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A systematic study on the utility of CHX-A''-DTPA-NCS and NOTA-NCS as bifunctional chelators for ^{177}Lu radiopharmaceuticals

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ABSTRACT

This paper describes the evaluation of [(R)-2-Amino-3-(4-isothiocyanatophenyl)propyl]-trans-(S,S)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A''-DTPA-NCS) and 2-S-(4-isothiocyanatobenzyl)−1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA-NCS) as bifunctional chelators for ^{177}Lu. While ^{177}Lu-CHX-A''-DTPA-NCS could be obtained in high yields at equimolar ratios of lutetium to CHX-A''-DTPA-NCS, > 95% yield of ^{177}Lu-NOTA-NCS could be achieved at 1:2 M ratio of lutetium to NOTA-NCS. Trace metals reduced the yields of ^{177}Lu-NOTA-NCS significantly as compared to ^{177}Lu-CHX-A''-DTPA-NCS. *In vitro* stability of ^{177}Lu-CHX-A''-DTPA-NCS was also superior to ^{177}Lu-NOTA-NCS. It could be concluded from this study that among the two chelators evaluated, CHX-A''-DTPA-NCS is more appropriate for preparation of ^{177}Lu radiopharmaceuticals.

1. Introduction

Growing interest in the development of ^{177}Lu radiopharmaceuticals could be attributed to the suitability of its nuclear properties for therapeutic use and its well-understood complexation chemistry. Use of ^{177}Lu radiopharmaceuticals enables real-time follow up and dosimetry, as ^{177}Lu emits gamma radiations of low energy and less abundance along with medium energy β^- radiations (78.6% of 497 keV) (Srivastava, 1996; Dash et al., 2015). As ^{176}Lu has a high thermal neutron absorption cross section (2100 b), neutron irradiation of enriched ^{176}Lu targets in medium flux nuclear reactors produces large quantities of ^{177}Lu in adequate specific activity (Das et al., 2007; Chakraborty et al., 2016). The β^- radiations emitted by ^{177}Lu are suitable for targeting small tumors and micrometastases (Michel et al., 2005; Cremonesi et al., 2006; Cutler et al., 2013). Supply to hospitals distant from the production facility without much loss due to decay is viable due to the 6.7 day half life of ^{177}Lu. Besides, the half life of ^{177}Lu is also well-matched with the *in vivo* localization properties of biomolecules such as monoclonal antibodies (Ray et al., 2012).

Bifunctional chelators (BFCs) serve as linkers between the radionuclide and targeting biomolecule and therefore play a crucial role in the success of targeted radionuclide therapy. The BFC is expected to impart high *in vivo* stability to the radiolabeled biomolecule translating to minimum radiation dose to non-target organs. The choice of BFC is therefore governed by the thermodynamic and kinetic stability of the radiometal complexes (Liu, 2008). The macrocyclic ligand DOTA (1,4,7,10-tetraazacyclododocane-1,4,7,10-tetraacetic acid) and its analogs are the most favored BFCs for radiolabeling biomolecules with radiometals such as ^{90}Y and ^{177}Lu (Price and Orvig, 2014; Liu, 2008). However, complexation of radiometals with DOTA often needs to be carried out at elevated temperatures as its macrocyclic structure needs to adapt itself to the coordination site geometry of the radiometal (Kang et al., 2012; Parus et al., 2015). Hence, the quest for BFCs capable of room temperature radiolabeling, especially for temperature-sensitive biomolecules such as monoclonal antibodies, has led to the evaluation of a number of acyclic and macrocyclic ligands. For example, there are few publications on the evaluation of bimodal ligands NETA (2-(4,7-biscarboxymethyl[1,4,7]triazacyclonona-1-yl-ethyl)carbonyl-methylamino]acetic acid) and DETA [2-{[5-(4-nitrophenyl)−1-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]pentan-2-yl]amino} acetic acid] for complexation with ^{90}Y and ^{177}Lu (Kang et al., 2012; Chong et al., 2015) while we had reported on the utility of PCTA-NCS for preparation of ^{177}Lu radiopharmaceuticals [Pandey et al., 2015]. Besides these, backbone-restricted DTPA analogs such as tiuxetan (1B4M-DTPA) and [(R)-2-Amino-3-(4-isothiocyanatophenyl)propyl]-trans-(S,S)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A''-DTPA-NCS) have been employed for preparation of receptor-targeted radiopharmaceuticals (Lee et al., 2005; D'Huyvetter et al., 2012; Kameswaran et al., 2015). The newer DTPA analogs impart enhanced stability to the radiometal complexes due to the rigidity conferred by the modified backbone (Parus et al., 2015). In 1B4M-DTPA, the propane 1,2-diamine moiety replaces one of the flexible ethane 1,2-

