Preparation of ^{188}Re-HYNIC-Bombesin as a novel agent for prostate cancer therapy

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Abstract

The Bombesin (BBN) peptide has a high affinity to bind to gastrin-releasing peptide receptors (GRPRs) which is highly expressed in the prostate cancer. Radionuclide therapy with rhenium-188 (^{188}Re), due to its proper physical properties, as well as its availability as the ^{188}W/^{188}Re generator, creates advantages that can be well used to develop this labeled compound for therapeutic goals. In this study, preparation, quality control and biostribution of ^{188}Re-HYNIC-Bombesin were studied. The results of quality control showed radiochemical purity above 95% using high-performance liquid chromatography (HPLC) and Radio thin layer chromatography (RTLC). This complex was observed to be stable in blood serum over 24 h. The results of biological distribution showed that this radiopharmaceutical is significantly absorbed in the prostate and can be considered as a possible radiopharmaceutical agent for prostate cancer therapy.

Keywords: Carrier-free, HYNIC-Bombesin, Radiolabeling, Targeted therapy.

Introduction

Prostate cancer is one of the most common malignancies among men and is considered as one of the leading causes of cancer death worldwide[1]. Conventional treatments for prostate cancer are surgery, cryotherapy for localized prostate cancer, hormone therapy, gene therapy, chemotherapy, and radiotherapy [2-5]. Various prostate cancer therapies have severe side effects, and the proper treatment is one that strikes a balance between deteriorating quality of life and treatment. Early-stage prostate cancer therapy includes surgery or radiotherapy [2]. Recently, targeted radionuclide therapy has been used as an important alternative to conventional therapeutic methods for primary and metastatic tumors treatment because this method can specifically deliver radiation doses to tumor cells while protecting healthy tissues and organs.

There are some of beta-emitting radionuclides which is used for targeted prostate cancer therapy, like ^{90}Y [6,7], ^{177}Lu[8,9], ^{131}I [10], ^{161}Tb[11], and ^{188}Re (Delavari tez).. Among these radionuclides, ^{188}Re with proper nuclear properties (half-life: 16.98 hours, maximum beta energy: 2.12 MeV (71.1%), and gamma energy: 155keV (14.9%)), and its availability with high specific activity from the generator is considered as one of the promising radionuclides. The maximum penetration depth is about 3.5 mm in the soft tissue.

In the present research, we prepared (^{188}Re-Hynic-BBN) by labeling BBN with ^{188}Re via HYNIC as bi-functional chelating agent and evaluated ^{188}Re-Hynic-BBN as a novel agent for PRRT of prostate cancer.

Experimental

Material and Methods

Tricine and EDDA were purchased from Merck and Fluka companies, respectively. The HYNIC-BBN was supplied from Pars Isotope Company. ^{188}Re-perrhenate was obtained according to standard procedures using the ^{188}W/^{188}Re generator in our institute. All other chemical solvents of analytical grade were obtained from Sigma-Aldrich Company. Radio chromatography was performed by counting of the Whatman no.1 using a thin-layer chromatography scanner (Raytest-GITA scanner). Analytical reversed-phase high performance liquid chromatography (RP-HPLC) was performed on a Sykam S7131 HPLC system

Radiolabeling of HYNIC- BBN with ^{188}Re

The HYNIC-BBN stock solution with a concentration of 1 µg/µL was prepared in a 0.1 M phosphate buffer. First, 15 mg EDDA was dissolved in 400 µL of 0.1 M NaOH. 30 mg of Tricine dissolved in a 0.1 M phosphate buffer was then added to it dropwise. After that, 50 µg

of peptide was added. In the next stage, 600 µg of $SnCl_2$ dissolved in 0.1 M HCl was added and the acidity was adjusted between pH 4 to 5. Finally, 2-5 mCi of ^{188}Re was added to the reaction vial in a lead cell and the vial was heated for 45 min at 95ºC.

Quality control of ^{188}Re-HYNIC-BBN

The labeling yield and radiochemical purity of ^{188}Re-Hynic-BBN are determined using RTLC. For this purpose, 5µL of the final solution is stained on Whatman no.1 as the stationary phase and acetonitrile/water (1:1) was used as mobile phase. To confirm the RTLC results, the radiochemical purity, and labeling yield of the ^{188}Re-HYNIC-BBN compound were also determined using HPLC. To this purpose, 10-30 µl of the cooled final solution was injected into the HPLC column with a syringe. HPLC was performed by 0.1% trifluoroacetic acid (TFA)/water (Solvent A) and acetonitrile (Solvent B) as a mobile phase with the following gradient: 100 % A: 0 % B for 3 min, 50 % A: 50 % B for 7 min , 0 % A: 100 % B for 5 min, with a flow rate of 2.6 mL min^{-1} for a total time of 30 minutes.

