Immuno-PET Imaging of ^{89}Zr-Pertuzumab in Breast Cancer Model

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1. Introduction

Molecular imaging, especially positron emission tomography (PET), is of increasing importance for the diagnosis of various diseases. Currently, immuno-positron emission tomography (immuno-PET) imaging has shown great potential in cancer imaging. It can play a pivotal role in drug development and molecular characterization of tumors for personalized anti-cancer treatment. For PET imaging of monoclonal antibodies (immuno-PET), an appropriate positron emitter, with a half-life($t_{1/2}$) that is compatible with the time needed to achieve optimal tumor-to-background ratios, has to be securely labeled to targeting molecule. Therefore, zirconium-89 (^{89}Zr; $t_{1/2}$: 78.4 h) was selected as a possible candidate radionuclide. For imaging purpose, immuno-PET imaging is preferred over immune-SPECT imaging because of the higher resolution, sensitivity and more accurate image quantification [1-3].

The human epidermal growth factor receptor 2 (HER2) transmembrane oncoprotein is overexpressed in many human tumors, especially 15~20% of breast cancer, and several HER2-targeting agents have entered clinical practice. Trastuzumab was the first clinically approved anti-HER2 antibody for breast cancer patients. Pertuzumab is another HER2-targeting mAb approved by the FDA after a survival benefit was achieved for patients with HER2-positive metastatic breast cancer. Importantly, pertuzumab binds to a HER2 binding site distinct from that of trastuzumab, augmenting the binding and treatment efficacy of each other [4].

In this study, we prepared desferrioxamine (DFO)-pertuzumab, anti-HER2 humanized antibody, conjugate and labeled with ^{89}Zr and evaluated the immuno-PET imaging and biodistribution in breast cancer xenografted model.

2. Methods and Results

2.1 Preparation of ^{89}Zr-Pertuzumab

Pertuzumab (Perjeta) were purchased from Roche (South San Francisco, CA). Deferoxamine-p-benzyl-isothiocyanate (DFO-Bz-NCS) was purchased from Macrocyclics (Dallas, TX). HER2−expressing breast cancer cell lines JIMT-1 were purchased from the Korean Cell Line Bank (Seoul, South Korea) and and cultured in Dulbecco's Medium (DMEM) containing 10% fetal bovine serum (FBS) and anti-biotic/-mycitic agents and in a humidified incubator with 5% CO2 at 37℃.

Pertuzumab (10 mg/mL) was conjugated to 10-fold molar excess of DFO-Bz-NCS dissolved in dimethyl sulfoxide in 0.1 M sodium bicarbonate buffer (pH 8.5) at room temperature for 2 h and overnight incubated at 4℃. The resulting DFO-pertuzumab conjugate was purified and buffer exchanged into 20 mM HEPES buffer (pH 7.0) using ultrafiltration (MWCO 50 kDa, Sartorious). DFO-pertuzumab was radiolabeled with $^{89}ZrCl_4$ at 37°C for 60 min for a final pH between 6.8−7.2. ^{89}Zr-pertuzumab with radiochemical yields ≥ 98% (Fig. 1) as determined by instant thin-layer chromatography was used for *in vitro* and *in vivo* studies without further purification.

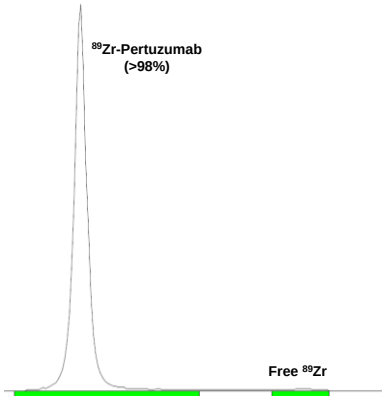

Fig. 1. Instant thin layer Radiochromatogram of ^{89}Zr-pertuzumab.

2.2 Immuno-PET imaging of ^{89}Zr-Pertuzumab in breast cancer xenografted model

Female athymic BALB/c mice (NARA Biotech, South Korea), aged 6 weeks, were used in all experiments. 1×10^7 of JIMT-1 cells were injected subcutaneously in the right flank of each mouse. Biodistribution was conducted when each tumor reached 100 mm^3 at 6~8 weeks after tumor implantation.

To evaluate the tumor targeting of ^{89}Zr-pertuzumab and *in vivo* HER2 expression level, immuno-PET imaging was performed in JIMT-1 tumor-bearing mice. ^{89}Zr-pertuzumab (2.2~2.5 MBq, 50 ug) was intravenously injected into the mice and static scans were acquired for 30 min at 1, 5 and 7 day post-injection using a small animal PET scanner (microPET R4, Concorde). Quantitative data were expressed as injected radioactivity dose per a gram (%ID/g). Image

visualization was performed using the ASIPro display software (microPET, Concorde Microsystems).

