Factors Affecting Chemistry of Reduction - Mediated ^{99m}Tc-Labelling of Monoclonal Antibodies and Polyclonal Human Immunoglobulins

VIPA BOONKITTICHAROEN, Ph.D.*, DUANGPEN PUCHINDA, M.Sc.*,
ANNA NGONRATH NA AYUDHYA, B.Sc.*, PUANGTONG KRAIPHIBUL, M.D.*

Abstract

In developing a new method for preparing a radiopharmaceutical for clinical investigation, a thorough understanding of reaction stoichiometry is crucial in optimizing the labelling chemistry. Factors determining labelling efficiency of the 2 - mercaptoethanol (2-ME) - mediated ^{99m}Tc-labelling of antibody molecules were elucidated using anti-tumor monoclonal antibodies of different IgG subclasses (i.e. IOR-CEA(IgG_1), M170(IgG_1), 3F8(IgG_3) and EMD (IgG_{2a})) and polyclonal human immunoglobulins (Sandoglobulin). Antibodies which were sensitive to 2-ME reduction (i.e. required 500-1000 molar excess of 2-ME) could tag ^{99m}Tc with high efficiency since they possessed abundant reactive sites (i.e. sulfydryl groups) for ^{99m}Tc binding. Reduction sensitivity of antibodies was unlikely to be affected by IgG subclass and could be rated as follows : Sandoglobulin > IOR-CEA > 3F8 > M170 > EMD. Concentrations of the reduced antibodies for effective labelling appeared to be related to the reduction sensitivity, i.e. 0.2, 0.4 and 0.6 mg/ml were required for labelling of IOR-CEA, 3F8 and M170 respectively. In addition, susceptibility to 2-ME reduction seemed to reflect the rate of antibody labelling. For 2-ME resistant molecules, i.e. M170 and EMD, successful labelling could be achieved by using a slow ^{99m}Tc reducing agent such as $SnCl_2$ instead of SnF_2 which reacted more rapidly. Since 2-ME generates reactive sulfhydryl groups that are distal to antigen binding sites, the immunoreactivity of the modified antibody was not affected by the effect of reduction.

Key word : ^{99m}Tc-Labelling, Labelling Chemistry, Anti-Tumor Monoclonal Antibody, Human Immunoglobulin

BOONKITTICHAROEN V, PUCHINDA D, NGONRATH NA AYUDHYA A, KRAIPHIBUL P
J Med Assoc Thai 2001; 84: 545-553

* Division of Radiotherapy and Nuclear Medicine, Department of Radiology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

^{99m}Tc-labelled immunoglobulins are established radiopharmaceuticals for radio-immunoscintigraphy of malignant diseases and infection/inflammation processes[1,2]. Immunoglobulins used in nuclear medicine studies are classified as non-specific polyclonal immunoglobulins (i.e. human immunoglobulin G, HIG) which are used for infection/inflammation imaging[2] and specific monoclonal antibodies against tumor associated antigens[1] or inflammatory cells such as granulocytes and lymphocytes[2]. ^{99m}Tc is a radionuclide of choice for labelling immunoglobulins for several reasons[3] : (1) It has superior imaging properties because of its high photon flux and ideal emitted energy (i.e. 140 keV) which matches the spectral sensitivity of gamma camera. (2) Relatively short half-life (6 hours) and lack of beta emission that greatly reduces radiation doses to normal organs especially those involved in degradation and excretion pathways. (3) It is inexpensive and readily available in most nuclear medicine centers.

The immunoglobulin molecules may be labelled with ^{99m}Tc either by direct binding to thiolate groups in the antibody molecules or by indirect binding to a synthetic chelating agent which is usually conjugated to the protein before radiolabelling[4,5]. In contrast to the indirect methods which require complex synthetic procedures, chemistry of direct labelling is simple and the labelling process uses inexpensive reagents. This makes direct labelling a more popular technique. In generating reactive sites for ^{99m}Tc-labelling, the direct labelling technique employs organic reducing agents such as 2-mercaptoethanol or dithiothreitol to reduce some interchain disulfide bridges possibly at the hinge region to induce free thiol groups which are then tagged with ^{99m}Tc by transcomplexation from a weak ligand such as methylene diphosphonate (MDP)[4,6]. High labelling efficiency (>95%) can be achieved within 10 minutes[6]. Simple reaction pathway and rapid reaction rate make the direct labelling technique feasible for instant kit formulation. High labelling efficiency is particularly attractive in working with the short-lived radionuclide like ^{99m}Tc. Since the time consuming process for post-labelling purification can be omitted.

