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Optimization of labeling and metabolite analysis of copper-64-labeled azamacrocyclic chelators by radio-LC-MS

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Abstract

The cross-bridged tetraamine ligand 4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (H_2CB-TE2A) allows formation of a radio-copper complex with higher in vivo stability than that of the corresponding non-cross-bridged analog 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA). The structure of the ^{nat}Cu(II) complex of CB-TE2A has been previously determined by X-ray crystallography; however, direct high-pressure liquid chromatography (HPLC) characterization of the corresponding ^{64}Cu complex was inaccessible due to the inability to detect the complex by ultraviolet absorbance at the radiotracer level. A reverse-phase HPLC separation of a series of ^{nat}Cu(II)-tetraazamacrocyclic complexes, both traditional and cross-bridged, was developed and applied toward characterization and assessment of the purity of the corresponding no-carrier-added ^{64}Cu-labeled complexes. Verification of the identity of copper-64-labeled compounds was also achieved by coupling this HPLC method with mass spectrometry. The radio-liquid chromatography/mass spectrometry methodology was further extended to study the in vivo metabolic fates of ^{64}Cu-azamacrocyclic complexes.

Keywords: Copper-64; Azamacrocycle; HPLC; Radio-LC-MS; Impurity identification; Metabolite analysis

1. Introduction

Copper-64 is a useful diagnostic and therapeutic radionuclide in nuclear medicine due to its half-life ($t_{1/2}$=12.7 h), decay characteristics (β^+,7.4%; β^-, 39%) and the capability for large-scale production with high specific activity on a biomedical cyclotron [1,2]. Increased use of ^{64}Cu and other Cu radioisotopes in both positron emission tomography and targeted radiotherapy applications has created a need for copper chelators with high in vivo stability. The development of optimal chelators for Cu(II) is of considerable importance when designing systems for the in vivo delivery of copper radioisotopes.

Polyaminopolycarboxylate macrocyclic ligands are commonly used for complexation of metals for radiopharmaceutical applications. However, the Cu(II) complexes of these ligands are susceptible to metal dissociation in vivo, releasing copper ions that readily bind proteins. The commercially available macrocyclic ligands 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (H_4TETA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (H_4DOTA) (Fig. 1) have been extensively used for chelation of copper radionuclides in clinical imaging and therapy studies involving both antibodies and peptides [3–9]. However, the in vivo dissociation and subsequent binding of radiometals to proteins have been demonstrated in normal rats [10,11]. The macrocyclic ligands 4,11-bis(carboxy-

Abbreviations: HPLC, high-pressure liquid chromatography; PDA, photodiode array; UV, ultraviolet; λ_{max}, wavelength of maximal absorbance; RP, reverse phase; LC-MS, liquid chromatography/mass spectrometry; ESI, electrospray ionization; ES^+, electrospray (positive ionization mode); PET, positron emission tomography; H_2CB-TE2A, 4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane; H_4TETA, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid; H_2CB-DO2A, 4,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane; H_4DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; H_3TE3A, 1,4,8,11-tetraazacyclotetradecane-1,4,8-triacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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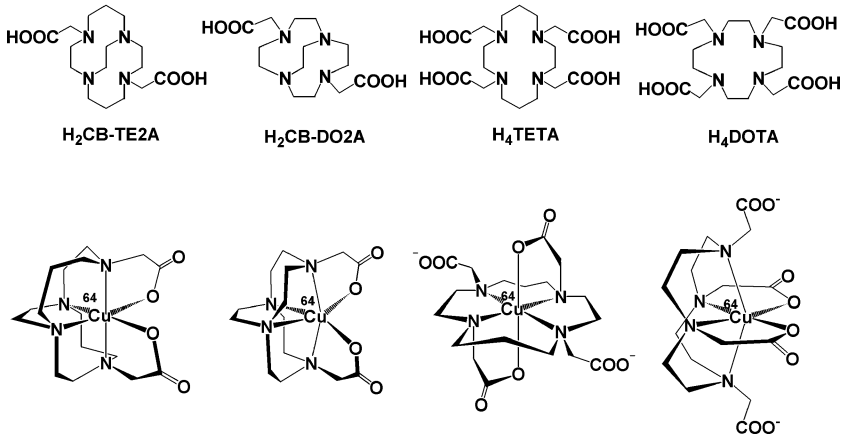

