Reduction - Mediated ^{99m}Tc - Labeling of Antitumor Monoclonal Antibodies : Effect of Increasing Specific Activity on Antibody Binding Kinetics

VIPA BOONKITTICHAROEN, Ph.D.*, DUANGPEN PUCHINDA, B.Sc.*,
PUTHIPORN CHOUPLYWECH, B.Sc.*, WIRAWAT JALAYONDEJA, B.Sc.*

Abstract

Reduction-mediated ^{99m}Tc-labeling of antibodies has gained widespread acceptance in preparation of tumor imaging agents. Increased specific activity to enhance detection signals has raised the question of whether such an attempt would cause change in antibody binding kinetics. To answer this question, two antitumor monoclonal antibodies, i.e. IOR-CEA (IgG_1) and EMD (IgG_{2a}) were labeled with ^{99m}Tc to yield specific activities ranging from 549-4414 MBq/mg. Regression analysis of the binding data revealed that the binding kinetics of IOR-CEA were shifted from monovalent to bivalent binding upon increasing the specific activities. This phenomenon of affinity enhancement was confirmed by the dissociation study where we found soluble CEA had greater difficulty in extracting the cell-bound IOR-CEA labeled at higher specific activity. The bivalent binding was further supported by the finding that IOR-CEA with higher specific activities delivered less than expected radioactivity to tumor targets despite their immunoreactivities being well preserved.

For EMD, the kinetics seemed to be shifted from bivalent to monovalent interaction. At higher specific activities, adverse changes in immunoreactivity were recognized. Breakage of EMD into ^{99m}Tc-Fab fragments was likely to occur and was supported by the observation that EMD delivered more than expected radioactivities to target cells upon increasing specific activity. Precaution should be taken when one deals with high specific activity labeling since this might alter the antibody binding kinetics either favorably or unfavorably.

Recent successes in oncologic as well as infectious/inflammation radioimmuno-scintigraphy are greatly attributed by tremendous development of ^{99m}Tc-labeling chemistry of immunoglobulins which either involves bifunctional chelating agents or endogenous sulfhydryl groups[1,2]. Reduction-mediated direct conjugation of ^{99m}Tc to antibody molecule has received widespread interest for two

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

main reasons, namely : (1) The use of inexpensive organic reducing agents such as 2-mercaptoethonol (2-ME)[3], ascorbic acid[4], etc. to generate a high affinity site for effective conjugation of ^{99m}Tc enabling the development of an instant kit for convenient labeling. (2) The method allows effective labeling of antibodies with other sulfur avid radionuclides such as $^{186}Re/^{188}Re$[5,6] and ^{67}Cu[7] for radioimmuno-therapy and ^{64}Cu for radioimmunodetection[7].

This technique of direct labeling involves the reduction of disulfide bridges in antibody molecules for ^{99m}Tc bindings[3,4]. Disulfide chain reduction is believed to occur at the hinge region [3,8,9] which is known to control Fab arm rotation during antigenic recognition[8]. Increased loading of ^{99m}Tc into this region may interfere with the rotational movement of Fab arms and thus alter antibody binding kinetics.

It is quite an obvious need to prepare a radiolabeled antibody with high specific activity. The specific activity which describes the amount of radioactivity labeled per unit mass of antibody is one key factor in determining the magnitude of radioactivity targeting[10] that will eventually enhance the sensitivity of tumor detection[11] or govern the success in tumor treatment[6]. Recently, the Division of Nuclear Medicine at Ramathibodi Hospital has established and standardized the reduction-mediated technique for ^{99m}Tc-labeling of antitumor monoclonal antibodies. This study was conducted to test whether the increase in specific activity of ^{99m}Tc-labeled antibody would change the behavior of two antitumor monoclonal antibodies, i.e. IOR-CEA (IgG_1) and EMD (IgG_{2a}) in reacting with their tumor targets.

MATERIAL AND METHOD

Antibodies

IOR-CEA and EMD are murine monoclonal antibodies (MAbs) of IgG_1 and IgG_{2a} subclasses respectively. IOR-CEA was a gift from the Center of Molecular Immunology (Havana, Cuba). The antibody recognized carcinoembryonic antigen (CEA). EMD was kindly donated by Dr Baum (Goethe University Hospital, Germany). It reacts with epidermal growth factor receptor (EGFR).

Radioisotope

Technetium-99m was obtained from a generator (Amertec II, Amersham, UK). The concentration used for labeling was greater than 1110 MBq/ml.