Instant kits for several monoclonal antibodies of IgG_1 subclass have been developed and used for clinical investigations, for instance PRIA3 [7] and BW 431/26[8] for detection of colorectal cancers, BW 250/183[9] for imaging of infectious / inflammatory foci. Because IgG of different subclasses contain an unequal number of hinge interchain disulfide bonds[10], questions have often been raised as to whether the stoichiometric parameters which are optimal for labelling IgG_1 will also be effective for other subclasses or for polyclonal HIG which contains mostly IgG_1 and IgG_2[5]. The present study investigated the stoichiometric factors in labelling four antitumor monoclonal antibodies of three different subclasses, i.e. IgG_1 (IOR-CEA, M170), IgG_{2a}(EMD) and IgG_3(3F8), as well as one polyclonal HIG. Reduction sensitivity, concentration of a reduced antibody and type of stannous compounds were found to govern labelling efficiency. Experiments were also conducted to understand the effect of 2-ME reduction on antibody immunoreactivity.

MATERIAL AND METHOD

Monoclonal and polyclonal antibodies

Four antitumor monoclonal antibodies of different subclasses were used as models for study. IOR-CEA[11] (Center of Molecular Immunology, Havana Cuba) is an anti-CEA antibody of IgG_1 subclass. M170 of IgG_1 subclass (reactive against cytokeratin in human adenocarcinoma) was obtained from Biomira Inc. (Edmonton, Alberta, Canada). EMD (IgG_{2a}) is reactive against the human epidermal growth factor (EGF) receptor was kindly donated by Dr Baum (Goethe University Hospital, Gemany). 3F8[12], a murine monoclonal IgG_3 reactive with GD_2 antigen on neuroblastoma, was a gift from Dr Cheung (Memorial Sloan-Kettering Cancer Center, New York, U.S.A.) The polyclonal Sandoglobulin ($IgG_1$60.5%, IgG_2 30.2%, IgG_3 6.6% and IgG_4 2.6%) was purchased from Sandoz Pharma Ltd (Basle, Switzerland).

Tumor cell line and target cell preparation

A human squamous cell carcinoma from the pharynx (FaDu) purchased from the American Type Culture Collection (ATCC, Rockville, U.S.A.) was used for this study because of its reactivity to EMD[13]. The cell line was maintained in a 25 cm^2 flask and cultured in RPMI 1640 supplemented with 5 per cent fetal calf serum (FCS). Subculture and feeding were performed weekly.

In target cell preparation, FaDu was cultured in a 75 cm^2 flask with 10 per cent FCS supplemented RPMI 1640 until confluence. Suspen-

sion of single cells was prepared by treating the culture with a 1:1 mixture (by volume) of 0.25 per cent trypsin and 0.03 per cent EDTA. The number of the tumor cells was counted by a haemocytometer. The tumor cell suspension was adjusted to a concentration of 7.5 x 10^5 cells/ml. Aliquots of 200 µl of cells were pipetted onto a 96-well plate. The cells were cultured for 24 hours in a CO_2 incubator and then were fixed with 2 per cent formalin for 1 hour at room temperature. The fixed cells were stored under 4°C for antibody assay.

Antibody reduction

One mg antibody samples in phosphate buffered saline (PBS), pH 7.2-7.4, with concentrations between 5 - 10 mg/ml were reduced with freshly prepared 2-mercaptoethanol (2-ME) at varying molar ratios from 500:1 - 6000:1 (2ME: IgG) at room temperature for 30 min. The reduced antibody was purified by Sephadex G25 M presaturated with 25 per cent human serum albumin (HSA) (PD-10, Pharmacia, Uppsala, Sweden) by using N_2-purged PBS as mobile phase. For the eluate fraction of 1 ml, the antibody peak was recovered from the 4th to 6th fractions (~ 90% recovery)[14].The concentration of reduced antibody was adjusted to 0.5 mg/ml. Aliquots of 250 µl samples were pipetted into 1.8 ml polypropyrene tubes and were stored at - 20°C for radiolabelling study.