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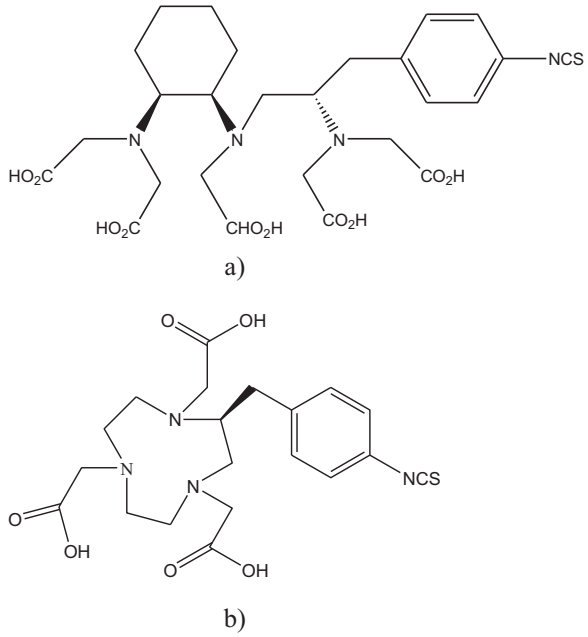

Fig. 1. Chemical structures of CHX-A''-DTPA-NCS and NOTA-NCS.

diamine units while cyclohexane 1,2-diamine units replace one ethane 1,2-diamine unit in CHX-A''-DTPA (Camera et al., 1994; Frullano et al., 2011). Another BFC which has been utilized for ^{177}Lu labeling of monoclonal antibodies and peptides is the macrocyclic chelator para-isothiocyanato benzyl 1,4,7-triaza cyclononane 1,4,7-tri acetic acid (NOTA-NCS) (Hwan Ju et al., 2010; Novy et al., 2014). However, reports on different aspects of complexation of ^{177}Lu with CHX-A''-DTPA-NCS and NOTA-NCS such as the metal to ligand ratios for maximum complexation yields, role of trace metal impurities in solution on the yields of the ^{177}Lu complexes and their *in vitro* stability are not available in literature, to the best of our knowledge. This paper focuses on these aspects of complexation of CHX-A''-DTPA-NCS (Fig. 1a) and NOTA-NCS (Fig. 1b) with ^{177}Lu which may have implications for their choice as BFCs towards future development of ^{177}Lu radiopharmaceuticals.

2. Experimental

2.1. Materials

Lutetium-177 (25.7 ± 1.4 Ci/mg at 24 h after irradiation) was produced at Radiopharmaceuticals Division, BARC by irradiation of enriched Lu_2O_3 target (82% enriched in ^{176}Lu) in the Dhruva reactor at a thermal neutron flux of $\sim 1.4 \times 10^{14}$ ncm^{-2} s^{-1} for 21 days (Chakraborty et al., 2013; Vimalnath et al., 2014). CHX-A''-DTPA-NCS and NOTA-NCS (purity ≥94%) were purchased from M/s. Macrocyclics, Dallas, USA. Anhydrous sodium acetate (≥99.999% pure, metal basis), $LuCl_3$, Ca(II), Cu(II), Fe(III) and Zn(II) salts were from M/s. Sigma, USA. All the reagents were prepared using HPLC grade water (Merck, India).

Counting of radioactivity during the radiolabeling experiments was carried out on a well-type NaI(Tl) counter (Electronic Corporation of India Limited, India). HPLC was performed on a dual pump system (JASCO, Japan) which was equipped with a reversed phase C18 column (5 μm, 4 × 250 mm). The system was connected to a UV/visible detector (JASCO, Japan) and a NaI(Tl) detector (Raytest, Germany). Paper chromatography was carried out on Whatman 3 mm chromatography paper (20 mm width) purchased from M/s. Whatman International, UK. All the glassware were acid washed in order to minimize the presence of competing trace metallic impurities.