Tumor cell lines

Two tumor cell lines, LS 174T (human adenocarcinoma from the colon) and FaDu (human squamous cell carcinoma from the pharynx), were purchased from American Type Culture Collection (ATCC, Rockville, U.S.A.). LS174T is known to express CEA on the cell surface[12]. FaDu was selected against several other malignant histologies from the head and neck for its good reactivity with EMD (our study). The cell lines were cultured in media as recommended by ATCC. The media were supplemented with 5 per cent fetal calf serum (FCS) for culture maintenance and 10 per cent FCS for experimental work.

Antibody reduction

At concentrations between 5-10 mg/ml in phosphate buffered saline (PBS) pH 7.2-7.4, IOR-CEA and EMD were respectively reduced with 2-ME at 1000 :1 and 6000:1 in molar excess. The reactions were allowed to proceed at room temperature for 30 minutes. At the end of reduction, the antibodies were purified on a prechilled Sephadex G-50 (medium) column (Pharmacia, Uppsala, Sweden) using PBS as eluent. The protein peak was searched with a UV-spectrophotometer at wavelength 280 nm (Spectronic 1001 plus, Milton Roy, U.S.A.) and the samples were divided into 1 mg aliquots with concentrations between 0.55-0.65 mg/ml. The head space of antibody vial was filled with sterile N_2 gas and the sample was stored under -60°C until use.

Radiolabeling

The frozen antibody solution was thawed and allowed to warm up to room temperature. A desirable ml of medronate kit was added into the antibody vial, followed by sodium [^{99m}Tc] pertechnetate of predetermined activity. The radiolabeling process took 15 minutes to complete. In the labeling of IOR-CEA, the medronate kit contained 1 mg/ml of methylene diphosphonate (MDP) and 0.068 mg/ml of SnF_2. It required at least 0.132 µg of SnF_2 for successful labeling with 37 MBq of ^{99m}Tc. Another medronate kit was used for labelling EMD. The kit contained MDP at 1 mg/ml and $SnCl_2 2H_2O$ at 0.162 mg/ml. The amount of $SnCl_2 2H_2O$ required for 37 MBq of ^{99m}Tc was

0.951 μg. The specific activities varied between 450 to 4500 MBq/mg.

Radiolabeling efficiency was determined by instant thin layer chromatography (ITLC). One μl of labeled antibody solution was spotted onto an ITLC-SG strip, silica gel impregnated glass fiber strip (Gelman Sciences, Ann Arbor, MI) and developed in normal saline solution. MDP and pertechnetate moved along with the solvent front and left the labeled antibody and colloid at the origin. The magnitude of colloid was determined by using HSA-impregnated ITLC-SG and mixture of EtOH : NH_4OH : H_2O = 2 : 1 : 5. The strip retained colloid at the origin[13]. After subtracting percentages of all radiochemical impurities from 100 per cent, it yielded the percentage of radioactivity incorporated into the antibody.

Immunoreactivity measurement

Antibody immunoreactivity was assessed by enzyme-linked immunosorbant assay (ELISA technique) using formalin-fixed tumor cells. The target cells were prepared as follows : Fixed number of tumor cells (3.3 x 10^5 cells of LS174T with plating efficiency 97.3% and 1.5 x 10^5 cells of FaDu with plating efficiency 99.1%) was plated onto each well of the 96-well plate. Cells were cultured for 24 hours and then fixed with 2 per cent formalin for 1 hour at room temperature. The fixed cells were stored under 4°C for antibody assay (shelf life ~ 1 year).

For antibody assay, nonspecific binding sites on cells were saturated with 1 per cent bovine serum albumin and followed by incubating 200 μl of antibody solutions at varying concentrations, i.e. 0.1-1 μg/ml for IOR-CEA and 0.01-0.15 μg/ml for EMD, with their target cells. Reactions were allowed to undergo at 4°C for 1 hour. Target cells with bound activities were washed 3 times with PBS and then incubated with 200 μl of horse radish peroxidase (HRP) -conjugated rabbit antimouse IgG (Zymed Labaratory, U.S.A.). Quantitate HRP - conjugated IgG with 150 μl of 0-phenylene diamine (OPD) and H_2O_2 (1 ml of 0.1 M citrate buffer pH 5, contained 1.08 mg of OPD and 0.03% H_2O_2). The reaction was stopped with 50 μl of 2N H_2SO_4 after 15 minutes of incubation under subdued lighting at room temperature. The solution in each well was transferred to another plate for optical density (OD) measurement at 490 nm by a microplate reader (EL311S Autoreader, Biotek, U.S.A.). Slope from the double inverse plot between antibody concentration and OD was obtained from the least-squared regression line[10]. The ratio between the slopes from native and modified antibody binding curves yielded the immunoreactivity index. Significant differences between slopes were analysed by *t*-test.

