Small animal PET imaging of TAG-72 expressing tumor using ^{68}Ga-NOTA-3E8 Fab radioimmunoconjugate

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1. Introduction

The tumor-associated glycoprotein TAG-72 is express ed in the majority of human adenocarcinomas but is rarely expressed in most normal tissues, which makes it a potential target for the diagnosis and therapy of a variety of human cancer [1, 2]. 3E8 is anti-TAG-72 humanized antibody. Antibody fragments have some advantages such as improved pharmacokinetics and reduced immunogenicity compared to whole IgG. ^{68}Ga is a short-lived positron emitter ($t_{1/2}$ 68 min; β^+, 88%) that is produced, independent from a cyclotron, by a ^{68}Ge/^{68}Ga generator. The parent nuclide ^{68}Ge has a long half-life (270.8 day), allowing its use as a generator for more than 1 year. A ^{68}Ga is labeled with antibodies through bifunctional chelators, which allows possible kit formulation and which wide availability of the nuclear imaging agents [3, 4]. In this study, Fab fragment of anti-TAG-72 humanized Ab (3E8) was conjugated with 2-(p-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4, 7-triacetic acid (p-SCN-Bn-NOTA) and radiolabeled with ^{68}Ga and acquire small animal PET image.

2. Methods and Results

2.1 Conjugation of p-SCN-Bn-NOTA with antibody

Antibody was used 3E8 Fab fragments. p-SCN-Bn-NOTA and 3E8 Fab was allowed to react for overnight at 4℃. Conjugation molar ratio of NOTA and 3E8 Fab was 10:1 and conjugation buffer was used 0.1 M sodium borate buffer [5]. NOTA conjugated 3E8 Fab was purified by dialysis and purity was check by SDS-PAGE.

2.2 ^{68}Ga labeling with NOTA-3E8 Fab

^{68}Ga was eluted from the ^{68}Ge/^{68}Ga generator with 0.1 N HCl [6, 7]. ^{68}Ga (18.5 MBq in 100 ul of 0.1 N HCl) was added to various concentration of NOTA-3E8 antibod ies (1, 5, 10, 20, 50, 100 ug in sodium acetate buffer) prepared and the optimum pH was adjusted with NaOH and sodium acetate buffer. After incubation for 30 min at room temperature, labeling efficiencies were checked by ITLC-SG with 0.1 M citrate buffer. Radiolabeling yiels of ^{68}Ga-NOTA-3E8 Fab showed

difference patterns as changes of antibody concentration. Radio labeling yields of ^{68}Ga-NOTA-3E8 Fab were such as 4.59, 52.04, 68.17, 85.15, 99.04, and 99.13 % in 1, 5, 10, 20, 50 and 100 ug antibody concentrations. Radio labeling yield was >99% in case of antibody concentration of >50 ug. Stability of ^{68}Ga-NOTA-3E8 Fab (18.5 MBq ^{68}Ga / 50 ug 3E8 Fab) was in human serum condition at 10, 30, 60, 120 min. ^{68}Ga-NOTA-3E8 Fab showed stable during 2 hr in human serum.
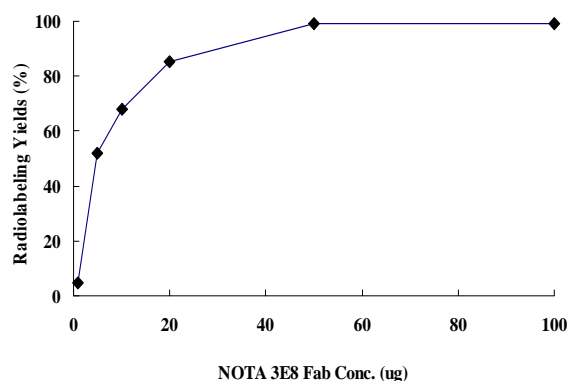

Fig. 1 Radiolabeling yields of ^{68}Ga-NOTA-3E8 Fab as various antibody concentrations.
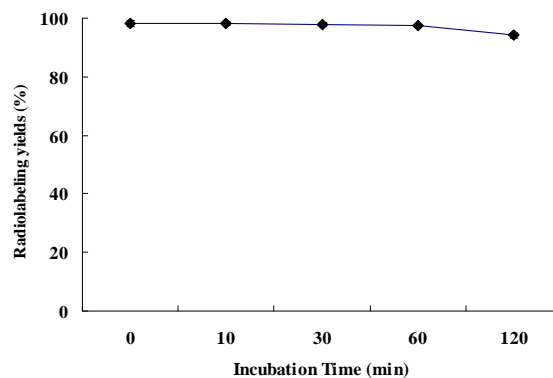
Fig. 2 Stability of ^{68}Ga-NOTA-3E8 Fab in human serum condition as various incubation time.

2.3 Small Animal PET Imaging

Small animal PET scans and image analysis were performed using a microPET R4 rodent model scanner (rodent R4 microPET scanner; Concorde Microsystems Inc). Nude mice received xenografts of 5×10^6 LS174T colon cancer cells and tumors were grown for 2 weeks. 7.4 MBq/100 ul ^{68}Ga-NOTA-3E8 Fab was injected through a tail vain. Tumor bearing animal model was anesthetized with 2% isoflurane at 1, 2, 3 h after injection, and small animal PET images were obtained for 30 min static images. The images were reconstructed by a 2-dimensional ordered-subsets expectation maximum (OSEM) algorithm [8]. Small-animal PET revealed rapid excretion through the urine and high levels of tumor, liver and kidney uptake (T/B ratio =4.97).
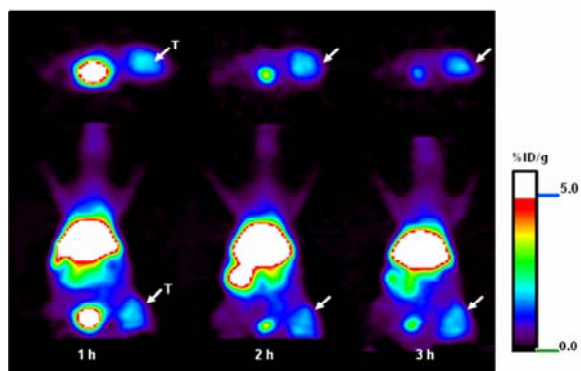

Fig. 3.Small Animal PET images of ^{68}Ga-NOTA-3E8 Fab in LS174T Tumor bearing mice. (T; LS174T tumor xenograft)

3. Conclusions

^{68}Ga-NOTA-3E8 Fab was prepared with high labeling yield and showed specific uptake to TAG-72 expressing tumor and rapid renal excretion. ^{68}Ga-NOTA-3E8 Fab could be used as a promising radioimmunoconjugate for tumor imaging in TAG-72 expressing tumor.

4. References

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