Radiolabelling

The 250 µl of reduced antibody sample as mentioned in the previous section was thawed at room temperature. Five µl of MDP (methylene diphosphonate) -SnF_2 (containing 1 mg of MDP and 0.068 mg SnF_2 in 1 ml solution) was added to the antibody sample having high reduction sensitivity. While the antibody sample was more resistant to 2-ME reduction, it required 15 µl of MDP-$SnCl_2$ (containing 1 mg MDP and 0.162 mg of $SnCl_2 2H_2O$ in 1 ml solution). A total of 100 MBq ^{99m}Tc was then added into the reaction tube which was kept in a lead container. The labelling was performed behind a lead shield equiped with a lead glass window. The reaction proceeded at room temperature for 15 min.

Radiolabelling efficiency was determined by a two-strip ITLC (instant thin layer chromatography) technique. One µl of the labelled sample was spotted onto a 1x8 cm strip for developing and then the strip was cut into two halves for radioactive counting. The first ITLC-SG strip, silica gel impregnated glass fiber strip (Gelman Sciences, Ann Arbor, MI) was developed in normal saline solution where $^{99m}TcO^-_4$ and MDP-^{99m}Tc migrated along the solvent front. The second strip was ITLC-SG impregnated with HSA and was developed in a mixture of EtOH:NH_4OH:H_2O = 2:1:5. This would leave the hydrolysed reduced ^{99m}Tc to remain at the origin. After subtracting all radiochemical impurities from 100 per cent, we obtained the labelling efficiency which was the percentage of radioactivity incorporated into the antibody molecules.

Under the optimal reduction condition, efficiency in labelling the reduced antibody at varying concentrations was also investigated.

Immunoreactivity measurement

Effect of 2-ME reduction on antibody immunoreactivity was assessed by an enzyme-linked immunosorbant assay (ELISA) using formalin-fixed tumor cells on a 96-well plate. EMD was used as a model for study. Before assay, the plate was saturated with 1 per cent bovine serum albumin to block the nonspecific binding sites on target cells. Two hundred µl of EMD with a concentration ranging from 0.01-0.15 µg/ml was incubated with the target cells at 4°C for 1 hour. The unbound antibody was aspirated and the cells were washed three times with phosphate-buffered saline (PBS). The cells with bound antibody were incubated for another hour at 4°C with the peroxidase-conjugated rabbit antimouse IgG at 1:2000 dilution (Zymed laboratory, U.S.A.) The amount of primary antibody reacted to FaDu cells was in direct proportion to the amount of the secondary antibody which reacted against the primary antibody. Bound peroxidase-conjugated antibody was assayed by its substrate reaction, i.e O-phenylenediamine (OPD) reacted with H_2O_2 to form a color product in the liquid phase. One hundred µl of substrate solution containing OPD 0.4 mg/ml and 0.01 per cent H_2O_2 in 0.1 M citrate buffer pH5 was allowed to react under the catalysis of the peroxidase conjugated secondary antibody and the reaction was stopped in 10 min by adding 100 µl of 2N H_2SO_4.

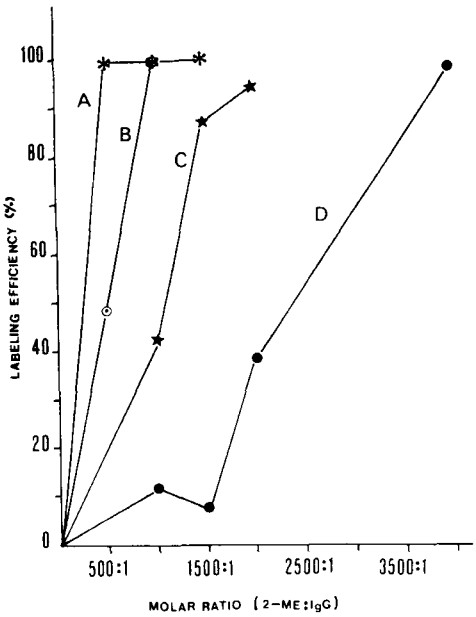

Fig. 1. **Reduction sensitivity of different monoclonal and polyclonal antibodies, which is indicated by labelling effeciency of each antibody sample. A. Human immunoglobulins (polyclonal IgG), B. IOR-CEA (an anti-CEA monoclonal antibody of IgG_1 subclass), C. M170 (an anti-cytokeratin monoclonal antibody of IgG_1 subclass), D. EMD (an anti-epidermal growth factor receptor monoclonal antibody of IgG_{2a} subclass).**