3. Methods

3.1. Complexation of ^{177}Lu with CHX-A''-DTPA-NCS and NOTA-NCS

Complexation of ^{177}Lu with CHX-A''-DTPA-NCS and NOTA-NCS was carried out at various Lu to BFC molar ratios (1:1, 1:2, 1:4 and 1:10) so as to determine the optimum metal to ligand molar ratio at which radiolabeling yield exceeds 95%. Reactions were carried out in 0.1 M sodium acetate solution (pH 4.5–5) containing 37–74 MBq of $^{177}LuCl_3$ and 4 μg of inactive carrier $^{176}LuCl_3$ (23 μM – corresponding to 100 mCi of $^{177}LuCl_3$) to which the corresponding amount of CHX-A''-DTPA-NCS or NOTA-NCS solution (1 mg/mL in HPLC grade water) (23 μM, 46 μM, 92 μM and 230 μM) was added such that the Lu to BFC molar ratios are 1:1, 1:2, 1:4 and 1:10 respectively. Reaction volume was made up to 1 mL and the reaction mixtures were incubated for 15 min at room temperature. Percentage radiolabeling yield was determined by HPLC and paper chromatography techniques. HPLC characterization was carried out by injecting 20 μL of the reaction mixture into a C18 reverse phase column which was eluted using a gradient elution of water (Pump A) and acetonitrile (Pump B), both containing 0.1% trifluoroacetic acid at a flow rate of 1 mL/min (0–4 min 5% B, 4–20 min 5–95% B, 20–30 min 95–5% B). Radioactivity eluting out of the column was monitored using a flow-through NaI(Tl) scintillation detector and was quantified using the GINASTAR software. Paper chromatography was performed on Whatman 3 mm chromatography paper with a 1:1 (v/v) mixture of water and acetonitrile as mobile phase to differentiate between the free and BFC-bound ^{177}Lu.

3.2. Determination of log P value

To determine the octanol to water partition ratio (log P) of ^{177}Lu-CHX-A''-DTPA-NCS complex, 0.1 mL of the complex was added to a biphasic mixture of 0.9 mL of water and 1 mL of *n*-octanol. The mixture was shaken well and allowed to settle, after which the aqueous and octanol phases were separated. Equal aliquots of both phases were counted in a NaI(Tl) well counter. The experiment was repeated by mixing 0.1 mL of the octanol phase with 0.9 mL of octanol and 1 mL of water. Logarithm of the ratio of the counts in octanol phase to the counts in the aqueous phase gave the value of log P of ^{177}Lu-CHX-A''-DTPA-NCS complex. The procedure was repeated for determination of log P of ^{177}Lu-NOTA-NCS.

3.3. Influence of trace metallic impurities on the yields of ^{177}Lu-CHX-A''-DTPA-NCS and ^{177}Lu-NOTA-NCS

Complexation of biomolecules with radiometals such as ^{177}Lu and ^{90}Y are carried out using ultrapure chemicals and reagents, as many of the trace metals in solution can also complex with the BFCs employed for radiolabeling. Hence, it is imperative to know the interference from competing trace metal ions on the yields of ^{177}Lu-CHX-A''-DTPA-NCS and ^{177}Lu-NOTA-NCS. Therefore, influence of trace metal ions such as Ca(II), Cu(II), Fe(III) and Zn(II) on the yields of ^{177}Lu-CHX-A''-DTPA-NCS and ^{177}Lu-NOTA-NCS was studied by performing the radiolabeling in presence of deliberately added trace metal ions. Previously, ICP-AES analysis of decayed ^{177}Lu samples confirmed the absence of trace metal ions in $^{177}LuCl_3$ solution (Chakraborty et al., 2014). Standard solutions of Ca(II), Cu(II), Fe(III) and Zn(II) salts were prepared which were serially diluted corresponding to trace metal to ^{176}Lu molar ratios of 0.1:1, 1:1 and 10:1 respectively. 20 μL of $^{177}LuCl_3$ solution (37–74 MBq) was added to 0.1 M sodium acetate solution containing 23 μM of $^{176}LuCl_3$ carrier along with the corresponding trace metal solution (2.3 μM or 23 μM or 230 μM). Thereafter, 23 μM solution of CHX-A''-DTPA-NCS or 46 μM solution of NOTA-NCS was added (Lu to CHX-A''-DTPA-NCS molar ratio of 1:1 and Lu to NOTA-NCS molar ratio of 1:2). Reaction volume was kept constant at 1 mL and the reaction mixture (pH 4.5–5) was incubated at ambient temperature for 15 min.