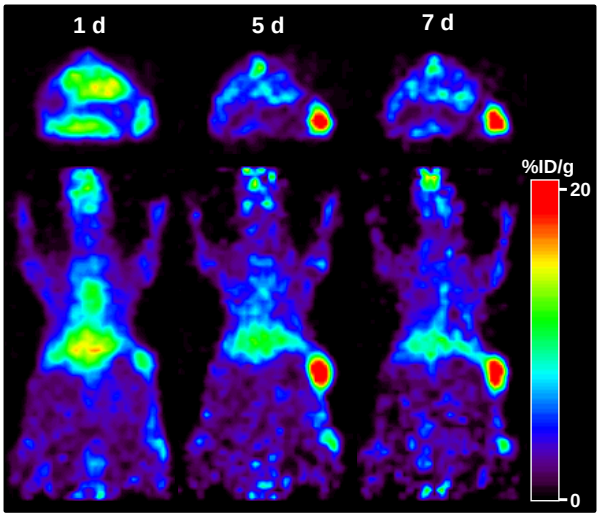

Fig. 2. Small animal PET imaging of ^{89}Zr-pertuzumab in breast cancer xenografted model.

The small animal PET transverse and coronal images of ^{89}Zr-pertuzumab were acquired at 1 d, 5 d and 7 d post-injection. At 1 d post injection, ^{89}Zr-pertuzumab was accumulated at liver and JIMT-1 tumor and also circulated in blood vessel deduced from the radioactivity in heart region. JIMT-1 tumor uptake increased and liver uptake decreased as time dependent manner, respectively. ^{89}Zr-pertuzumab was selectively localized in HER2 expressing breast cancer. These data suggest that ^{89}Zr-pertuzumab could evaluate the HER2 expression level in tumors as non-invasive and quantitative manners.

2.3 Biodistribution of of ^{89}Zr-Pertuzumab in breast cancer xenografted model

The biodistribution of ^{89}Zr-pertuzumab was evaluated in JIMT-1 tumor-bearing mice. Each tumor-bearing mouse was intravenously injected with 2.2~2.5 MBq (50 ug) of ^{89}Zr-pertuzumab. The mice (n = 3) were sacrificed at 7 day post-injection. The blood was collected by cardiac puncture and organs and tissues were excised. Samples were weighed and the amount of radioactivity was assessed in a gamma counter (Wizard 1480). The accumulated activity represented as the percentage of the injected radioactivity dose per a gram of tissue (%ID/g). Tumor-to-blood (T/B) ratio, tumor-to-muscle (T/M) ratio and tumor-to-liver (T/L) ratio were also calculated.

^{89}Zr-pertuzumab was accumulated in JIMT-1 HER2 expressing tumor with 18.1 ± 4.4 %ID/g at 7 day post-injection. Blood radioactivity was fast cleared at 7 day to 5.0 ± 1.4 %ID/g. The radioactivity of major organ or tissues were relatively low. However, there was moderate uptake in femur due to free ^{89}Zr is bone seeking radionuclide. Tumor-to-blood (T/B), tumor-to-muscle (T/M) and tumor-to-liver (T/L) ratios of ^{89}Zr-pertuzumab in JIMT-1 tumor bearing mice were 3.68 ± 0.61, 13.08 ± 2.26 and 2.47 ±0.41, respectively.

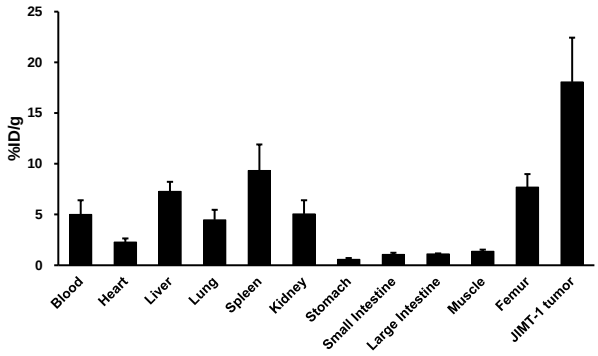

Fig. 3. Biodistribution of ^{89}Zr-pertuzumab in breast cancer xenografted model.

These data suggest that ^{89}Zr-pertuzumab could be used for evaluating the HER2 expression level in HER2 expressing tumor as non-invasive and quantitative approach by PET imaging. However, there was some need for a new chelating agent to reduce in vivo demetallation and to achieve more stable complexation with ^{89}Zr.

3. Conclusions

We successfully prepared ^{89}Zr-DFO-pertuzumab with high radiolabeling yield and radiochemical purity. ^{89}Zr-DFO-pertuzumab could be used to evaluate HER2 expression level in vivo and select the pertinent patient for HER2 targeted therapy in clinic.

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