Immunoreactivity and Radioimmunoscintigraphy of 4-Lysine Single Chain (Fv) Lym-1 Antibody for the Radiometal Chelation

[a]Jae Ho Jung, [a]Tae Hyun Choi, [a]Tae Sup Lee, [a]Gwang Sun Uh, [a]Wee Sup Chung, [a]Eun Jung Kim, [b]Gi Jeong Cheon, [b]Chang Woon Choi, [b]Sang Moo Lim.

[a] *Lab. of Nuclear Medicine, Korea Institute of Radiological and Medical Sciences (KIRAMS),*

[b]*Department of Nuclear Medicine, KIRAMS,*

Gongleung-Dong 215-4, Nowon-Gu, Seoul, 139-706.

1. Introduction

Small size of recombinant scFv, composed of VH and VL region of IgG, has many advantages such as faster blood clearance, improved tumor localization and reduced human anti-mouse antibody (HAMA) response. On the other hand, owing to small size, number of amino group, which was not involved in binding site, of ScFv lym-1 was insufficient in conjugation with CITC-DTPA chelator for radiometal labeling. The goal of this study is to introduce 4-lysine tag to the end of ScFv lym-1 sequence for radiometal conjugation and to evaluate the immunoreactivity and radioimmunoscintigraphy of chelator conjugated 4-lysine taq scFv lym-1 (4-lys scFv).

2. Methods and Results

To perform cell binding assay with Human Burkitt`s Lympoma Raji cell, 4-lys scFv lym-1 labeled with I-131 by Iodo bead. The radiochemical purity of labeld products was evaluated by TLC-SG with acetone. And at the same time to performed radiolabeling with another isotope for radioimmunoscintigraphy.

Table 1. Radiolabeling yields of scFv Lym-1 and 4-lys scFv Lym-1 antibody

	Radiolabeling yield (%)			
	I-124	I-125	I-131	Tc-99m
scFv lym-1	-	64.5	> 99	-
Lysine scFv lym-1	> 99	-	>99	>99

Radioiodinated antibodies were measured for immunoreactivity using Lindmo method and radioimmunoscintigraphy was obtain with using gamma camera.

Immunoreactivity of I-131 labeled IgG, scFv and 4-lys scFv lym-1 antibodies were determined with HLA-DR antigen expressed Raji cell line. Immunoreactivity indicated that 4-lys scFv lym-1 antibody showed a potential binding activity against the Raji lymphoma cell (Table 2).

Table 2. Immunoreactivity of I-131 labeled IgG, scFv and 4-lys scFv lym-1 antibodies

	IgG	ScFv	4-Lysine ScFv
Immunoreactivity (%)	54	53.7	61

Immunoreactivity IgG, scFv and 4-lys scFv lym-1 antibodies were such as 54%, 53.7% and 61%.

Raji cell was injected in to the C57BR/cdJ SCID mice. Cells were grown routinely in RPMI1640 supplemented with 10% fetal bovine serum, antimicrotics, antibiotics and sodium bicarbonate. At 5-6 weeks of age, they received s.c. injection in a right thigh. with 2.5 x 10^6 Raji cell grown in tissue culture. When tumors were approximately 0.4 - 0.5 g in size (day 25-28, in the experiments reported here), mice received i.v. injections with radiolabeled antibodies. For the tumor growth antibodies, tumor size was estimated as length x width x depth. Gamma camera imaging of I-131 labeled scFv and 4-lys scFv lym-1 antibodies were taken time point at 1, 8, 24, and 48 hr. And in case of Tc-99m labeled 4-lys scFv lym-1 antibody was taken time point at 0.5, 1, 12, and 24 hr because half-life of Tc-99m. For obtain large size image was used 8 mm pinhole colimeter. Distence of mice and pinhole was 90 mm, and obtain counts 250,000 (Fig.1, 2).

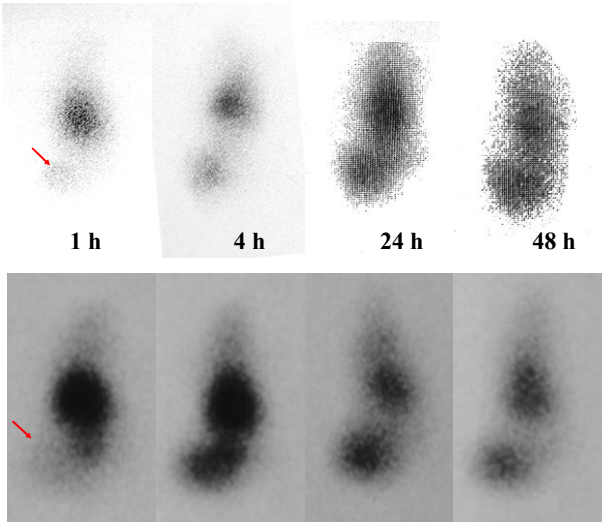

Fig. 3 Gamma camera image of I-131 labeled scFv (up) and 4-lysine scFv (down) lym-1.

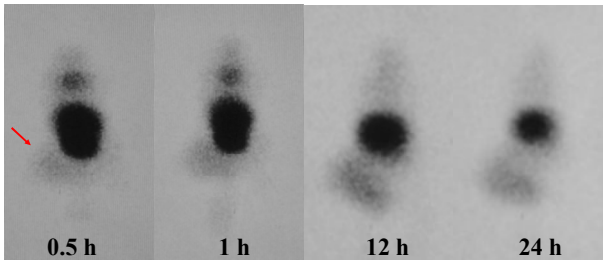

Fig. 4. Gamma camera image of Tc-99m labeled 4-lysine scFv lym-1.

3. Conclusions

ScFv lym-1 DNA was obtained from pCANTAB 5E. Radiolabeling yields of IgG lym-1 with I-125 was 64.2 %, but another isotopes were more than 99 %. Immunoreactivity of I-131 4-lys scFv lym-1 was about 61%. In vitro and in vivo properties of 4-lysine scFv lym-1 were similar to those of scFv lym-1. 4-Lysine scFv lym-1 showed fast blood clearance and tumor uptake. These results suggest that 4-Lysine scFv lym-1 antibody can be useful for radiometal chelator and tumor imaging agent.

4. References

1. Chung J-K, Yeo JS, Lee Ds, Park SY, Lee MC, Kim BK, et al. Bone marrow Scintigraphy using TC-99m anti granulocyte antiody in hematologic disorder. *J Nucl Med* 1996;30:978-82

2. Hong MK, Jeong JM, Yeo JS, Kim KM, Chang YS, Lee YJ, et al. In vitro pro-Perties and biodistribution of Tc-99m and Re-188 labeled monoclonal antibody CEA 79.4. *J Nucl Med* 1998;32:516-24