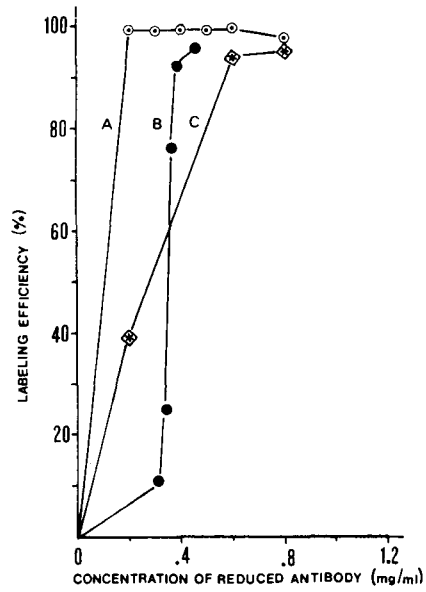

Fig. 2. **The dependence of labelling efficiency on the concentration of reduced antibodies.**
A. IOR-CEA (an anti-CEA monoclonal antibody of IgG_1 subclass),
B. 3F8 (an anti-GD_2 monoclonal antibody of IgG_3 subclass),
C. M170 (an anti-cytokeratin monoclonal antibody of IgG_1 subclass).

The solution in each well was transferred to another 96-well plate for measuring the optical density (OD) at 490 nm by a microplate reader (EL311S Autoreader, Biotek, U.S.A.). Slope from the double inverse plot between antibody concentration and OD representing antibody immunoreactivity was calculated by regression line analysis[13].

RESULTS

Sensitivity to 2-mercaptoethanol (2-ME) reduction

Different antibody molecules were reduced with 2-ME at different molar ratios (2-ME : IgG). The series of dose response relationships are presented in Fig. 1. Every immunoglobulin investigated here appeared to be unique in its susceptibility to reduction. The polyclonal HIG (Sandoglobulin) was most susceptible to reduction while the monoclonal EMD was the least. Reduction sensitivity did not seem to depend on IgG subclass. IOR-CEA and M170 which belonged to the same IgG_1 subclass were different in their reduction sensitivity. In another study which used 3F8, an anti-neuroblastoma monoclonal antibody of IgG_3 subclass, we observed nearly the same reduction sensitivity as IOR-CEA. To achieve the best labelling efficiency, Sandoglobulin, IOR-CEA, M170 and EMD had to be reduced at molar ratios of 500:1, 1000:1, 2000:1 and 6000:1 respectively.

Concentration of the reduced antibody

Since a very small fraction of disulfide bonds on the antibody molecule needed to be reduced for effective labelling with ^{99m}Tc[4], therefore concentration of the reduced antibody could be a factor governing the labelling effeciency. Fig. 2

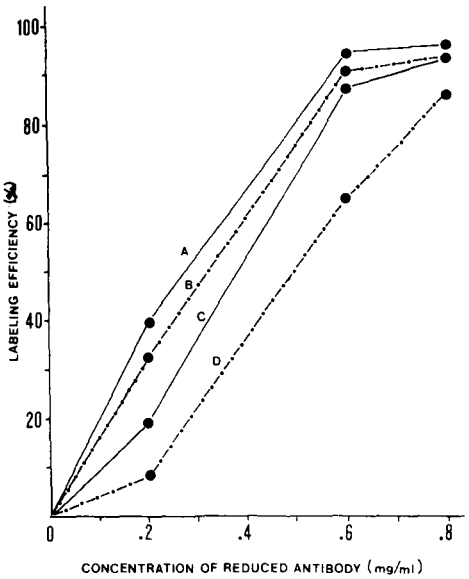

Fig. 3. The dependence of labelling efficiency on types of stannous compounds used in ^{99m}Tc reduction.
A. and B. represent the labelling characteristics of M170 respectively using $SnCl_2$ and SnF_2 for ^{99m}Tc-reduction. The antibody was priorly treated with 2-ME at 2000:1 in molar excess. C. and D. depict the labelling characteristics of M170 when using $SnCl_2$ and SnF_2 respectively in reducing ^{99m}Tc for antibody tagging. The antibody was priorly treated with 2-ME at 1500:1 in molar excess.

illustrates the dependence of labelling efficiency on the concentration of the reduced antibodies. The concentration curves reached plateau at different rates depending on the reduction sensitivity. Optimal concentrations for effective labelling were 0.2, 0.4 and 0.6 mg/ml for IOR-CEA, 3F8 and M170 respectively. IOR-CEA and 3F8 which had greater reduction sensitivity reached plateau sooner than the more resistant M170. Nevertheless, at concentrations greater than 0.5 mg/ml, all reduced antibodies could effectively trap ^{99m}Tc with high efficiency (i.e. > 95%).