Measurement of antibody binding kinetics

Fixed numbers of LS174T (2 x 10^6 cells) and FaDu (7.5 x 10^5 cells) were seeded onto each well of a 24-well plate. Formalin-fixed cells were prepared by the same method as described in the ELISA study. The reaction design, i.e. concentrations of antibody solution, time and temperature of incubation, was similar to that for ELISA except for the volume of antibody solution which was 1 ml instead of 0.2 ml. At the end of the reaction, solutions containing unbound antibodies were aspirated and the plate was washed 3 times with 0.5 per cent Tween 20 in PBS. Cells with bound activities were scraped, resuspended and transferred to 10 x 70 mm polystyrene tubes for gamma counting in a multi - crystal counter (Berthold, Germany). The total activities which added to the series of wells were measured and the counting efficiency of the gamma detector was quantitated.

The radioactivities were converted into units of nM. We performed double reciprocal transformation of the binding data, i.e. bound antibody [AgAb] versus total antibody [Ab] which approximated the free antibody, according to the equation below[10].

$$\frac{1}{[AgAb]} = \frac{1}{[Ag_T]} + \frac{1}{[Ag_T]K[Ab]} \quad (1)$$

The y-intercept would reflect the total antigenic sites $[Ag_T]$ by the number of antibodies which reacted when [Ab] was in infinite excess. When the number of tumor cells involving the reaction was known, N_{max}, the maximum number of antibodies reacted per tumor cell could be calculated. The affinity binding constant (K) was extracted from slope and y-intercept.

Dissociation of bound activities

Samples of IOR-CEA were labeled at 810 and 1558 MBq/mg. The antibody solutions with concentrations around 0.65 μg /ml were allowed to react with LS174T cells for 1 hour at

room temperature. At the end of the reaction, the solution with unbound antibodies was aspirated and the target cells were washed 3 times with 0.5 per cent Tween 20 in PBS. The target cells were then incubated in PBS and CEA solution (concentration 2 μg/ml). Activities which remained bound were measured periodically for 6 hours. In another parallel experiment, stability of the ^{99m}Tc-label was assessed by measuring the spontaneous release of ^{99m}Tc by ITLC technique.

RESULTS

Effect of increasing specific activities on antibody binding kinetics.

Each ^{99m}Tc-labeled antibody was allowed to react with its tumor target. The apparent binding constant K and the maximum number of antibody molecules which reacted per tumor cell N_{max} are presented in Table 1. Increasing specific activities of the radiolabels altered binding constants of the two antibodies in the opposite direction, i.e. binding affinity of EMD decreased while that of IOR-CEA increased. Interestingly, the change in N_{max} occurred in a different direction from the change in K. For fixed number of target cells, increase in K followed by the decrease in N_{max} would imply a shift from monovalent to bivalent binding or vice versa. None of the radiolabels were found to decrease in both K and N_{max} like the radiodinated antibody as observed in our previous study(10).

Radioactivity delivered to target cells

This experiment was conducted to demonstrate that if the radiolabeling did not alter antibody binding kinetics, the magnitude radioactivity targeting would be increased at the same rate as that in the increase of specific activity. ^{99m}Tc-labeled antibodies with various concentrations (0.1 - 1 μg/ml of IOR-CEA and 0.01-0.15 μg/ml of EMD) were allowed to react with their tumor targets and the magnitudes of bound counts per minute (cpm) were determined. Data were normalized to bound cpm per 10^6 cells to allow them to be comparable. Straight line curves describing the magnitudes of bound cpm as a function of antibody concentrations were obtained for each level of specific activity. The slope of each binding curve, i.e. bound cpm/ 10^6 cells/μg-ml^{-1}, was plotted against the specific activity. The dotted lines in Fig. 1 display the dependence of slopes of radioactive binding curves on specific activities. The solid lines belong to slopes expected from the increases in specific activities. It happened that IOR-CEA upon raising its specific activity delivered less than expected radioactivity to target cells, while EMD carried more.

