Applied Radiation and Isotopes 100 (2015) 96–100

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Tumoral fibrosis effect on the radiation absorbed dose of ^{177}Lu–Tyr^3-octreotate and ^{177}Lu–Tyr^3-octreotate conjugated to gold nanoparticles

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HIGHLIGHTS

- Fibrosis increases the radiation absorbed dose to the tumor.
- Fibrosis increases the radiopharmaceutical residence time in the tumor.
- The multimeric nature of the radiopharmaceuticals enhances the radiopharmaceutical retention.

ARTICLE INFO

Article history:
Received 2 September 2014
Received in revised form
8 September 2014
Accepted 9 September 2014
Available online 18 September 2014

Keywords:
Tumoral fibrosis model
Monte Carlo
Targeted radiotherapy
Gold nanoparticles
Lutetium-177
Octreotate

ABSTRACT

The aim of this work was to evaluate the tumoral fibrosis effect on the radiation absorbed dose of the radiopharmaceuticals ^{177}Lu–Tyr^3-octreotate (monomeric) and ^{177}Lu–Tyr^3-octreotate–gold nanoparticles (multimeric) using an experimental HeLa cells tumoral model and the Monte Carlo PENELOPE code. Experimental and computer micro-environment models with or without fibrosis were constructed. Results showed that fibrosis increases up to 33% the tumor radiation absorbed dose, although the major effect on the dose was produced by the type of radiopharmaceutical (112 Gy-multimeric vs. 43 Gy-monomeric).

1. Introduction

Cancer among other factors could be the result of a cronic inflamatory response initially triggered by external aggressions. The production and deposition of extracellular matrix proteins during the inflammatory process may lead to fibrosis. Cancer aggressiveness is usually related to the fibrosis of tumors (Wu et al., 2013).

Targeted therapy based on ^{177}Lu-radiolabeled octreotide peptides that specifically bind to somatostatin receptors overexpressed on cancer cells, provides a potent strategy for therapy of neuroendocrine tumors (Ezziddin et al., 2014). The conjugation of octreotide to gold nanoparticles (AuNP) produces biocompatible and stable multimeric systems with target specific recognition useful as theranostic radiopharmaceuticals (Mendoza-Nava et al., 2013). It has also been demonstrated that octreotide inhibits HeLa cell growth in cultures and animal models (Mascardo and Sherline, 1982, Evers et al., 1991).

The extent of fibrosis in tumoral tissues reduces the permeability of certain drugs (Hosoya et al., 2012), therefore it could affect the cumulated activity of radiopharmaceuticals in tumors. However, the presence of fibrosis in tumors is currently not considered for radiation absorbed dose calculations during nuclear medicine treatment planning.

The aim of this work was to evaluate the tumoral fibrosis effect on the radiation absorbed dose of the radiopharmaceuticals ^{177}Lu–Tyr^3-octreotate (monomeric) and ^{177}Lu–Tyr^3-octreotate–AuNP (multimeric) using an experimental HeLa cells tumoral model and the Monte Carlo PENELOPE code.

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http://dx.doi.org/10.1016/j.apradiso.2014.09.005
0969-8043/

2. Experimental methods

2.1. Preparation of ^{177}Lu –Tyr^3-octreotate (monomeric system)

A 5 μL aliquot of Tyr^3-octreotate (1 mg/mL) was diluted with 40 μL of 1 M acetate buffer at pH 5, followed by the addition of 10 μL of a $^{177}LuCl_3$ (~740 MBq, > 3 TBq/mg, ITG Isotope Technologies Garching GmbH, Germany) solution. The mixture was incubated at 90 °C in a block heater for 30 min. A radiochemical purity of > 98% was verified by TLC silica gel plates (aluminum backing, Merck); 10 cm strips were used as the stationary phase, and ammonium hydroxide:methanol:water (1:5:10) was used as the mobile phase to determine the amount of free ^{177}Lu (R_f=0) and ^{177}Lu–Tyr^3-octreotate (Fig. 1a), monomeric system (R_f=0.4–0.5).

2.2. Preparation of ^{177}Lu–Tyr^3-octreotate–AuNP (multimeric system)

Gold nanoparticles (20 nm) were obtained from Sigma-Aldrich (7×10^{11} particles/mL). Absorption spectra in the range of 400–800 nm were obtained with a Perkin-Elmer Lambda-Bio spectrometer using a 1 cm quartz cuvette to monitor the characteristic AuNP surface plasmon band at 520 nm (OD=0.9). To 1 mL of AuNP (20 nm), 3 μL (40 MBq) of ^{177}Lu–Tyr^3-octreotate (Fig. 1a) was added (0.25 μg of peptide; 1.89×10^{14} molecules;100 molecules per 20 nm AuNP), and the mixture was stirred for 5 min to form the ^{177}Lu–Tyr^3-octreotate–AuNP, multimeric system (Fig. 1b). No further purification was required since we have evaluated that the maximum number of peptides that can be bound to one AuNP (20 nm) is from 520 to 1701 depending of the peptide structure (Ocampo-García et al., 2011). The number of peptides per nanoparticle was calculated by UV–vis titration of peptides (8 μM) using increasing concentrations of gold nanoparticles (from 0 to 1 nM).

The chemical conjugation was confirmed by UV–vis, and IR spectroscopy. Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS) were also performed.

