Comparison of the immunoreactivity of Rituximab antibody labeled with either I-125 or Re-188 for Radioimmunotherapy

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1. Introduction

Monoclonal antibodies against tumor-associated antigens can be applied as delivery vehicles for radionuclides to treat tumors. The specificity of MAbs for tumor-associated antigens can be exploited to direct radionuclides selectively to tumor cells after systemic administration.

In radioimmunotherapy, therapeutic efficacy depends on the choice of the radionuclide. The chemical characteristics of radioiodine and radiometals (Re-188) differ significantly with respect to labeling procedure and consequently the specificity of monoclonal antibody can be affected due to discrepancy of labeling condition.

Rituximab is a genetically engineered, chimeric anti-CD20 monoclonal antibody with mouse variable and human constant region. The CD-20 itself plays an important role in human B-cell proliferation and is an effective target for immunotherapy (1)

In the present study, we compared the immunoreactivity of I-125-labeled Rituximab with Re-188-labeled Rituximab according to radionuclide-optimized labeling condition in cell binding assay of Lindmo method (2)

2. Methods and Results

2.1 labeling of Rituximab with I-125 and Re-188

The Na-I-125 was added into a vial containing Iodobead and incubated for 5 minutes at room temperature (RT). Then Rituximab was added to the vial and incubated for 20 minutes, RT. The radiochemical purity was evaluated by TLC-SG with ethanol for mobile phase.
The labeling of reduced Rituximab antibody with Re-188 was performed in the presence of stannous tartrate at RT for 2 hours. The radiochemical purity was evaluated by TLC. Both lanbeled antibodies were assayed for immunoreactivity measurements using Lindmo method

High labeling yield of I-125-Rituximab was obtained at more than 99% and free I-125 at less than 1%, while Re-188 gave 95% radiolabeling yield and 5 % perhenate (Table 1).

Table 1. Comparison of labeling of Rituximab with I-125 and Re-188

Radioisotope	Radiolabeling Yield (%)	Impurities (%)
I-125	99	1
Re-188	95	5

It was found that the optimum amount of antibody used for the assay were 40 ng for I-125-Rituximab and 10 ng for Re-188-Rituximab. Antigen source of the assay was metastatic B-limfosit Farage cell line. The assay was carried out to obtain binding percentage vs cell number as shown in Figure 3A and Figure 4A for I-125-Rituximab and Re-188-Rituximab respectively.. The immunoreactivity was obtained from 1/intercept of the inverse of binding percentage vs inverse of cell number shown in Figure 3B and 4B. The results were 51% for Re-188-Rituximab and 60% for I-125-Rituximab.

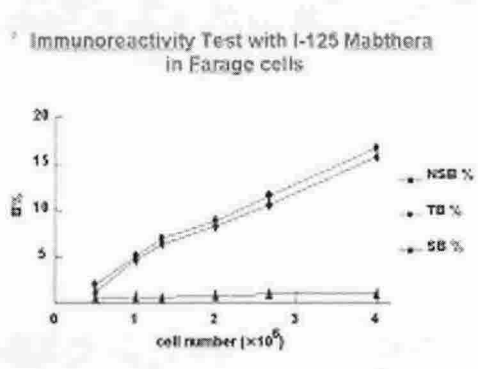

Figure 3APercent bound vs amount of cell on the cell binding assay for I-125-Rituximab and Farage cell

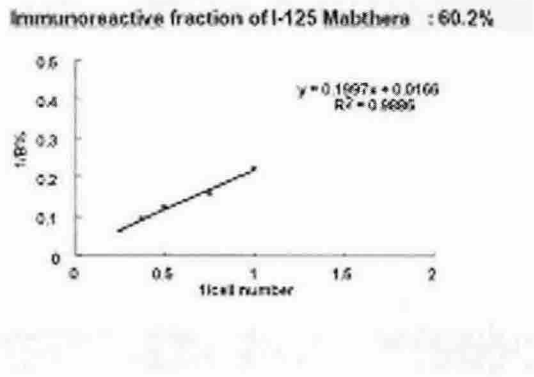

Figure 3B.Immunoreactivity test for I-125-Mabthera with Farage cells

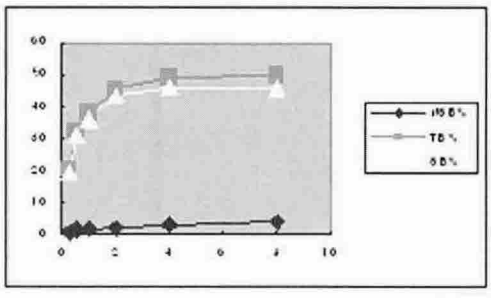

Figure 4APercent bound vs amount of cell on the cell binding assay for Re-188-Rituximab and Farage cell

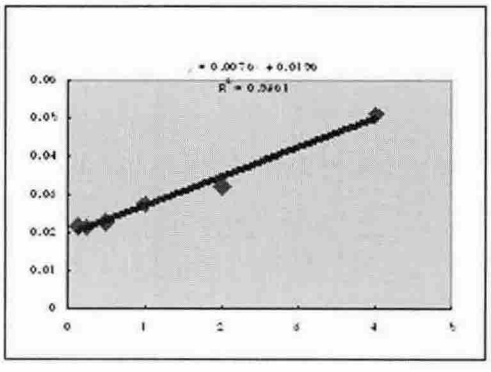

Figure 4BImmunoreactivity test for Re-188-Mabthera with Farage cells

Differences in immunoreactivity of both radiolabeled antibody possibly because of discrepancy of labeling condition. In addition, at the amount of $2x10^6$ cells, increasing binding percentage was observed for I-125-Rituximab, while the Re-188-Rituximab showed a saturation state. No increased binding was obtained. When using I-125-Mabthera, it was estimated that increasing amount of cell will gave higher immunoreactivity of the labeled monoclonal antibody. However, an unstable of radiometal Re-188-Rituximab was shown.

3. Conclusion

A higher labeling yield of Rituximab labeled with I-125 (99%) using iodobead method than labeled with Re-188 (95%) was obtained. The immunoreactivity of both labeled monoclonal antibody were 60% and 51% for I-125-Rituximab and Re-188-Rituximab respectively. This significant diference probably because of discrepancy of labeling condition.

REFERENCES

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