution studies on rats has shown significant uptake of ^{99m}Tc-complex (2.1% ID), 2 min after injection.

Introduction
The clinical usefulness of ^{99m}Tc-labeled complexes depends on their ability to rapidly cross cell membranes, high stability and rapid clearance from vital organs and tissues [1]. Among radiopharmaceuticals for imaging cerebral blood flow (CBF) by single-photon emission computed tomography (SPECT) in nuclear medicine, ^{99m}Tc-*d,l*-HMPAO (hexamethyl-propylene amine oxime) and ^{99m}Tc-*l,l*-ECD (ethyl cysteinate dimer), combine the best overall features of high brain uptake, fixed regional distribution within the brain and their distribution in the brain is proportional to CBF in a wide range [2]. Both the ligands form neutral and lipophilic complexes with ^{99m}Tc possessing an oxotechnetium core. The HMPAO belongs to the class of diamine-dioxime compounds, which are good chelating agents due to the presence four nitrogen atoms that coordinate with ^{99m}Tc(V) in aqueous media. The goal of this work was to examine the use of a new ligand, derivative of HMPAO, as a potential candidate for the brain-perfusion imaging in SPECT. Although the ligand shows stereoisomerism, the presented work is based on research of isomeric mixtures of the meso- and d,l- diastereoisomers without the diastereo-enantio separation.

Experimental

^{99m}Tc ($T_{1/2}$=6 h, E_γ=140 keV) was eluted from a ^{99}Mo-^{99m}Tc generator (The Vinča Institute of Nuclear Sciences, Belgrade, Serbia). All reagents obtained from commercial sources were of analytical grade and used without further purification. IR spectra were recorded on a Bomem MB 100 IR spectrophotometer using prepared KBr pellets. The ^{1}H and ^{13}C NMR spectral measurements were performed on a Varian Gemini 2000 (200 MHz) at RT with deuterated DMSO (d^6-DMSO) as solvents. The melting points were determined by Mel-Temp melting point apparatus (Laboratory Devices Inc., USA). Radioactivity measurements were done in a NaI(TI) well-type gamma counter (LKB Wallac Compu Gamma Counter, Finland). Spectrophotometric measurements were carried out using an Uvicon 810/820 spectrophotometer (Kontron Instruments, Austria) with 10 mm quartz cells.

Preparation of ligand. 4,7-diaza-3,8-diethyldecane-2,9-dione bis oxime (LH_2) was prepared using the method described in the literature [3]. Yield: 80%, mp: 133-134 °C. λ_{max}= 205 nm. Selected IR data (KBr, pallets, cm^{-1}): ν_{OH} 3264, $\nu_{(OH, NH)}$ 3212. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.76 (CH_3(C5), 3H, t, *J*=7.5 Hz), 1.35-1.50 (CH_2(C4), 2H, m), 1.62 (CH_3(C1), 3H, s), 2.34 (CH_2(C6), 2H, s), 2.90 (CH(C3), 1H, t, *J*=7.3 Hz), 10.32 (-OH, 1H, s). ^{13}C NMR (200 MHz, DMSO-d_6, δ ppm): 10.89 C(1), 157.56 C(2), 63.12 C(3), 25.83 C(4), 8.37 C(5), 46.50 C(6).

Results and Discussion

Radiolabeling procedure. Radiolabeling conditions were optimized to give the maximum yield of ^{99m}Tc-complex. Complexation studies of ligand with ^{99m}Tc(V) were carried out using $SnCl_2$ as a reducing agent. Dissolution of desire amount of LH_2 in oxygen-free double-distilled H_2O was achieved by acidification with HCl_{conc} to pH 1.5-2. The solution was bubbled with N_2 gas and cooled in an ice bath. Then, appropriate volume of $SnCl_2$ stock solution (5 mg of anhydrous $SnCl_2$ in 0.05 ml of HCl_{conc} diluted up to 10 mL of H_2O) was added and the pH adjusted to 9. After the addition of sodium pertechnetate ($^{99m}TcO_4^-$, 18.5-37 MBq/mL), the solution was gently shaken and allowed to stand 10 min at RT. The total reaction volume in vial was maintained at 4 mL. Sn(II):LH_2 mole ratio was 1:55.

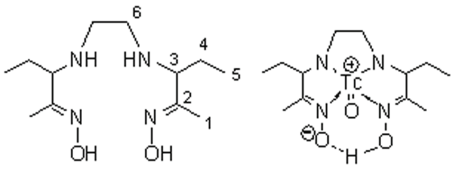

Figure 1. Structure of LH_2 and proposed structure of ^{99m}Tc-complex.

According to the literature data for similar diamino-dioxime ligands and their ^{99m}Tc(V)-labeled complexes [4], proposed structures of LH_2 and ^{99m}Tc-complex are shown in Fig.1. Numbering of C-atoms in Fig.1 was used for ^{1}H NMR and ^{13}C NMR spectra interpretation.

Radiolabeling yield. The labeling yield as well as the stability of the ^{99m}Tc-complex were determined using a combination of two chromatographic systems: paper chromatography on Whatman No.1 with acetonitrile:water (1:1) as the mobile phase and TLC on silica gel 60 strips with saline as the mobile phase. Stability of the ^{99m}Tc-complex was determined by investigation of radiochemical purity of the complex at different time intervals after labeling (Fig.2A). The content of free $^{99m}TcO_4^-$ and ^{99m}Tc-reduced-hydrolyzed in the form of $^{99m}TcO_2$ were considered as radiochemical impurities.

Biodistribution of ^{99m}Tc-complex. Healthy male 8-weeks old Wistar rats, 200-250 g body weight (n=3 per each time point) were intravenously (i.v.) injected through the tail vein with 0.1 mL of ^{99m}Tc-complex (approx. 0.5–1.0 MBq per animal). The animals were sacrificed *via* spinal cord dislocation at 2, 5, 15, 30 and 60 min after injection and the radioactivity in brain was measured. ^{99m}Tc-complex exhibits good uptake in the brain (2.10% of injected dose (%ID)) at 2 min after injection (Fig.2B.)

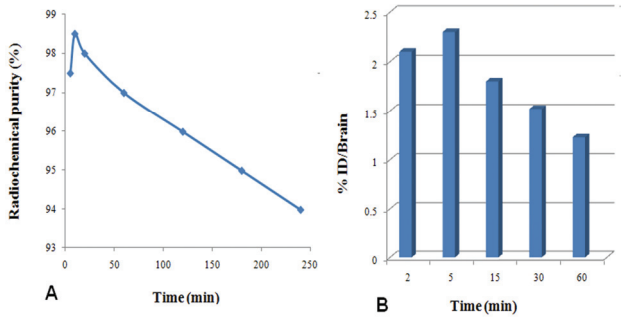

Figure 2. Radiochemical purity(A); Brain uptake(B) of the ^{99m}Tc-complex.

Conclusion

A new diamine-dioxime ligand, 4,7-diaza-3,8-diethyldecane-2,9-dione bis oxime (LH_2) were synthesized in high yields, characterized by UV-Vis, IR, ^{1}H, and ^{13}C NMR and successfully labeled with ^{99m}Tc(V). From the obtained results, it can be concluded that ^{99m}Tc(V) complex could be made easy with a ligand such as LH_2. This complex showed good stability, higher than 94% up to 4h during storage at RT. The present study suggests that complex exhibited favorable properties as promising candidates for cerebral perfusion imaging.

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