Immunoreactivity study

The immunoreactivities of the reduced and ^{99m}Tc-labeled antibodies were compared to that of the native antibody and were described in terms of immunoreactivity index. A significant drop in immunoreactivity index was observed for IOR-CEA labeled at the highest specific activity, i.e. 3924 MBq/mg (Table 2). EMD was more sensitive to adverse change which was caused by radiolabeling. Change in immunoreactivity occurred at a lower level of specific activity, i.e. 1097 MBq/mg. Without ^{99m}Tc-conjugation, both reduced antibodies did not react differently from their native molecules.

Table 1. The effect of specific activity on antibody binding kinetics.

Antibody	Tumor cell	Specific activity (MBq/mg)	N_{max} (Ab/cell)	Affinity constant, K (M^{-1})
IOR-CEA	LS174T	650	5.82×10^5	2.25×10^7
		1294	2.21×10^5	7.52×10^7
		2170	2.31×10^5	6.33×10^7
		3342	2.13×10^5	8.13×10^7
EMD	FaDu	549	2.84×10^5	2.12×10^8
		1097	2.64×10^5	2.18×10^8
		2292	9.02×10^5	5.73×10^7
		4414	4.71×10^5	1.61×10^8

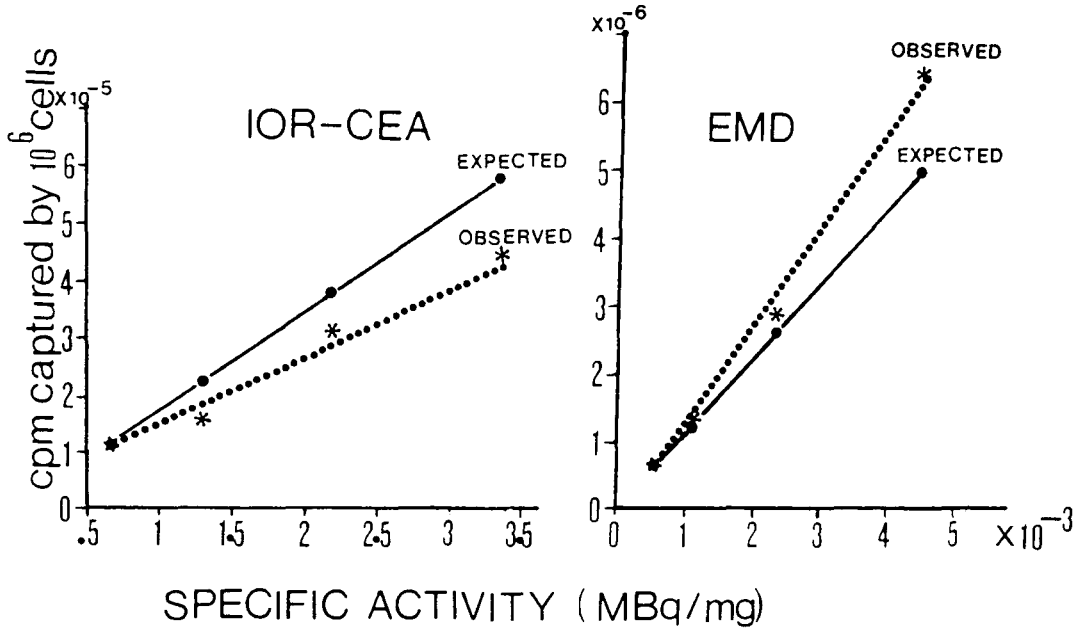

Fig. 1. **Increase in radioactivity delivery as a function of specific activity. The figure describes rate of binding, i.e. the magnitude of count per minute (cpm) captured by 10^6 target cells from 1 μg/ml of antibody solution labeled with different specific activities. The solid lines (———) display the rate of binding predicted from the rate of increase in specific activities. The dotted lines (.....) were obtained by direct observation.**

Table 2. Effects of antibody reduction and ^{99m}Tc - labeling on antibody immunoreactivity.

Antibody	Subclass	2ME:IgG	Specific activity (MBq/mg)	Immunoreactivity index	*t*-test
IOR-CEA	IgG_1	1000:1	0	1.061	P>0.5
(anti-CEA)			1392	0.979	P>>0.5
			2619	0.976	P>>0.5
			3924	0.719	P<0.02*
EMD	IgG_{2a}	6000:1	0	0.990	P>>0.5
(anti - EGFR)			549	0.842	P>0.5
			1097	0.734	P<0.001*
			2292	0.745	P<0.005*
			4414	0.716	P<0.001*

* Level of significance : $P \leq 0.05$

Stability of ^{99m}Tc-labeled IOR-CEA binding to target cell.