Stability studies

The stability of the labeled compound in saline and human blood serum (37 °C) was studied up to 24 hours after preparation. In order to investigate the stability of the radio-complex in saline, 100 µL of the final solution was incubated with 1cc of saline at room temperature and its stability was investigated at specific time intervals using RTLC. To study the stability of the radio-complex in human blood serum,100 µL of ^{188}Re(1mCi) HYNIC- BBN was added to 500 µL of fresh human serum and kept at 37ºC for 24 h. The radiochemical purity was determined at specific time intervals using RTLC technique.

Biodistribution studies

To investigate the biodistribution of ^{188}Re-HYNIC-BBN, Wistar rats weighing 150-200 g were used. Each rat was injected intravenously via a tail vein with 200µCi of ^{188}Re-HYNIC-BBN solution. The biodistribution was evaluated 1, 2, 4 and 12 hours post injection by sacrificing rats, dissecting their organs, and calculating percent injected dose per gram (%ID/g) by measuring the weight of each organ and the dose reached to it.

Results and discussion

Quality control

The results of quality control showed radiochemical purity above 95% using HPLC (Fig. 1).

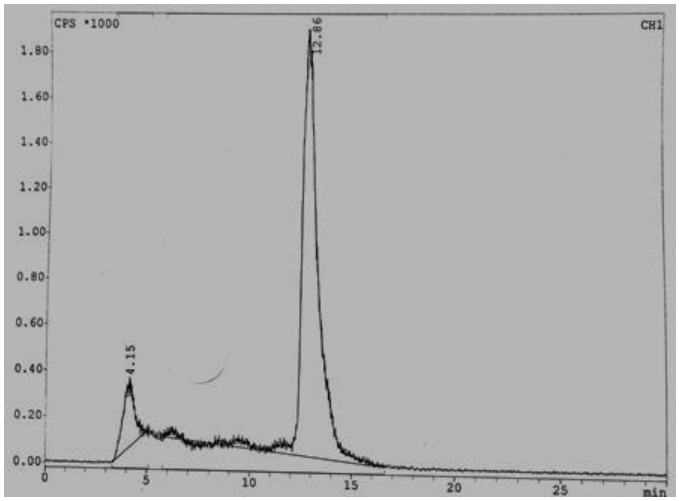

Fig.1. RP-HPLC chromatogram for the ^{188}Re-HYNIC-BBN compound.

Stability studies

The stability in normal saline and human blood serum was studied up to 24 hours after preparation. The obtained results showed that the labeled compound is stable in normal saline and human blood serum after 24 h and radiochemical purity is still above 95%.

Biodistribution of ^{188}Re-HYNIC-BBN in rats

The %ID/g of each organ was measured at different hours post injection (at 1, 2, 4, and 24 h post injection) and was shown in Fig.2.

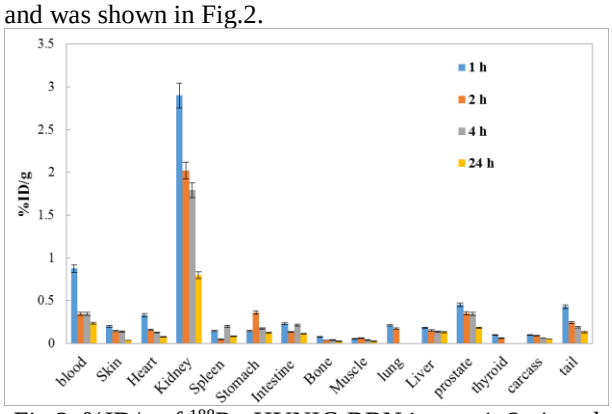

Fig.2. %ID/g of ^{188}Re-HYNIC-BBN in rats 1, 2, 4, and 24 h post injection

The obtained results showed that radiolabeled peptide was excreted mainly via the kidneys and this radio-complex had little tendency to be accumulated in the liver and excreted by hepatobiliary system. Rapid blood clearance was observed within 2h post injection. Moreover, it was significantly absorbed in prostate, indicating the expression of receptor in prostate because BBN peptide is an amphibian analogue of GRP that has a very high affinity for binding to GRP receptors.

Conclusions

In this research, radiolabeling of BBN analogue conjugated to *HYNIC* with ^{188}Re was investigated and evaluated for the treatment of prostate cancer. The obtained results showed that ^{188}Re-HYNIC-BBN has the adequate potential to be used in the prostate cancer therapy.

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