Use of ^{99m}Tc-MAG3-biocytin in Avidin/Biotin based Three Step Targeting

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

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

1. Introduction

A general point of concern in clinical radioimmuno-scintigraphy(RIS) and radioimmunotherapy(RIT) studies is the long residence time of radioimmuno-conjugates in the bloodstream, resulting in limited sensitivity and specificity in RIS and in myelotoxicity in RIT. A solution to this problem might be the use of so-called pretargeting strategies. The concept of pretargeting is based on the separation, in time, of antibody localization and radionuclide targeting[1,2].
Several ligand-receptor systems have been evaluated of which the biotin-(strept)avidin system is the most widely studied. The attractiveness of this system lies in the very high affinity of avidin and streptavidin for biotin[3].
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 feasibility of pretargeting strategy using biontinylated 3E8, avidin and ^{99m}Tc-MAG3-biocytin in LS174T tumor bearing mice model. We prepare biotinylated 3E8 antibody conjugates, perform radiolabeling and in vitro targeting of ^{99m}Tc-MAG3-biocytin and evaluate tumor pretargeting in tumor bearing nude mice model.

2. Methods and Results

2.1 Preparation of biotinylated 3E8

The 3.3 ml of 3E8 IgG (20 mg/ml, 1 equi.) dissolved in 50mM bicarbonate buffer pH=8.5 gently stirred on ice. 0.1ml of Sulfo-NHS-LC-Biotin(3.7 mg/ml, 5 equi) umole) dissolved in saline was added and reacted for 2 hr. The removal of free biotin by centrifugation was performed by three times with a Centricon-30 microconcentrator. The volume was made as low as possible with Centricon-30. The concentration was adjusted into 10mg/ml with 0.1M phosphate buffer pH 7.0. Aliquots were made at 1 mg/0.1 ml in Eppendorf tube.

2.2 Radiolabeling of S-benzoyl-MAG3-Biocytin with ^{99m}Tc

A 1 ml of $Na^{99m}TcO_4$ (10 mCi) in saline was added to the vial containing 20ul of S-benzoyl-MAG3-biocytin (10 mg /ml in DMSO) and sequentially added 10ul of stannous tartrate(dissolved in Ar purged D.W by addition of 100ul of 10N HCI) and 50ul of 1M sodium acetate (8.2mg/ml, dissolved in D.W). The reaction was performed for 30 min at 100℃ under a stream of Argon. After the reaction mixture was diluted with 5 ml H2O, loaded on Sep-pak C18 cartridges. The cartridge was subquently washed with 10ml H2O,10ml 5% EtOH in: 10mM PBS, pH=6.7. ^{99m}Tc-MAG3-biocytin was eluted with 1ml EtOH. The solvent was evaporated at room temp. under a stream of Argon, and ^{99m}Tc-MAG3-biocytin was dissolved in saline. Radiolabeling yield was evaluated by HPLC.
A more than 98% labeling yield and radiochemical purity were obtained(Fig 1).

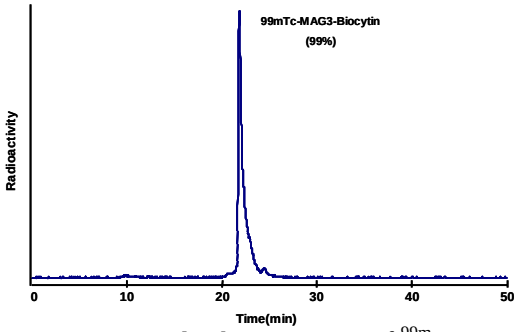

Figure 1. HPLC radiochromatogram of ^{99m}Tc-MAG3-biocytin.

2.3 In vitro evaluation of three step targeting

Specific binding between biotinylated 3E8 antibody and avidin was determined by incubation of a aliquot of ^{125}I-Avidin and biotinylated 3E8 and by analysis of bound fraction by size exclusion HPLC (Fig. 2).

Biotinylated 3E8 antibody was specifically bound to radiolabeled avidin and high molecular weight bound conjugate was pre-eluted in HPLC

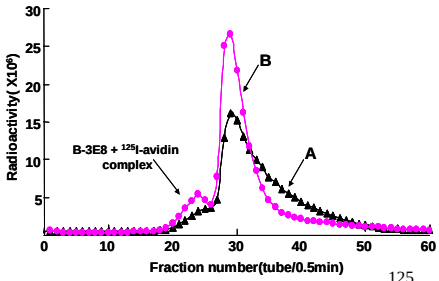

Figure 2. HPLC radiochromatograms of ^{125}I-Avidin (A) and ^{125}I-avidin by addition of biotinylated 3E8 (B).

