Tumor Targeting of Radiolabeled Antibodies using HYNIC Chelate

Tae Sup Lee, [b] Siti Darwati, [a] Wee Sup Chung, [a] Kwang Sun Woo, [a] Tae Hyun Choi, [a] Hye Kyung Chung, [a] Myong Jin Lee, [a] So Yeon Kim, [a] Jae Ho Jung, [a] Chang Woon Choi, [a] Sang Moo Lim.

a Lab. of Cyclotron Application, Korea Institute of Radiological and Medical Sciences (KIRAMS), Gongleung-Dong 215-4, Nowon-Gu, Seoul, 139-706, nobelcow@kcch.re.kr

b Radiopharmaceuticals, National Nuclear Energy Agency, Kawasan Puspiptek Serpong, Tangerang, 15310, Indonesia

1. Introduction

There is an increasing interest in the use of labeled antibodies for diagnosis of cancers as well as for therapy. Various radiolabeling methods have been used in order to obtain better tumor specific targeting for detection and therapy. It was generally used to tumor targeted immunotherapy and immunodetection that lym-1, mouse monoclonal antibody, was specific binding to surface antigen of Raji [1]. The 3E8 antibody was produced from humanized anti-TAG-72 monoclonal antibody (AKA) by amino acid change in 95-99 residues of heavy chain complementary determinant regions (HCDRs) 3 using phage displayed library technology.

In this study, we are investigating the usefulness of HYNIC chelate as a bifunctional chelating agent in radioimmunodetecton of tumor. Two types of antibodies, Lym-1 and 3E8, were used for the conjugation with HYNIC chelate. Lym-1 and 3E8 are specific antibodies to surface antigen of Non-Hogkin's lymphoma and TAG-72 antigen of colorectal carcinoma [2], respectively. We prepare HYNIC-antibody conjugates, determine radiolabeling yield with ^{99m}Tc and evaluate tumor targeting in tumor bearing nude mice model.

2. Methods and Results

2.1 Preparation of HYNIC-Lym-1 and -3E8

The solution A was made by dissolving 15.9 g of HYNIC (MW = 262) in dimethylsulfoxide (DMSO) into a final concentration of 64 mg/ml. The solution B was prepared by dissolving 10 mg of the 3E8 antibody in 1 ml of 0.1M sodium phosphate buffer (pH 7.8). A 20 times higher molar ratios of solution A was added into the solution B and vortexed in room temperature. Then mild stirring was carried out for 5 hours dark room. Dialysis with 10mM Sodium Citrate buffer (pH 5.2) was performed for 24hr with five buffer change. The volume was made as low as possible with Centricon-30. The concentration was adjusted into 10mg/ml with 10mM citrate buffer, pH=5.2. Aliquots were made at 1 mg/0.1 ml in Eppendorf tube.

2.2 Radiolabeling of HYNIC-Lym-1 and 3E8 with ^{99m}Tc

A 1 ml of $Na^{99m}TcO_4$ (25 mCi) in saline was added to the vial containing cold kit Tricine (36 mg Tricine and 0.04 mg $SnCl_2$ at pH 7.1)[3]. The radiolabeling yield was determined using ITLC-SG and methylethyl-ketone(MEK) and saline as mobile phase. This system gave radiolabeling yield at more than 99%, calculated by percentage of counts of the product from the total. When ITLC-SG was used as stationary phase and saline as mobile phase. about 1% free pertechnetate was obtained and giving radiochemical purity at more than 98%.

A 200 ul of this ^{99m}Tc-Tricine solution was added to already prepared HYNIC-Lym-1 and HYNIC-3E8 conjugates. The mixture was incubated in room temperature for 1 hour with mild stirring. The radiolabeling yield of ^{99m} Tc-HYNIC-Lym-1 and -3E8 were evaluated using ITLC-SG with 0.1M citrate buffer pH 5.2 as mobile phase. After addition of the ^{99m}Tricine into the HYNIC-Lym-1 and HYNIC-3E8 conjugate, the radiolabeling yield of monoclonal antibody obtained from was obtained in ITLC-SG and 0.1M citrate buffer pH 5.2 system. A more than 99% labeling yield was obtained.

2.3 Biodistribution of ^{99m}Tc-HYNIC-Lym-1 and -3E8

A BALB/c nude mice was injected with $1x10^7$ of Raji cells for Lym-1 targeting, LS174T cells for 3E8 targeting, subcutaneously on the left thigh of the animal. The prepared radiolabeled monoclonal antibodies (200 uCi/ 50 ug/ 0.1 ml) were intravenously injected into tumor bearing nude mice. At 2 h, 6 h and 24 h after administration, three mice per group were sacrificed. The organs and the tumor were removed, weighed and counted in gamma counter. The results were presented as percentage of injected radioactivity dose per gram of tissue (%ID/g).

In biodistribution of ^{99m}Tc-HYNIC-Lym-1 (Fig. 1), the kidney was major excretion route and radiolabeled lym-1 was metabolized in reticuloendothelial system of liver and spleen, because accumulation of radioactivity was higher than other organs. The blood radioactivity of 99mTc-HYNIC-Lym-1 was 18.41 and 7.51, respectively, in 6hr and 24hr. T/B and T/M ratio increased from 0.51 and 4.62 at 6hr to 1.15 and 4.38 at 24hr.

