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Synthesis, stabilization and formulation of [^{177}Lu]Lu-AMBA, a systemic radiotherapeutic agent for Gastrin Releasing Peptide receptor positive tumors

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Abstract

A robust formulation was developed for [^{177}Lu]Lu-AMBA (^{177}Lu-DO3A-CH_2CO-G-[4-aminobenzoyl]-QWAVGHLM-NH_2), a Bombesin-like agonist with high affinity for Gastrin Releasing Peptide (GRP) receptors. During optimization of labeling, the effect of several radiostabilizers was evaluated; a combination of selenomethionine and ascorbic acid showed superiority over other tested radiostabilizers. The resulting two-vial formulation maintains a radiochemical purity (RCP) of >90% for at least 2 days at room temperature. The method of stabilization should be useful for other methionine-containing peptide radiopharmaceuticals in diagnostic and therapeutic applications.

Keywords: ^{177}Lu-AMBA; Bombesin; Gastrin releasing peptide; Targeted radiotherapy; Radiolysis protection

1. Introduction

Interest in using radiolabeled bombesin derivatives as agents for diagnostic imaging and/or systemic radiotherapy of tumors (Smith et al., 2003; Zhang et al., 2004; Lantry et al., 2006 and references therein) has increased considerably because of the observation that Gastrin Releasing Peptide receptors (GRPr) are over-expressed in a variety of human tumor cells. Lantry et al. (2006) recently demonstrated that the [^{177}Lu]Lu-labeled Gastrin Releasing Peptide (GRP) derivative known as [^{177}Lu]Lu-AMBA (AMBA = (DO3A-CH_2CO-G-(4-aminobenzoyl)-QWAVGHLM-NH_2)) binds with nanomolar affinity to GRP receptors; preclinical studies with this Lu-labeled compound demonstrated therapeutic efficacy in a GRPr positive PC-3 human prostate tumor-bearing nude mouse model. [^{177}Lu]Lu-AMBA is now in clinical trials for the radiotherapeutic treatment of prostate cancer.

Radiopharmaceuticals for systemic therapeutic applications are designed to deliver a therapeutic dose of radiation to specific disease sites. The ionizing radiation (e.g., α- or β-particles) given off from such compounds can either damage cellular components in the target tissue directly, or indirectly via the free radicals (e.g., $OH^{\bullet}$, $H^{\bullet}$, $O_2^{\bullet -}$) formed by the interaction of ionizing radiation with water in the target tissue (Burton and Lipsky, 1957; Liu and Edwards, 2001; Liu et al., 2003; Pozzi and Zalutsky, 2005). However, the potentially destructive properties of a therapeutic radioisotope's emissions are not limited to their cellular targets. Radiation-induced damage to the radiolabeled compound itself is one of the most challenging aspects in the development of a therapeutic radiopharmaceutical. For peptides and proteins, Garrison (1987) has reported that radiation induced damage may include oxidation, hydroxylation, aggregation and/or bond scission.

Preliminary tests showed that [^{177}Lu]Lu-AMBA was very radiosensitive; in the absence of radiostabilizers, degradation occurred both during and after radiolabeling.

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In particular, the methionine residue of the targeting peptide was found to be readily oxidized to its methionine sulfoxide [Met(O)] form, one of the major radiolytic degradants of [^{177}Lu]Lu-AMBA. Biological studies demonstrated that the targeting capability of [^{177}Lu]Lu-AMBA was totally inactivated by this oxidization. Hence, use of a radiostabilizer or stabilizer combination in the [^{177}Lu]Lu-AMBA formulation was an absolute requirement.

The purpose of this study was to establish a robust formulation for [^{177}Lu]Lu-AMBA for use in Phase I clinical trials. The effect of pH, ligand concentration, and reaction time were determined, and several stabilizers were evaluated to identify a formulation yielding and maintaining high radiochemical purity (RCP).

2. List of abbreviations

Potential radiostabilizers evaluated included (a) amino acids: glycine (Gly), methionine (Met), cysteine (Cys), cysteine ethyl ester (CEE), tryptophan (Trp), and histidine (His); (b) naturally occurring selenium compounds: selenomethionine (Se-Met) and selenocysteine (Se-Cys); (c) sulfur-containing reducing agents: 2-mercaptoethanol (ME), dithiothreitol (DTT), and 1-pyrrolidinecarbodithioic acid (PDTC). The results were compared to those obtained with the commonly used radical scavengers such as ascorbic acid (AA), gentisic acid (GA), human serum albumin (HSA), and ethanol (EtOH).

