Preparation and Characterization of ^{177}Lu Labeled Antibody against Tyrosine Kinase Receptor Her2

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1. Introduction

The tyrosine kinase receptor Her2, also known in humans as erbB2, is a member of the epidermal growth factor receptor (EGFR or erbB1) family. The Her2 is highly expressed in many cancer types and overexpressed in approximately 30% of all primary breast cancer. Overxpression of Her2 is associated with a poor prognosis. Her2 is a suitable target because it involves an extracellular domain that can be targeted by antibodies produced by B cells. Based on these advantages, we tried to prepare the ^{177}Lu labeled Her2 antibody. This radioimmunoconjugate could act by not only blocking the Her2 signalling pathway using antibody but also killing the tumour cell using β energy of ^{177}Lu.

2. Methods and Results

2.1 Preparation of ^{99m}Tc labeled DTPA-NCS-IgG

For our study we chose DTPA-NCS as the bifunctional chelating agent, which can be linked to the antibodies via the isothiocynatobenzyl(NCS). The anti-Her2 antibody(santacruz) was conjugated with DTPA-NCS the molar ratio of 1:1 to 1:4 at room temperature and pH 7.4. Unbound DTPA-NCS was removed by molecular weight filteration system (Millipore). One hundred micromolar of the purified anti-Her2 antibody immunoconjugate was added to the 10MBq/ml of ^{177}Lu solutions resulting to a final pH was 5 to 5.5 and stood for 5min at room temperature. Radiolabelling yields were determined with Instant Thin-Layer Chromatography (ITLC) scanner (EG&G Berthold linear Analyzer) with silica gel paper (Gelman Science Inc.) using saline as the mobile phase.

The R_f values of the ^{177}Lu, ^{177}Lu -DTPA-NCS, and ^{177}Lu -DTPA-NCS- anti-Her2 antibody were 1, 0.8~0.9, and 0, respectively. The radiolabeling yield was 100% at 5 min. (Fig. 1)

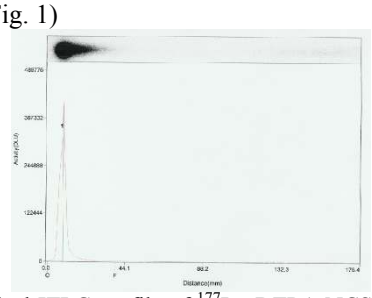

Fig.1. A typical ITLC profile of ^{177}Lu-DTPA-NCS- anti-Her2 antibody determined byITLC-SG using an autoradiographic system. The *x-axis* distance in cm from origin(left) the *y-axis* shows proportional activity count

The filmless autoradiographies of silica gel or polyacrylamide gel were carried out using Cyclone Storage Phosphor System (PerkinElmer, Wellesley). The radioactivity was measured using an ionizing chamber (Capintec 115R, BIODEX Atomlab 200) by setting the calibration value for ^{177}Lu that was corrected and calibrated by the manufacturer. The radioimmunoconjugate was analyzed by polyacrylamide gel electrophoresis. Ten ul of sample solution was mixed with 2 ul of the sample buffer (5 × containing 125 mM Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, and 1 ug/ml of bromophenol blue (BPB)). Polyacrylamide gel electrophoresis was carried out using a Hoefer Scientific Instruments electrophoresis apparatus and visualized by Coomassie brilliant blue R-250 staining. The gel was also analyzed by autoradiography. The radioactive bands corresponded to the bands of labeled with conjugated anti-VEGFR 1. That means no degradation products or other impurities resulted from the conjugation. (Fig.2)

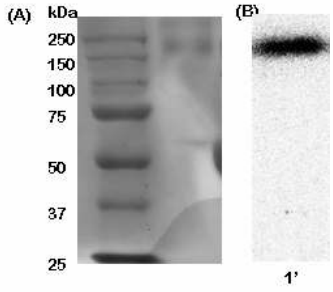

Fig.2. Electrophoresis and autoradiograpy of analysis ^{177}Lu-DTPA-NCS- anti-Her2 antibody using polyacrylamide gel. The gels were visualized by coomassie brilliant blue R-250 (A) and autoradiography (B). 1, 1' ; ^{177}Lu -DTPA-NCS -anti-Her2 antibody

3. Conclusions

The immunoconjugate of Her2 antibody (Ab) was optimized by reacting the DTPA-NCS with the Ab for 10min at room theperature at pH 7.4 with the molar ratio of 1:1. Then the radioimmunoconjugate was prepared by simple mixing of ^{177}Lu solution with the immunoconjugate at room temperature with high radiolabeling yield(≥ 98%). We also demonstrated the stability of the ^{177}Lu in DTPA-NCS-Her2 Ab by comparing the images of electrophoresis with autoradiography. In this study, we carried out a radioimmunoconjugation using ^{177}Lu with Her2 Ab to

apply as a potential radiotheraputic agent for breast cancer.

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