To evaluate competitive bining assay between avidin and ^{99m}Tc-MAG3-biocytin, avidin conjugated agarose diluted with sepharose 4B(Phamacia) to adjust the biotin binding capacity(about 0.1ug biotin binding/

100ul packed beads). One hundred ul of packed beads was diluted with 100ul of PBS. To this avidin-bead solution, a mixture of 200ul of biotin at amount up to 24.4ug and 100ul of ^{99m}Tc-MAG3-Biocytin (2.5ng) was added. After 1 hr incubation at room temp the test tubes were centrifuged at 2,500*g* for 30min. The supernatants were aspirated and the agarose beads were washed with 1ml of PBS. Bound radioactivity bound to agarose beads was measured in a gamma counter

The binding of ^{99m}Tc-MAG3-biocytin to avidin was shown to be specific by performing a competitive bind assay using cold biotin as a competitive ligand(Fig.3)

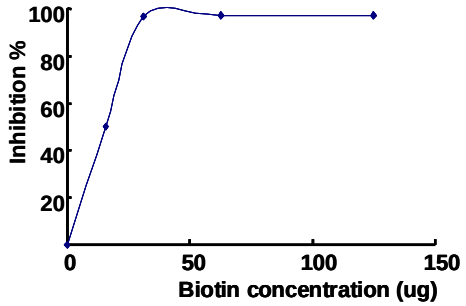

Figure 3. Competitive binding assay between avidin-agarose and ^{99m}Tc-MAG3-biocytin according to addition of biotin.

2.4 In vivo evaluation of three step targeting

For avidin/biotin pretargeting experiment, LS174T xenograft bearing nude mouse was prepared by subcutaneouly injected LS174T tumor cell(1 X 10^7 cell/0.1ml) into right thigh of nude mice. At first biotinylated-3E8(50 ug/0.1 ml/head) was injected intravenously. Two day later, avidin(250 ug/0.1 ml/head) was injected to clear the biotinylated 3E8 from the blood. Another 2 day later, ^{99m}Tc-MAG$_3$-biocytin(200 uCi /5 ug) injected. Gamma camera image was obtained at 6 hr post-injection of ^{99m}Tc-MAG$_3$-biocytin in LS174T tumor bearing nude mice.

^{99m}Tc-MAG3-biocytin was localized in LS174T tumor and major excretion route of ^{99m}Tc-MAG3-biocytin was GI tract.

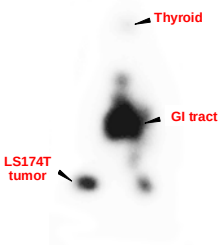

Figure 4. Gamma camera image of three step targeting using ^{99m}Tc-MAG3-biocytin in LS174T tumor bearing mice at 6 h post-injection.

3. Conclusion

Radiolabeling yield and radiochemical purity of ^{99m}Tc-MAG3-biocytin were above 98%. In size exclusion HPLC analysis, biotinylated 3E8 antibody specifically bound to avidin and in competitive binding assay, The binding of ^{99m}Tc-MAG3-biocytin to avidin was shown to be specific. In *in vivo* three step targeting, ^{99m}Tc-MAG3-biocytin was selectively localized in LS174T tumor.

Based on these results, it is suggested that avidin/biotin based three-step pretargeting using ^{99m}Tc-MAG$_3$-biocytin showed higher tumor-to-non-target ratio. Three-step avidin/biotin based antibody pretargeting using ^{99m}Tc-MAG$_3$-biocytin can be useful for tumor targeting in radioimmunoscintigraphy and also could be used as radioimmunodetection method for the tumor detection and excision in clinical radioimmuno-guided surgery.

REFERENCES

[1] Goldenberg DM. Targeted therapy of cancer with radiolabeled antibodies. J Nucl Med. Vol 43, No 5, pp693-713, 2002

[2] van Gog FB, Visser GW, Gowrising RW, Snow GB, van Dongen GA. Synthesis and evaluation of 99mTc/99Tc-MAG3-biotin conjugates for antibody pretargeting strategies. Nucl Med Biol. Vol 25, No 7,pp611-619, 1998

[3] Boerman OC, van Schaijk FG, Oyen WJ, Corstens FH. Pretargeted radioimmunotherapy of cancer: progress step by step. J Nucl Med. Vol 44, No 3, pp400-411, 2003