Types of stannous compounds for ^{99m}Tc reduction

In radiolabelling the antibody molecules, ^{99m}Tc pertechnetate ($^{99m}TcO^-_4$) must first be reduced by stannous ion to an oxidation state which is fit for antibody tagging[16]. There are two forms of stannous compounds, i.e. SnF_2 and $SnCl_2$, that are used by most hot laboratories. For antibodies with high reduction sensitivity (Sandoglobulin and IOR-CEA), SnF_2 and $SnCl_2$ were equally effective in mediating ^{99m}Tc-labelling. Antibodies which were less susceptible to 2-ME reduction (M170 and EMD), only $SnCl_2$ was found to be effective. Fig. 3 demonstrates this concept using M170 as a model for study. It is noteworthy that $SnCl_2$ greatly enhanced the labelling yield even at sub-optimal reduction.

Effect of 2-ME reduction on antibody immunoreactivity

Because of the availability of the tumor targets, two monoclonal antibodies, IOR-CEA and EMD, were tested as to whether 2-ME reduction would change their immunoreactivity. Under optimal reduction, immunoreactivities were well preserved for both IOR-CEA (reduced at 1000:1 molar excess) and EMD (at 6000:1). In comparing with their native molecules, immunoreactivity indicies for IOR-CEA and EMD were 1.061 and 0.990 respectively. These indicated the immunoreactivity of the reduced antibodies was well preserved. Table 1 demonstrates the independence of immunoreactivity (derived in terms of Line Weaver Burk slope) on the concentration of 2-ME. EMD was used as the model for study. Although, changes in immunoreactivity were recognized at some concentrations of 2-ME, the magnitude of changes did not significantly correlate with the changes in concentration of 2-ME ($0.2 > p > 0.1$). Biological variation among the antibody samples and technical error might be the causes of fluctuation.

DISCUSSION

^{99m}Tc-labelling of immunoglobulins by reduction-mediated technique, in fact, is based on the principle of ligand exchange[16]. This involves, first, the generation of sulfhydry group in the antibody molecule by 2-ME reduction. In radiolabelling, ^{99m}Tc must be reduced by stannous ion to an oxidation state which is highly reactive for antibody tagging. The reduced ^{99m}Tc is stabilized by MDP in the form of weak complex and is subsequently transchelated to the sulfhydryl groups to form a more stable complex. Reaction sequences can be described as follows: -

$$\text{IgG} + 2\text{-mercaptoethanol} \rightleftharpoons \text{IgG - SH} + \text{Di - }\beta\text{ - hydroxyethanol disulfide} \quad (1)$$

$$^{99m}\text{TcO}^{-}_{4} + \text{Sn}^{2+} \rightleftharpoons \text{Reduced } ^{99m}\text{Tc} + \text{Sn}^{4+} \quad (2)$$

$$\text{Reduced } ^{99m}\text{Tc} + \text{MDP} \rightleftharpoons ^{99m}\text{Tc - MDP} \quad (3)$$

$$^{99m}\text{Tc - MDP} + \text{IgG - SH} \rightleftharpoons ^{99m}\text{Tc - IgG} + \text{MDP} \quad (4)$$

Table 1. Effect of 2-mercaptoethanol (2-ME) reduction on the immunoreactivity of EMD.

2-ME Reduction		Line Weaver Burk Slope	Immunoreactivity (%)
Molar Ratio	Concentration (mM)		
0:1	0	0.0721	100
1000:1	32.68	0.0972	74.18
1500:1	49.02	0.0866	83.27
2000:1	65.36	0.0998	72.24
4000:1	130.72	0.0765	94.25
6000:1	196.08	0.0750	96.13

In this study, all IgG molecules regardless of their clonality (polyclonal or monoclonal) and subclasses (IgG_1, IgG_{2a}, IgG_3) could be successfully labelled with ^{99m}Tc by direct approach using different reaction stoichiometry. Sufficiently reduced IgG molecules had to be readily available for transchelating the reduced ^{99m}Tc once the MDP complex was formed otherwise reoxidation occurred and the reduced ^{99m}Tc was converted back to $^{99m}TcO^{-}_{4}$ which was no longer reactive to antibody molecules. The molar ratio (2-ME : IgG) for reduction and the concentration of the reduced IgG were factors that determined the number of sulfhydryl groups for labelling. Antibodies that displayed high reduction sensitivity were labelled more easily than those which showed low sensitivity (M170, EMD).

