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Gold nanoparticle probes: Design and in vitro applications in cancer cell culture

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ABSTRACT

A new architecture has been designed by the conjugation of [^{18}F]2-fluoro-2-deoxy-D-glucose (^{18}F-FDG), gold nanoparticles (AuNPs), and anti-metadherin (Anti-MTDH) antibody which is specific to the metadherin (MTDH) over-expressed on the surface of breast cancer cells. Mannose triflate molecule is used as a precursor for synthesis of ^{18}F-FDG by nucleophilic fluorination. For the conjugation of ^{18}F-FDG and AuNPs, cysteamine was first bound to mannose triflate (Man-CA) before synthesizing of ^{18}F-FDG which has cysteamine sides (^{18}FDG-CA). Then, ^{18}FDG-CA was reacted with $HAuCl_4$ to obtain AuNPs and with $NaBH_4$ for reduction of AuNPs. At the end of this procedure, AuNPs were conjugated to ^{18}F-FDG via disulphide bonds (^{18}FDG-AuNP). For the conjugation of Anti-MTDH, 1,1′-carbonyl diimidazol (CDI) was bound to the ^{18}FDG-AuNP, and Anti-MTDH was conjugated via CDI (^{18}FDG-AuNP-Anti-MTDH). This procedure was also performed by using Na^{19}F to obtain non-radioactive conjugates (^{19}FDG-AuNP-Anti-MTDH). Scanning electron microscopy (SEM) images demonstrated that synthesized particles were in nano sizes. ^{18}FDG-AuNP-Anti-MTDH conjugate was characterized and used as a model probe containing both radioactive and optical labels together as well as the biological target. The ^{18}FDG-AuNP-Anti-MTDH conjugate was applied to MCF7 breast cancer cell line and apoptotic cell ratio was found to be increasing from 2% to 20% following the treatment. Hence, these results have promised an important application potential of this conjugate in cancer research.

1. Introduction

Nanoparticles (NPs), are widely used in drug delivery, cellular imaging [1,2], and biomedical diagnostics and therapeutics [3,4] because of their small (10–100 nm) sizes and specific properties, and are very suitable for manipulations at the molecular level; for example, they have site-selective effects for targeting and imaging when they are bound to cell-receptors; expression systems decorated with NPs may be suitable for perceiving at nm scale.

Another field of investigation with regards to AuNPs is radiotherapy. Radiotherapy is a prominent tool in oncology, but it can be responsible for important biological damages, as ionizing radiations also induce degradation of healthy tissues. For example, following pelvic radiotherapy, 20–40% of patients report that gastrointestinal symptoms severely affect their quality of life [5]. It is the reason why since more than five decades, great efforts have been devoted to increase its efficiency and tolerance, such as better dose fractionation schedules or tomotherapy. In spite of these advances, normal tissue toxicity remains a dose-limiting factor in clinical radiation therapy. The dose enhancement by high-*Z* materials is known in the literature since long time [6]; nevertheless, in this study it is estimated that AuNPs will be able to increase specifically the radio-sensitization of cancer cells.

Positron emission tomography/computational tomography (PET/CT) technique is an imaging technique in early detection of cancer and ^{18}F-FDG is widely used in PET. ^{18}F-FDG, an analogue of glucose, is taken up by cells and phosphorylated by hexokinase to ^{18}F-FDG-6-phosphate. Since, ^{18}F-FDG-6-phosphate is not a substrate for further glycolysis, it is not readily dephosphorylated, and is remained trapping in the cell [7].

There are increased numbers of reports about properties of AuNPs and their biological affinities, but less report about radionuclide labeled NPs [8,9]. Namavari synthesized [^{18}F]-fluoro benzaldehyde and conjugated it with an affibody and this was used in PET experiments for tumor imaging [10]. On the other hand, El-Sayed reported that AuNPs have less affinity to cancer cells, but in the case of its conjugation to specific antibodies, incorporation rate may be increased up to 600% comparing to normal cells [11].

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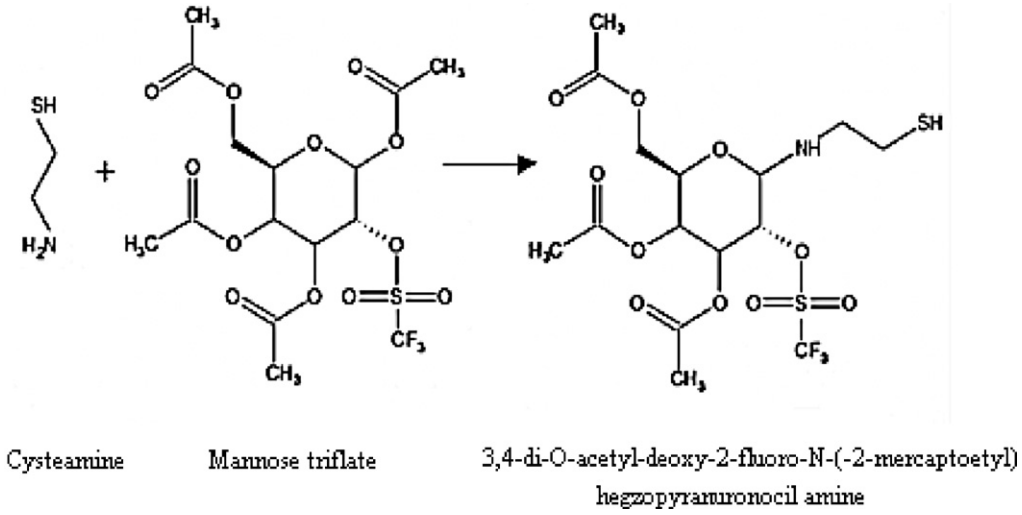

Scheme 1. Synthesis of Man-CA (mannose triflate-cysteamine).