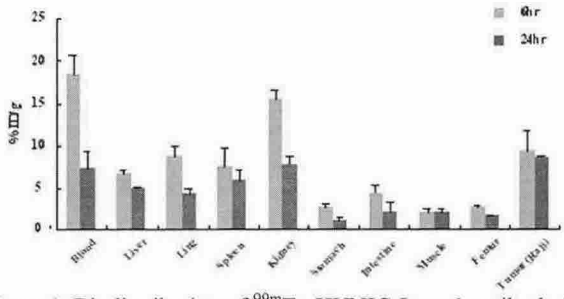

Figure 1. Biodistribution of ^{99m}Tc-HYNIC-Lym-1 antibody in Raji xenograft tumor bearing nude mice at 6h and 24h post injection.

In biodistribution of ^{99m}Tc-HYNIC-3E8 (Fig. 2), the labeled monoclonal antibody showed high tumor uptake. The spleen and kidney were also showed higher accumulation of radioactivity than other organ (16 and 14 %ID/g, respectively), but these were non specific uptake. The ratio of tumor-to-blood (T/B) was increased from 0.28 at 2 h to 1.47 at 24 h, and tumor-to-muscle (T/M) was increased from 3.86 at 2 h to 5.20 at 24 h as time dependent manner.

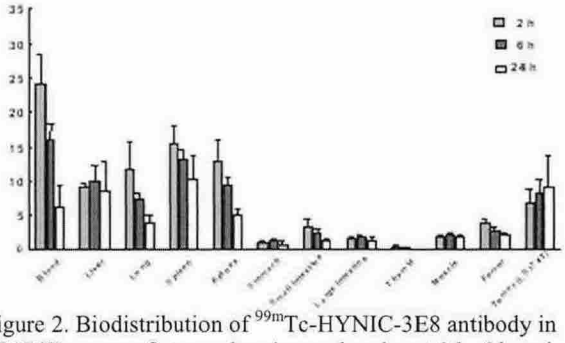

Figure 2. Biodistribution of ^{99m}Tc-HYNIC-3E8 antibody in LS174T xenograft tumor bearing nude mice at 2 h, 6 h and 24h post injection.

2.4 Gamma camera image of ^{99m}Tc-HYNIC-Lym-1 and -3E8

In Lym-1 targeting experiment, Raji xenograft bearing nude mouse was intravenously injected into tail vein with ^{99m}Tc-HYNIC-Lym-1 at 200 uCi/ 0.1ml per head of animal. Gamma camera images at 24 h post injection were observed at 1,000,000 counts by gamma camera. ^{99m}Tc-HYNIC-Lym-1 was selectively localized in Raji tumor at 24 h in gamma camera image (Fig. 3).

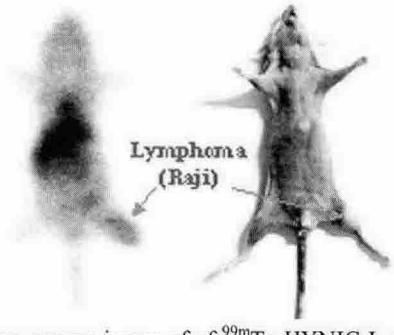

Figure 3. Gamma camera imags of of ^{99m}Tc-HYNIC-Lym-1 antibody in Raji xenograft tumor bearing nude mice at 24 h post injection.

In 3E8 targeting experiment, LS174T xenograft bearing nude mice were intravenously injected into tail vein with ^{99m}Tricine-HYNIC-3E8 at 200 uCi/ 0.1ml per head of animal. Gamma camera images at 6 h and 24 h post injection were observed at 1,000,000 counts by gamma camera. ^{99m}Tc-HYNIC-3E8 was localized in tumor at 6 h and 24 h images (Fig. 4). Heart and liver uptake was decreased from 6 h to 24 h, but LS174T tumor uptake was increased.

Both radiolabeled antibodies using HYNIC chelate showed specific tumor accumulation in xenograft nude mice model.

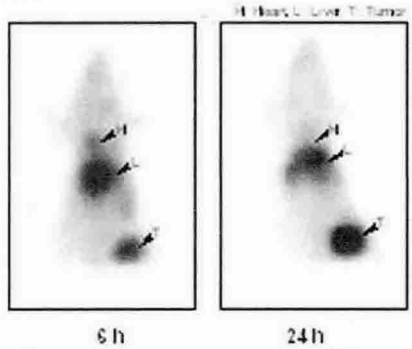

Figure 4. Gamma camera imags of of ^{99m}Tc-HYNIC-3E8 antibody in LS174T xenograft tumor bearing nude mice at 6 h and 24 h post injection.

3. Conclusion

Radiolabeling method of tumor specific antibody using HYNIC chelate was so easy and convenient and short preparation time was required and high yield and radiochemical purity were obtained on the labeling of monoclonal antibodies, Lym-1 and 3E8 with ^{99m}Tc using HYNIC as bifunctional chelator and Tricine as coligand. The high tumor uptake was obtained in nude mice bearing Raji or LS174T xenograft.

Based on these results, it is suggested that ^{99m}Tc-HYNIC-Lym-1 and ^{99m}Tc-HYNIC--3E8 can be used as radioimmunodetection in tumor specific antigen expressing tumors and also HYNIC conjugate of various biomolecules can be used for tumor detection in clinical radioimmunoscintigraphy.

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