Site of reduction is believed to occur at interchain disulfide bridges in the hinge region[4,6,16] which is a loosely folded structure and is easily accessible by various proteolytic enzymes and reducing agents[17]. Different numbers of hinge interchain disulfide bridges are reported for human and murine IgG[10]. Two, 4, 11 and 2 are the numbers of inter-heavy chain disulfide bonds in human IgG_1, IgG_2, IgG_3 and IgG_4 respectively. In mouse IgG, three hinge inter-heavy disulfide chains are observed in IgG_1 and IgG_{2a} whereas the IgG_3 is reported to have 2[17]. Results from our study suggested that the number of hinge disulfide bonds was not the factor governing reduction sensitivity of IgG molecules. Reduction sensitivity for the immunoglobulins investigated here could be ranked as follows : Sandoglobulin (IgG_1 60.5%, IgG_2 30.2%, IgG_3 6.6%, IgG_4 2.6%)[19] > IOR-CEA (IgG_1) > 3F8(IgG_3) > M170(IgG_1) > EMD(IgG_{2a}). Steric hindrance could be one factor[20] that accounted for the difference in reduction sensitivity among the antibodies studied here. Pathway of biosynthetic assembly of IgG molecule has also been observed to relate to the reduction sensitivity of immunoglobulin interchain disulfide bonds[21]. Disulfide bonds that are most resistant to reduction are the first to be found in antibody producing cells. In mouse IgG_{2a}, the first disulfide bonds formed are those that link the two heavy chains of the IgG molecule[22]. These disulfide bridges are also extremely resistant to reduction[22].

The use of different stannous compounds (SnF_2 and $SnCl_2$) for ^{99m}Tc reduction provided an insight into the reactivity of antibody towards the reduced ^{99m}Tc. Fluoride ion, in general, has higher electronegativity than the chloride ion[23]. This indicates a higher reaction rate for SnF_2 than $SnCl_2$ in reducing ^{99m}Tc. Experimentally, it required 0.1 μg of Sn^{2+} in form of SnF_2 and 0.5 μg of Sn^{2+} in form of $SnCl_2$ to induce optimal reduction of 37 MBq (1 mCi) of ^{99m}Tc. For antibodies with high reduction sensitivity (Sandoglobulin, IOR-CEA), SnF_2 and $SnCl_2$ were equally effective in mediating the radiolabelling. For M170 which had an intermediate reduction sensitivity, $SnCl_2$ was

more effective than SnF_2. With the most resistant antibody like EMD, $SnCl_2$ was the only effective reducing agent. The antibodies which were resistant to 2-ME reduction could in nature be slow reacting molecules. ^{99m}Tc should therefore be reduced at a rate slow enough to allow the capture by these slow reacting molecules. If fast reacting SnF_2 was used instead of $SnCl_2$, reoxidation of ^{99m}Tc occurred. Since these antibody molecules were too slow to transchelate the reduced ^{99m}Tc once it formed a weak complex with MDP. (i.e the reaction continuingly reversed from steps 3 to 2).

The merit of a novel labelling method depends not only on high labelling efficiency but also counts on the preservation of the immunoreactivity of the modified antibody molecules. Because the sulfhydryl groups serving as ^{99m}Tc binding sites located at the hinge region which is distal to hypervariable region for antigenic recognition[24]. Like many other investigators,[4,6-9,13,16] we also observed that immunoreactivity was unlikely to be affected by the action of 2-ME. Nevertheless, extensive insertion of ^{99m}Tc atoms into the sensitive hinge area of Fc region might impair the nonspecific binding to Fc receptors[25] on tumor cells rather than altering the antigenic recognition. This hypothesis supported by our previous findings involving the effect of increasing specific activity on immunoreactivity[13].

ACKNOWLEDGEMENT

This study was partly supported by World Health Organization (WHO) and the International Atomic Energy (IAEA).

