Hybrid Nanoparticles Targeted by Antitumor Antibodies and Emitting Alpha-particles

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ABSTRACT

Here we describe a new therapeutic nanoparticle's design, which consists of three parts: targeting, effecter and linker. Targeted radionuclide therapy with the use of alpha-emitting radionuclides is currently one of the most promising and rapidly expanding methods for treating oncology diseases. The alpha-emitters are applied for labeling the monoclonal antibodies, synthetic polypeptides, albumin micro spheres etc. Such complexes provide alpha-emitter delivery to the certain cancerous cell.

Keywords: Tumor-targeted nanoparticles, biodistribution, AFM, radiopharmaceuticals.

1 MATERIALS AND METHODS

Targeting part: an anti-HER2/neu mini-antibody protein. The anti-HER2/neu mini-antibody could be used to deliver radioisotope to HER2/neu-positive cells and provide its penetration into the target cells, as HER2/neu is a ligand-internalizing receptor. This construct has potential applications to both radioisotope and antibody itself therapies of cancer, because many tumor cells are HER2/neu-positive, breast cancer for example.

Effecter: Tumor targeted alpha-particles can result in high cancer-cell killing with minimal normal-tissue irradiation because of their high energy deposition and short range. Generator for alpha-particle therapy: it decays and generates three alpha-particle-emitting daughters.

Linker: synthetic strategies for construction of hybrid nanoparticles under study based on chelating agents.

2 EFFECTER PART

Due to short track of alpha-particles (tens of microns), a high local irradiation dose in a malign cell is produced, by 1-2 orders of value exceeding the dose for currently applied beta-emitters. Among perspective α-emitters, 212Bi (t1/2=60 min), 223Ra (t1/2=11.4 d), 225Ac (t1/2=10.0 d), and 211At (t1/2=7.2 h) are of major interest. However, considering the combined nuclear, physical, chemical and biological properties, 213Bi (t1/2=45.6 min) holds the lead. The ongoing clinical trials with 213Bi have demonstrated its effectiveness (high performance) in treatment of oncologic diseases. It is especially important, that the radionuclide 213Bi can be used at early stages of treatment of practically all cancer types, as well as in combination with other methods (surgery, chemotherapy).

213Bi and its precursor, 225Ac are the decay products of long-lived 229Th (t1/2=7400 y). In turn, 229Th is a decay product of 233U, which can be obtained from old stocks of this very long-lived and fissile isotope of uranium. As pointed out, 225Ac is also of interest for radioimmunotherapy. The decay process, starting with 233U from which 229Th is currently produced, continues through two generator systems involving four intermediate radioisotopes and finally results in 213Bi.

A few nuclear processes of 213Bi reception are known up to now. Some of these processes were experimentally studied or are currently under investigation, which resulted in received samples of target radioisotopes. Processing of 233U old stocks is of major interests.

233U (T1/2 = 1.56·105 years) decays into 229Th (T1/2 = 7340 years). In a decay chain of 229Th are present 225Ra (T1/2 = 14.8 days) and 225Ac (T1/2 = 10 days) of rather long half-lives. Decay chain of 225Ac ends with formation of 213Bi (T1/2 = 45.6 min).

Thorium-229 radiochemically separated from 233U serves as a mother radionuclide in 229Th/225Ac generator. One milligram of 229Th provides (after 40-90 day holding) reception of 7.45·106 Bq (0.20 mCi) of 225Ac and 6.8·106 Bq (0.18 mCi) of 225Ra, from which after 17-18 day holding can be received in addition 3.01·106 Bq (0.08 mCi) of 225Ac. Actinium-225 serves as a mother radionuclide in 225Ac/213Bi generator. From the loaded 225Ac generator it is possible during one - two months to elute every few hours a portion of 213Bi. This last operation is carried out on a place of 213Bi use - at the medical centres.

Extraction separation of ^{229}Th from ^{233}U

Uranium is dissolved in 6M HCL with the 30 % addition of hydrogen peroxide (30 % H_2O_2).

Isotopic structure of uranium in a solution:

233U 93.6 %;
Other uranium isotopes 6.4 %;
^{232}U impurity 0.0023%.

Isotopic structure of thorium in a solution:
^{229}Th 6.81 %
^{230}Th 0.08 %
^{228}Th traces (< 0.02 %)
^{232}Th 93.11 %

Activity ratio of ^{228}Th and ^{229}Th in a solution makes 12.8.