3. Materials and methods

Glacial acetic acid (Ultrapure) and sodium acetate trihydrate (USP) were purchased from J.T. Baker. L-(+)-Selenomethionine (Se-Met) was obtained from Sabinsa Corp. Amino acids, ammonium sulfate, trifluoroacetic acid (TFA), acetonitrile and methanol were bought from EMD Chemicals, Inc. Bacteriostatic 0.9% Sodium Chloride Injection (USP) was purchased from Abbott Laboratories. ASCOR L500® Ascorbic Acid Injection (USP) [containing 500 mg/mL Ascorbic acid and 0.025% (w/v) Edetate disodium] was obtained from McGuff Pharmaceuticals, Inc. [^{177}Lu]$LuCl_3$ in 0.05 N HCl was purchased from Missouri University Research Reactor (MURR). ITLC SG strips were from Pall Life Sciences. Deionized water was used for all solutions containing water, including HPLC mobile phases.

3.1. Peptide synthesis

AMBA [(DO3A-CH_2CO-G-(4-aminobenzoyl)-QWAVGHLM-NH_2), DO3A = (1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-cyclododecyl)-acetyl] was synthesized using solid phase peptide synthesis chemistry, as described by Lantry et al. (2006). The compound structure was confirmed by LC/MS, amino acid sequence and elemental analysis. The proposed chemical structure of its lutetium complex is shown in Fig. 1. An authentic sample of AMBA-Met(O), a mixture of the two unresolvable methionine oxide epimers of the AMBA ligand was prepared using the appropriate Met(O) containing protected amino acids. This methionine oxidized compound mixture and its Lu complex [Lu-AMBA-Met(O)] were characterized by MS and HPLC.

3.2. Radiochemistry

3.2.1. Standard procedure for preparation of [^{177}Lu]Lu-AMBA

To a lead-shielded 7-mL vial containing 120 μg of AMBA and 1 mg Se-Met in 1 mL of 0.2 M (pH 4.8) sodium acetate (NaOAc) buffer, was added 4.07 ± 0.37 GBq [^{177}Lu]$LuCl_3$ in 0.05 N HCl (radioconcentration ~37 GBq/mL, specific activity 103.6–151.3 GBq/μmol). The mixture was heated at 100 °C in a heating block for 10 min. After cooling to ambient temperature in a water-bath for ~2 min, the reaction solution was diluted by adding 4 mL of ascorbate dilution solution [a 9:1 mixture of Bacteriostatic 0.9% Sodium Chloride Injection USP and ASCOR L500® Ascorbic Acid Injection USP (final ascorbic acid concentration, 40 mg/mL)] yielding a final radioconcentration of ~814 MBq/mL (22 mCi/mL). Any possible non-incorporated ^{177}Lu remaining in the reaction solution was converted to [^{177}Lu]Lu-EDTA by the EDTA contained in the Ascorbic Acid Injection. The radiocomplex was then characterized by HPLC. In some cases, this reaction was performed at a 1/10th or 1/5th scale, maintaining the same concentrations as described above, but using a 2-mL reaction vial.

3.2.2. Effect of buffer pH and ligand concentration

For the effect of buffer pH on [^{177}Lu]Lu-AMBA incorporation, studies were performed at 1/10th of the full scale formulation as described above, but using 0.2 M

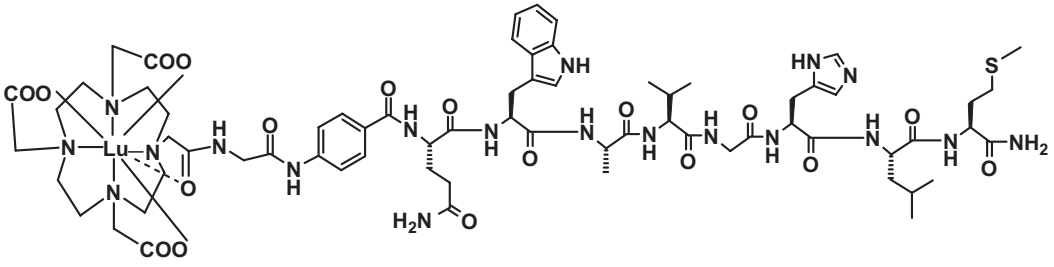

Fig. 1. The proposed chemical structure of Lu-AMBA.