(Received for publication on October 13, 1999)

REFERENCES

1. Britton KE. Towards the goal of cancer-specific imaging and therapy. Nucl Med Commun 1997; 18: 992-07.
2. Chianelli M, Mather SJ, Martin-Comin J, Signore A. Radiopharmaceuticals for the study of inflammatory processes : A review. Nucl Med Commun 1997; 18: 437-55.
3. Lever SZ. Technetium and rhenium compounds. In: Wagner HN, Szabo Z, Buchanan JW, eds. Principles of Nuclear Medicine. Philadelphia : WB Saunders Company, 1995; 1: 213-20.
4. Reilly RM. Immunoscintigraphy of tumours using ^{99m}Tc-labelled monoclonal antibodies : a review. Nucl Med Commun 1993; 14: 347-59.
5. Buscombe J. Radiolabelled human immunoglobulins. Nucl Med Commun 1995; 16: 990-01.
6. Mather SJ, Ellison D. Reduction-mediated technetium-99m labelling of monoclonal antibodies. J Nucl Med 1990; 31: 692-7.
7. Granowska M, Britton KE, Mather SJ, et al. Radioimmunoscintigraphy with Tc-99m labelled monoclonal-antibody, 1A3, in colorectal cancer. Eur J Nucl Med 1993; 20: 690-8.
8. Baum RP, Hertel A, Lorenz M, Schwarz A, Encke A, Hor G. ^{99m}Tc-labelled anti-CEA monoclonal antibody for tumor immunoscintigraphy: first clinical results. Nucl Med Commun 1989; 10: 345-52.
9. Becker W, Goldenberg G, Wolf F. The use of monoclonal antibodies and antibody fragments in the imaging of infectious lesions. Semin Nucl Med 1994; 24: 1-3.
10. Kerr MA, Thorpe R. Immunochemistry. The LABFAX series. Oxford : Blackwell Scientific Publications, 1994.
11. Tormo BR, Gavilondo JV, Dominguez C, Freyre M, Rodriguez T, Biberfeld P. CEA in colonic adenocarcinomas and precancerous lesions. An immunohisto-chemical study with a novel monoclonal antibody. APMIS 1989; 97: 1073-80.
12. Cheung NKV, Saarinen JE, Landmeier NB, Donovan MD, Coccia PF. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res 1985; 45: 2462-9.
13. Boonkitticharoen V, Puchinda D, Chouplywech P, Jalayondeja W. Reduction-mediated ^{99m}Tc-labelling of antitumor monoclonal antibodies : Effect of increasing specific activity on antibody binding kinetics. J Med Assoc Thai 1998; 81: 741-9.
14. Boonkitticharoen V, James H, Cheung NKV, Larson SM, Finn RD. Optimizing the recovery of chemically reduced monoclonal antibody for site-specific technetium-99m radiolabeling. BioFeedback 1993; 14: 706-7.
15. Robbins PJ. Chromatography of Technetium-99m Radiopharmaceuticals-A Practical Guide. New York: The Society of Nuclear Medicine, Inc., 1984.
16. Rhodes BA. Direct labelling of proteins with ^{99m}Tc. Nucl Med. Biol 1991; 18: 667-76.
17. Nezlin R. Internal movements in immunoglobulin molecules. Adv Immunol 1990; 48: 1-40.
18. Roitt I, Brostoft J, Male D. Immunology. New York: Mosby, 1985.
19. Romer J, Morgenthaler JJ, Scherz R, Skvaril F. Characteristics of various immunoglobulin preparations for intravenous application : I. Protein composition and antibody content. Vox Sang 1982; 42: 67-73.
20. Cecil R, McPhee JR. The sulfur chemistry of proteins. Adv Protein Chem 1959; 24: 255-389.
21. Bevan MJ, Parkhouse RME, Williamson AR, Askonas BA. Biosynthesis of immunoglobulins. Progr Biophysics Molec Biol 1972; 25: 133-62.
22. Askonas BA, Williamson AR. Interchain disulphidebond formation in the assembly of immunoglobulin-G. Biochem J 1968; 109: 637-43.
23. Marrison RT, Boyd RN. Organic Chemistry. New Jersey: Prentice Hall International, Inc, 1992; 23.
24. Goodman JW. Immunoglobulin structure and function. In : Stites DP, Terr AI, eds. Basic Human Immunology. Connecticut : Prentice-Hall International Inc, 1991.
25. Dormehl IC, Louw WKA, Hugo N. Biodistribution and accumulation in inflammatory lesions of different thiol reduction-mediated ^{99m}Tc-IgG preparations in baboon models. Nucl Med Commun 1994; 15: 475-82.