To confirm that IOR-CEA labeled at specific activity of 1558 MBq/mg had higher binding affinity for LS174T cell than the sample labeled with specific activity of 810 MBq/mg, we exploited the fact that antibody which bound bivalently dissociated more slowly than that bound monovalently(14,15).

Table 3 presents the percentage of radioactivity which remained bound after challenging the bound activities with PBS and CEA solution. The difference between the activities remaining bound in the presence of PBS and CEA reflected the proportions of radioactivities transferred to CEA. In PBS, both radiolabels dissociated approximately 10-15 per cent of radio-activities within the first hour of incubation and reassociated a few hours later. The sample with lower specific activity rebounded better than the higher one. Steric hindrance could be the likely cause of poor rebinding. There were two possible explanations for this observation. The first was that IOR-CEA labeled at 1558 MBq/mg would tag more than one ^{99m}Tc atoms per molecule of antibody. The second was because the bivalent binding which occupied the great majority of the antigenic sites made the rebinding become more difficult. From the average number of ^{99m}Tc atoms tagged per IgG, i.e. 0.006 for 810 MBq/mg and 0.021 for 1558 MBq/mg, the chances of incorporating 2 or more ^{99m}Tc atoms per IgG were zero(16) for both preparations. This made the first explanation unlikely. Target saturation by bivalent binding should be the cause of difficulty in rebinding of the sample labeled with 1558 MBq/mg.

When CEA was present, the soluble antigen seemed to retard the dissociation of loosely bound antibodies and at the same time it competed with the cell surface CEA for more tightly bound antibodies. This was evidenced by the delayed dissociation at early hours and progressive release of bound activities thereafter. Bound activities which transferred to soluble CEA were greater and faster for antibody with lower specific activity. It was clear at this stage that IOR-CEA labeled with 810 MBq/mg bound more loosely probably monovalently to target cells than the sample labeled with 1558 MBq/mg that might react bivalently. Throughout the entire study, stability of ^{99m}Tc that tagged to antibody molecules was also assessed and it was found to be reasonably stable.

DISCUSSION

High count-rate from ^{99m}Tc-labeled antitumor antibody allows the great ease in distinguishing regions of specific from nonspecific uptake(11). On this basis, ^{99m}Tc-labeled SM3 with respect to its ^{111}In- and ^{123}I-labels renders the most accurate results in diagnosis of ovarian tumors by radioimmunoscintigraphy(17). Specific activity which determines the magnitude of count-rate carried by radiolabeled antibody has become another crucial point of concern in preparing a new radiopharmaceutical for tumor imaging.

Table 3. Extraction of bound activities, i.e. ^{99m}Tc-labeled IOR-CEA, from tumor target by soluble CEA.

Specific activity (MBq/mg)	Time (hour)	Stability of ^{99m}Tc-IOR-CEA(%)	Activity remained bound(%)		Activity transferred to CEA (%)
			PBS	CEA	
810	0	100.00	100.00	100.00	0.00
	0.5	97.42	90.25	96.65	0.00
	1	97.42	89.20	83.64	5.56
	2	96.52	108.54	81.70	26.84
	4	96.83	99.31	74.75	24.62
	6	91.48	97.46	68.49	28.97
1558	0	100.00	100.00	100.00	0.00
	0.5	97.30	88.03	102.47	0.00
	1	96.23	85.84	93.21	0.00
	2	96.07	90.52	87.28	3.24
	4	95.64	90.33	79.11	11.22
	6	95.21	83.88	69.45	14.43

Our hypothesis for the possible effect of ^{99m}Tc-conjugation on antibody binding kinetics rest on the concept that the most probable sites of reduction and radiolabeling resided at the hinge region[3,8,9] which is part of the immunoglobulin structure playing a significant role in controlling the rotational motion of Fab arms during antigenic binding[8]. Mild reduction of hinge interchain disulfide bridges although rendering the molecule fragile[18] does not impair the ability of the antibody in antigenic recognition[3,5,6,18-20] or cause any chain separation[3,5,6,28-20]. In fact, the effect of reduction enhances antibody flexibility which facilitates the antibody to react bivalently[21]. The two antibodies used in this study exhibited different resistivity to 2-ME reduction. It required 0.38 M of 2-ME to generate sulfhydryl groups in EMD for effective conjugation of ^{99m}Tc while 0.06 M was needed for IOR-CEA. Nevertheless, neither antibodies revealed any changes in target cell bindings compared to their unreduced controls.