Size-exclusion chromatography and ultrafiltration were used as radiochemical control methods for the final radiopharmaceutical solution. A 0.1 mL sample of ^{177}Lu–Tyr^3-octreotate–AuNP was loaded onto a PD-10 column and injectable water was used as the eluent. The first radioactive and red eluted peak (3.0–4.0 mL) corresponded to radiolabeled Tyr^3-octreotate–AuNP. The free radiolabeled peptide (^{177}Lu–Tyr^3-octreotate) appeared in the fraction that eluted at 5.0–7.0 mL, and $^{177}LuCl_3$ remained trapped in the column matrix. Upon ultrafiltration (Centricron YM-30 regenerated cellulose 30,000 MW cut off, Millipore, Bedford, MA, USA), the ^{177}Lu–Tyr^3-octreotate–AuNP remained in the filter, while free ^{177}Lu–Tyr^3-octreotate and $^{177}LuCl_3$ passed through the filter. In the radio-HPLC size exclusion system (ProteinPak 300SW, Waters, 1 mL/min, injectable water), the t_Rs for the ^{177}Lu–Tyr^3-octreotate–AuNP and ^{177}Lu–Tyr^3-octreotate were 4–5 and 8 min, respectively.

2.3. Fabrication of three-dimensional multilayer in vitro model of a fibrotic tumor

The HeLa human cervical cancer cell line was originally obtained from ATCC (American Type Culture Collection, USA). The cells were routinely grown at 37 °C with 5% CO_2 and 85% humidity in minimum essential medium eagle (MEM, Sigma-Aldrich Co) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin).

Multilayers of HeLa cells alternated with collagen (8 mg/mL, extracted in house from pig skin) and pig skin gelatin (2%) were cultured on transwell inserts (Corning, Co.) to complete a five layer by layer (LBL) culture to mimic tumor fibrotic microenvironment. The LBL cultures were fixed and stained with toluidine blue and observed using a bright field microscope (MT6000, MEIJI Techno) with 40 × objective lenses to corroborate the absence or presence of collagen fibers in the not fibrotic and fibrotic models, respectively.

2.4. Nanoparticle delivery and calculation of residence times

LBL HeLa cell cultures were exposed during 24 h to: a)$^{177}LuCl_3$ or b)^{177}Lu–Tyr^3-octreotate–AuNP or c) ^{177}Lu–Tyr^3-octreotate (without nanoparticles) with an initial activity of 26 MBq. Radiopharmaceutical retention was calculated by measuring retained activity in the transwell inserts (radiopharmaceutical retained by

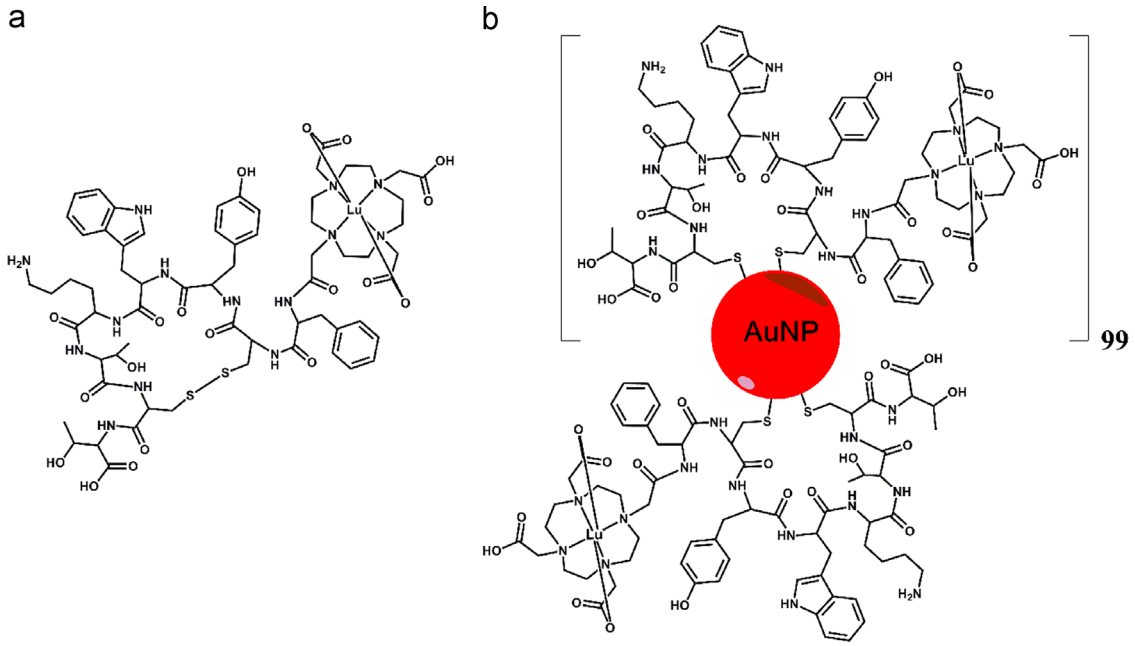

Fig. 1. Scheme of the (a) ^{177}Lu–Tyr^3-octreotate (monomeric system) and (b) ^{177}Lu–Tyr^3-octreotate–AuNP (multimeric system) structures.