In another report, it is claimed that AuNPs can be used as PET/CT contrast agents [12], but there is no any report in the literature on ^{18}F labeled AuNPs as a molecular probe in cancer detection.

In addition, Anti-MDTH which is specific to MDTH protein that is over-expressed on the breast cancer cells [13], was conjugated to the AuNPs and ^{18}F-FDG. Briefly, Anti-MDTH antibody which is specific to breast cancer cells and AuNPs are bound together via ^{18}F-FDG. This conjugate (^{18}FDG-AuNPs-Anti-MTDH) may also be used for computational tomography (CT) applications as agents for increasing the image quality.

In this study, a practical method was applied for preparation of AuNPs with considerably small sizes starting from mannose triflate and showed the application capability of this approach. Thus, the generation of a wide range of gluco- and manno-oligosaccharides containing AuNPs and their use in vitro bio-assays. Finally, following the characterization of this new architecture consisted of ^{18}F-FDG, AuNPs, and Anti-MTDH and this was preliminarily tested on MCF7 breast cancer cell lines.

2. Experimental

2.1. Materials

^{18}F was kindly donated from Eczacibasi-Monrol Nuclear Products Company. Anti-metadherin (100 μg protein/400 μL) was purchased from Zymed. CDI (1,1′-carbonyl diimidazol) which is used for the activation of hydroxyl groups of ^{18}F-FDG, chloroform ($CHCl_3$) (≥99%), Dowex 50 cation exchange resin, Eosin Y, Azur A, Malachite Green, Methylene blue, $NaBH_4$, cysteamine (2-aminoetanethiol), $HAuCl_4$ (tetrachloroauric acid), RIPA buffer were purchased from Sigma Chem. Co. Amberlite anion exchange resin, Ambersep 900 OH ion exchange resin, K_2CO_3, mannose triflate (1,3,4,6-tetra-O-acetyl-2-O-triflate-β-D-mannopyranose), NaF, $NaBH_3CN$ (sodium cyanoborohydride) were supplied from Fluka. Methanol (99%) and Kryptofix 222^{TM}, dimethylformamide (DMF), hydrochloric acid, K_2HPO_4, Na_2HPO_4 were purchased from Merck. Dioxan was supplied from Carlo Erba and C-18 cartridge column was purchased from Sep-Pak Cartridges.

MCF7 (human breast adenocarcinoma cell line) was obtained from the American Type Culture Collection (Manassas, VA). MEM Eagle (minimum essential medium eagle), Trypsin, PBS (phosphate buffer saline at pH 7.2), RPMI, Triphane blue were obtained from Bio. Ind.

2.2. Equipment

HPLC (high performance liquid chromatography) chromatograms were obtained using a Cd(Te) detector equipped with a RAD 501 single-channel analyzer and HPLC (with LC-10ATvp quaternary pump and SPD-10A/V UV detector and a syringe injector equipped with a 1.0-mL loop and 7.0-μm reversed-phase (RP)-C-18 column 250 × 21 mm I.D., Macherey-Nagel) in Ege University Institute of Nuclear Sciences. MALDI-TOF spectra were taken using a Bruker-Daltonix Flex Analyzer and Scintigraphic studies were performed using a PET/CT camera (Philips Gemini TF) at the Department of Nuclear Medicine, Dokuz Eylul University, Izmir, Turkey). Particle size and morphology were measured with Scanning Electron Microscope (SEM, Phillips XL-30 S FEG). The crystalline structure of NPs was characterized by X-ray diffractometer (XRD, Phillips X'Pert Pro). Zivac-Tandem Gold LC–MS/MS was used for analyses. Radioactivity measurements were obtained by using a multichannel analyzer (Canberra).

2.3. Synthesis procedures

Synthesis of ^{18}F-FDG-AuNP-Anti-MTDH involves the following steps:

(A) *Synthesis of mannose triflate-cysteamine (Man-CA)*. The synthesis was carried out according to Babu et al. [14]. Briefly, 46.4 mg of mannose triflate was dissolved in 2.0 mL of distilled water at 90 °C (solution A). 199.5 mg of cysteamine was dissolved in 250 μL of pure water in another test tube and pH was adjusted to 7.5 with 1.0 M HCl. Then, 100 mg of $NaCNBH_3$ was added to the solution (solution B). Solution A was then mixed with the solution B at 90 °C for 1 h. Then, the reaction product was precipitated and allowed to dry at the lyophilizator and dissolved in dimethyl formamide (1.0 mL). The procedure is summarized in Scheme 1.

(B) *^{18}F substitution of mannose triflate-cysteamine (^{18}FDG-CA)*. Radioflorination reaction was performed according to Gillies et al. [15] and is summarized in Scheme 2. Briefly, 700 μL of Man-CA solution which is prepared as described above, 100 μL of Kryptofix solution (2.0 mg/mL in DMF), 100 μL of K_2CO_3 aqueous solution (8.0 mg/mL), 300 μL of DMF, and 200 mCi $Na^{18}F$ were mixed in a reaction tube and heated in 90 °C water bath for 20 min. When the reaction was completed, the product was purified by passing through the following