Uranium is extracted with 60 % tributyl phosphate (TBP) in decane from ^{233}U solution in 6M HCL, that has allowed removing from a solution at least 99.9 % of ^{233}U. Then uranium is restored by weak (up to 0.5M) solution of a hydrochloric acid. From the received solution uranium is deposited by ammonia, the deposit is filtered, dried and annealed up to uranium oxide-protoxide at a temperature of 830^0 C.

Thorium extraction. The water phase after uranium extraction (refinery and washing solutions) is extracted twice by 50 % 2D-ethylhexyl phosphorous acid in decane.

After washing of extract and holding in it of thorium during 30 and more days the following elements are extracted from 2D-ethylhexyl phosphorous acid:

Radium by 0.1 and 0.5M HCl solutions;

Actinium by 4M HCl solution;

Thorium by 20 % ammonium carbonate (NH_4CO_3).

The thorium solution is conditioned by removing the uranium and organics traces. Then a solution is evaporated, and its acidity is corrected. The received thorium solution is held for accumulation of daughter decay products (DDP). After 30-40 day holding it is possible to take a new portion of actinium.

Radium solution is held not less than 17.5 days, then with the help of 2D-ethylhexyl phosphorous acid an additional portion of ^{225}Ac is extracted (more than 30 % from ^{225}Ra initial activity).

In the received ^{225}Ac sample ^{233}U, ^{229Th} and ^{228}Th impurity radionuclides and long-live isotopes Np, Am, Pu and Po were not found within the limits of the measuring equipment sensitivity (< 0,005 % of ^{225}Ac activity). This procedure ends a complete work cycle of "raw" actinium reception in the extraction scheme.

Cleaning of Ac-225 from impurity of metals. An initial ^{225}Ac solution passed through a column with cation exchanger Dowex 50×4 for sorption of ^{225}Ac. Then 0.1 M HC is passed through a column, and then - 8-9 M $HClO_4$. ^{225}Ac is desorbed by 4M HCL, eluate is evaporated up to damp salts and diluted by 0.5M HCL. The received solution passed through a column with anion exchanger Dowex 1×8, then it is washed out with 0.5M HCL. ^{225}Ac is desorbed with 1M HNO_3 and transferred into solution by 0.1M HCl.

Reception of Bi-213 solutions. A mother radioisotope ^{225}Ac is sorbed from 0.1M HCl solution in a column filled with cation exchanger Dowex 50×4 (200-400 mesh), then a column is washed out with 0.1M HCL. ^{213}Bi is eluted by a volume of 0.5M HCL.

3 PREPARING OF CONJUGATE

Mini-antibodies to an antigene HER2 (clone 4D5) were received from Institute of Bioorganic Chemistry by Schemyakin and Ovchinnikov (Moscow). For preparation of conjugates are used helating agents of the company Macrocyclics (USA) production: p-SCN-Bn-DTPA (cat. # B-305) and DOTA-NHS-ester (cat. # B-280). For reception of buffer solutions are used salts, acids and alkalis of the firm Sigma (qualification puriss.), or the firm Merck (qualification GR for analysis) production. Arsenazo III (cat. # 11090), $YCl3 \cdot 6H_2O$ (cat. # 46,431-7) and DMSO (cat. # D8779) were acquired at the firm Sigma. For preparation of all solutions is used only deionized water. Dialysis is carried out in dialyzing bags of the firm Sigma (cat. # D9277) according to the manufacturer recommendations. Electrophoresis of a mini-antibody preparation is carried out in 15 % polyacrilamid gel by the standard technique. A set of low-molecular markers LMW of firm GE Healthcare production is used as markers of molecular weight.

4 RESULTS

1. It was proven by experiments with breast cancer cells in-vitro, that anti-HER2/neu mini-antibody conjugate created do bind effectively with tumor cells.
2. Stability of nanoparticles was proven by AFM measurements in vitro.

Hybrid nanoparticles designed are being evaluated by in-vivo studies in animals (nude mouse) model.

5 CONCLUSIONS

Tumor-targeted nanoparticles with conjugated specific antitumor antibodies are promising tools for the reduction of malignant tumors. Our results form basics for creation of a new targeted radiopharmaceuticals.