ปัจจัยที่มีอิทธิพลต่อปฏิกิริยาการติดฉลากเทคนีเชียม-99เอ็ม แก่แอนติบอดีย์และอิมมูนโกลบุลิน ด้วยวิธีการรีดัคชั่น

วิภา บุญกิตติเจริญ, Ph.D., ดวงเพ็ญ ภู่จินดา, วท.ม.*,*
อันนา งอนรถ ณ อยุธยา, ภบ., พวงทอง ไกรพิบูลย์, พ.บ.**

การเรียนรู้ถึงพฤติกรรมและปัจจัยที่มีผลต่อปฏิกิริยาการติดฉลาก มีความสำคัญอย่างยิ่งต่อการปรับปรุงประสิทธิภาพของการเตรียมเภสัชรังสีเพื่อการวินิจฉัยโรค งานวิจัยนี้ได้ศึกษาปัจจัยที่มีผลต่อการติดฉลากแอนติบอดีย์ด้วย ^{99m}Tc โดยวิธีรีดัคชั่น ที่ใช้ 2-เมอร์แคปโตเอ็ททานอล (2-ME) แอนติบอดีย์ที่ใช้ในการศึกษาได้แก่ แอนติบอดีย์ต่อมะเร็งที่เป็น IgG ต่างชนิดกัน (IOR-CEA(IgG_1), M170(IgG_1), 3F8(IgG_3) และ EMD(IgG_{2a})) และอิมมูนโกลบุลิน (Sandoglobulin) พบว่าแอนติบอดีย์ที่ไวต่อรีดัคชั่น (นั่นคือต้องการ 2-ME 500-1000 เท่าของ IgG) จะได้ผลการติดฉลากที่ดีมีความบริสุทธิ์สูง ทั้งนี้เนื่องจาก 2-ME เหนี่ยวนำให้เกิดกลุ่ม sulfhydryl ที่ไวต่อการจับ ^{99m}Tc ได้มาก ความไวต่อ 2-ME ไม่ขึ้นกับ ชนิดของ IgG อันดับความไวต่อการเกิดรีดัคชั่นเป็นดังนี้ Sandoglobulin > IOR-CEA>3F8 > M170 > EMD นอกจากนี้ ความไวต่อ 2-ME ยังเป็นปัจจัยที่บ่งชี้ถึงความเร็วของแอนติบอดีย์ในการจับ ^{99m}Tc ได้อีกด้วย ทั้งนี้ ยืนยันได้จากการทดลองโดยใช้ M170 และ EMD ซึ่งค่อนข้างดื้อต่อ 2-ME แอนติบอดีย์ ดังกล่าวนี้จะจับกับ ^{99m}Tc ได้ดีต่อเมื่อใช้ $SnCl_2$ ในการออกซิไดซ์ ^{99m}Tc ให้มี oxidation state ที่เหมาะต่อการติดฉลากได้อย่างช้าๆ หากใช้ SnF_2 ซึ่งเกิดปฏิกิริยาเร็วกว่าจะให้ผลไม่ดี การรีดิวซ์แอนติบอดีย์ด้วย 2-ME จะไม่ส่งผลเสียต่อคุณภาพของแอนติบอดีย์ เพราะ 2-ME เหนี่ยวนำให้เกิดกลุ่ม sulhydryl ในตำแหน่งที่ห่างไกลจากบริเวณที่เกิดปฏิกิริยาทางอิมมูน

คำสำคัญ : การติดฉลากด้วยเทคนีเชียม-99เอ็ม, ปฏิกิริยาการติดฉลาก, แอนติบอดีย์ต่อมะเร็ง, อิมมูนโกลบุลิน

วิภา บุญกิตติเจริญ, ดวงเพ็ญ ภู่จินดา,
อันนา งอนรถ ณ อยุธยา, พวงทอง ไกรพิบูลย์
จดหมายเหตุทางแพทย์ ฯ 2544; 84: 545-553

* หน่วยรังสีรักษาและเวชศาสตร์นิวเคลียร์, ภาควิชารังสีวิทยา, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400