Fig. 1. Structural comparison of H_2CB-TE2A, H_2CB-DO2A, H_4TETA and H_4DOTA (top), and structural representations of the corresponding ^{64}Cu-labeled complexes based on solved crystal structures (bottom).

methyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (H_2CB-TE2A) and 4,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (H_2CB-DO2A) [12] are analogs of H_4TETA and H_4DOTA, respectively, where two of the acetate arms present have been replaced by ethylene bridges between nonadjacent nitrogens (Fig. 1). These ligands have been shown to form Cu(II) complexes [12,13] with superior kinetic stability and improved biological behavior compared to their nonbridged analogs [11,14].

Development of a chromatographic separation of these azamacrocyclic complexes would allow for the determination of radiochemical purity and would also provide a means for the detection of metabolites from biological samples. Accomplishing these goals would facilitate the evaluation of azamacrocycles as carriers of copper radionuclides in radiopharmaceutical applications. In studies reported previously, an ion-exchange chromatographic technique was developed for studying the lability of ^{64}Cu complexes with ethylenediaminetetraacetic acid (EDTA) and other acyclic chelators in an aqueous system [15]. A reverse-phase (RP) separation of a radiolabeled ^{153}Gd azamacrocyclic complex was developed using a C-18 column and a buffered aqueous eluant [16]. Herein we report the development of a RP method for the separation of ^{64}Cu-labeled azamacrocyclic complexes.

Mass spectrometry coupled to high-pressure liquid chromatography (HPLC) (LC-MS) has proven to be a valuable tool for the analysis of compounds being developed as radiopharmaceuticals, allowing for detection of trace-level impurities [17], determination of specific activity [18] and identification of metabolites [19–21]. An LC-MS methodology for separation of ^{64}Cu-labeled azamacrocyclic complexes would be advantageous because of the requirement of high sensitivity for detection and characterization of radiometal complexes at or near the tracer level. Incorporation of ^{64}Cu into the desired ligand may be confirmed by comparing the retention times of radiolabeled species with the appropriate characterized ^{nat}Cu complexes detected by LC-MS. This radio-LC-MS approach was used to confirm the identities of ^{99m}Tc Sestamibi [22] and other ^{99m}Tc radiopharmaceuticals [23,24], a process that is usually achieved at the tracer level only indirectly by assessment of RP-HPLC retention times. The experiments described herein were directed toward confirmation of the formation of desired ^{64}Cu-azamacrocyclic complexes at the tracer level, identification of ^{64}Cu-labeled impurities and investigation of the extent of ^{64}Cu-azamacrocyclic complex metabolism in vivo.

2. Materials and methods

2.1. General procedures and materials

Cold copper salts and solvents were commercial grade. Cross-bridged ligands H_2CB-TE2A and H_2CB-DO2A were prepared as trifluoroacetate salts by modifications of published methods [12,13]. H_4TETA obtained from Aldrich Co. (Milwaukee, WI) was used to collect the data shown in Fig. 7; H_4TETA obtained from Macrocyclics (Dallas, TX) was used to collect the data shown in Figs. 2, 8 and 9); H_4DOTA was obtained from Macrocyclics.

^{nat}Cu(II)·TETA·2H_2O [25], ^{nat}Cu(II)·DOTA [25], [^{nat}Cu(II)·CB-TE2A·Na(H_2O)ClO_4]$_2$·H_2O [13] and ^{nat}Cu(II)-CB-DO2A [11] were prepared according to published methods. Samples for injection were prepared by dissolving the crystals in mobile phase prior to injection; typical injection volumes were 50 or 100 μl. A solution containing all four ^{nat}Cu complexes, each at a concentration of 1.25 mg/ml, was analyzed to obtain the data for Fig. 2. Analysis of a 5 mg/ml solution of crude ^{nat}Cu-CB-TE2A gave the data shown in Fig. 3. Analysis of a 5-mg/ml solution of recrystallized ^{nat}Cu-TETA provided the data shown in Fig. 7.

2.2. Radiochemistry

Copper-64 was prepared on the Washington University Medical School CS-15 cyclotron by the ^{64}Ni(p,n)^{64}Cu nuclear reaction at a specific activity range of 50–200 mCi/μg as previously described [1]. No-carrier-added ^{64}Cu-