^{68}Ga labeled acyclic chelator HBED-CC conjugated folic acid for colorectal cancer diagnosis

Pyeong Seok Choi[1,2], Jun Young Lee[1], Taek-Soo Kim[1], Gun Gyun Kim[1,2], Jung Hoon Park[1], Sang Wook Kim[2]

[1]Radiation Instrumentation Research Division, Korea Atomic Energy Research Institute, Jeongeup,Jeollabuk-Do - 56212 Republic of Korea
[2]Department of Advanced Materials Chemistry, Dongguk University-Gyeongju, Gyeongsangbuk-Do – 38066, Republic of Korea
cps@kaeri.re.kr; ljy01@kaeri.re.kr kts0338@kaeri.re.kr; ggk@kaeri.re.kr; parkjh@kaeri.re.kr; interkim3@hanmail.net

To utilize as PET diagnostic radiopharmaceuticals for colorectal cancer, conjugated folic acid was used as a tartget ligand which has high affinity with folate receptor expressed on cell membrane of the various cancer. Acyclic Ga(Ⅲ) chelator HBED-CC was introduced instead of DOTA, NOTA chelator because of high stability and fast chelation with Gallium ions in R.T. This report describes the synthesis of precursor, labeling with Ga-68, measurements of radiochemical stability, in vitro cell uptake assay and in vivo analysis of microPET imaging of ^{68}Ga-HCEF. The cell uptake properties of ^{68}Ga-HCEF showed specifically on CT26 cells and microPET imaging was obtained distinctly for CT26 tumor.

I. Introduction

Folate receptor was overexpressed in various cancer cells and deficiently expressed in normal cells. They have been used as active target for delivery of drugs because of high affinity with folic acid.[1] CT26 cells of mouse colorectal cancer have been known to express folate receptor on cell membrane. The folate-based conjugates labeled with positron emitters are bound to the folate receptor and they are transported into the CT26 cells by endocytosis. Ga-68 [$T_{1/2}$: 68 min, $E_{\beta+}$: 740 keV (89%)] known as positron emitter which is attracted for the development of radiotracers for positron emission tomopraphy (PET) was typically labeled with bifunctional chleator (BFC) such as DOTA, NOTA conjugated small molecules like folic acid, peptide like RGD, TATE, TOC and antibodies.[2] In this study, heteroacyclic chelator N,N´-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N´-diacetic acid (HBED-CC) was chosen instead of DOTA, NOTA chelators due to beneficial properties that has much faster labeling time at ambient temperature and high stability with trivalent gallium ions at physiological pH. HBED-CC was introduced as a lipophilic side chain into the hydrophilic target ligand folic acid to interact with folate receptor. It is suitable to conjugate of hydrophilic target ligand for adjustment of total lipophilicity of the radiotracers influenced cell binding properties.[3] We synthesized precursors, HBED-CC-EDBE-Folate (HCEF) through amide coupling reaction between HBED-CC chelator and folic acid attached with hydrophilic linker 2,2'-(ethylenedioxy)-bis-(ethylamine) (EDBE) utilizing HBTU reagents and labeling with Ga-68 into the precursor before evaluation for stability, lipophilicity. In vitro evaluation of ^{68}Ga-HCEF was performed using CT26 cells cultured in folic acid free RPMI1640 media compared with excess folic acid addition on CT26 cells for blocked folate receptor. In vivo assay, we measured PET imaging for a Balb/C mouse model bearing CT26 tumor to confirm that ^{68}Ga-HCEF was effectively accumulated in CT26 tumor based on folate receptor targeting mechanism.

I.A. Experimental

I.A.1. Preparation of ^{68}Ga-HCEF

111 ~ 148 MBq of $^{68}GaCl_3$ in 0.05 M HCl from ^{68}Ge/^{68}Ga generator was added in 500 μL of 0.1M HEPES buffer (pH 4) and 100 μL of Precursor of HCEF (1 mg/1mL in 0.5 M NaOH). The reactant was stirred at room temperature for 5 min. The pH was controlled and adjusted in the range of 3.6 ~ 4.0. After the reaction, ^{68}Ga-HCEF was purified by semi-preparative reverse phase HPLC passing gradually 0.1% TFA in water and 0.1% TFA in acetonitrile to the C-18 column.

I.A.2. Measurement of radiochemical stability and lipophilicity

Radiochemical stability of ^{68}Ga-HCEF in PBS (pH 7.4) and human serum at 37 ℃ during each 5, 15, 30, 60, 120 min was determined by radio-TLC using pH 4 sodium acetate buffer as a mobile phase. To evaluate lipophilicity, 0.37 ~ 1.85 uCi of ^{68}Ga-HCEF was added into test tube containing 600 uL of each 1-octanol and PBS (pH 4). The tube was shaken for 3 min and centrifuged at 8,000 rpm for 5min to separate layer. The radioactivity of each layer was measured by gamma counter and calculated of lipophilicity (Log P = radioactivity in 1-octanol/racioactivity in PBS)

I.A.3. Biological evaluation for ^{68}Ga-HCEF

In vitro cell uptake study, CT26 cell line overexpressed folate receptor was cultured in folic acid free RPMI1640 with 10% fetal bovine serum and $1x10^5$ cells seeded in 24-well cell culture plates 24 h before treatment of radiotracer. After washing with PBS, the cells were incubated with the ^{68}Ga-HCEF (0.185 MBq/1 well) during 15, 30, 60, 120 min, respectively. To determine specific cell uptake, cells were blocked with excess folic acid (225 uM). Cellular uptake was terminated by washing 2 times with 1 mL of cold PBS and lysed using 200 uL of 0.1% SDS solution. The fractions of radioactive cells were measured in gamma counter. For the microPET studies, 3.7 MBq of ^{68}Ga-HCEF in saline was injected via a lateral tail vein into balb/c mouse bearing CT26 tumor xenografts. The anesthetized mouse was placed in PET scanner to perform a dynamic microPET scan starting at 15 min after post-injection during 30, 60, 90, 120 min, respectively.

I.A.4.Results and discussions

Radio chemical yield of >98% and radio chemical purity of >99% was obtained by measurement of reverse phase HPLC. ^{68}Ga-HCEF exhibited high stability in PBS (pH 7.4) and human serum at 37 ℃ during 120 min. Measured lipophilicity of ^{68}Ga-HCEF was relatively hydrophilic (Log P = – 1.06) and the more lipophilic than ^{68}Ga-NOTA-Folate (Log P = – 1.92).[5] In vitro assay, cell uptake of ^{68}Ga-HCEF for CT26 cells showed relatively higher binding affinity in folic acid free RPMI1640 media than post-treatment of excess folic acid. PET imaging of ^{68}Ga-HCEF for mouse bearing CT26 tumor showed fast blood and organ clearances and selectively specific uptake after injection of 3.7 MBq of ^{68}Ga-HCEF during 120 min.

II. CONCLUSIONS

The synthesized precursor, HCEF was effectively labeling with Ga-68 in ambient conditions and the overall radiochemical yield and purity for this radiotracer were quantitative with total synthesis time of less than 18 min. ^{68}Ga-HCEF has high radiochemical stability and suitable lipophilicity for human body environments. In the biology study, the results showed specific interaction on CT26 tumor. It could be promising candidates as selectively targetable PET radiotracers related to folate receptors expressing organs and tumors with HBED-CC chelator.

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