Changes in immunoreactivity occurred when the antibodies were labeled with a large quantity of ^{99m}Tc. Quite interestingly, the binding kinetics of IOR-CEA seemed to be shifted from monovalent interaction at specific activity of 650 MBq/mg to bivalent interaction when the specific activities were raised up to 1294 and 2170 MBq/mg at which levels the immunoreactivities were still well preserved (i.e. 97.9% *versus* 97.6%). This phenomenon of affinity enhancement was confirmed by antibody dissociation study where we found IOR-CEA with higher specific activity dissociated slowly upon CEA challenge as opposed to the sample labeled with lower specific activity. The shift to bivalent binding could also be demonstrated by radioactive targeting study. Bivalent binding would readily saturate the antigenic sites and thus permitted fewer attachments of radioactive molecules to tumor cells. Experimentally, IOR-CEA labeled at specific activities higher than 650 MBq/mg delivered less than expected radioactivities to target cells.

For EMD, despite the difference in immunoreactivity of the antibody labeled at 549 MBq/mg (i.e. 84.20%) and 1097 MBq/mg (i.e. 73.40%), both antibody samples displayed almost the same binding kinetics. However, at specific activities of 2292 MBq/mg (immunoreactivity = 74.50%) and 4144 MBq/mg (immunoreactivity = 71.60%), decreases in binding affinity K were observed along with the increases in N_{max}. This would imply the labeled antibodies rather reacted monovalently than bivalently. At these levels of specific activities, the antibodies delivered more than expected radioactivities to target cells which further supported the postulation of monovalent interaction. However, the drops in immunoreactivities at high specific activities suggested that the fragile IgG might be separated into ^{99m}Tc-Fab fragments to interact the antigenic sites more easily and more often than the intact form but with reduced affinity.

Why did IOR-CEA and EMD respond differently to increasing specific activities. Difference in subclass, i.e. IgG_1 for IOR-CEA and IgG_{2a} for EMD, might be one of the influencing factors. Since it has long been reported that IgG_1 has a shorter and more rigid hinge region than IgG_{2a}[22]. The technique of reduction-mediated ^{99m}Tc-labeling of antibody has been demonstrated to be a simple and efficient method. The adoption of the technique in labeling antibodies with other sulfur avid radionuclides such as ^{186}Re / ^{188}Re[5,6] and ^{64}Cu / ^{67}Cu[7] has been well received. Because of the lower rates of decay in these radionuclides than that of ^{99m}Tc, much larger quantities of radionuclides must be loaded into the antibody molcules to achieve the useful specific activities. Our study suggested that precaution should be taken when one involved the radiolabeling which demanded high radionuclide load where alteration in antibody binding kinetics would be expected and thus this would profoundly influence *in vivo* biodistribution.

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การติดฉลาก ^{99m}Tc กับแอนติบอดีต่อมะเร็งด้วยวิธีการรีดัคชั่น : ผลการเพิ่มความแรงจำเพาะต่อจลศาสตร์การเกิดปฏิกิริยาของแอนติบอดี

วิภา บุญกิตติเจริญ, Ph.D., ดวงเพ็ญ ภู่จินดา, ภ.บ.*,*
พุทธิพร เชื้อพลายเวช, วท.บ., วีรวัฒน์ ชลายนเดชะ, วท.บ.**

การรีดิวซ์แอนติบอดีสำหรับการติดฉลากด้วย ^{99m}Tc เพื่อใช้ในการถ่ายภาพรังสีของมะเร็งเป็นวิธีที่ได้รับการยอมรับอย่างแพร่หลาย การเพิ่มความแรงจำเพาะ (specific activity) ของสารเภสัชรังสีเพื่อเพิ่มประสิทธิภาพในการตรวจจับรอยโรคนั้น ก่อให้เกิดคำถามขึ้นว่า การกระทำดังกล่าวจะนำไปสู่การเปลี่ยนแปลงจลศาสตร์ในการเกิดปฏิกิริยาของแอนติบอดีหรือไม่ เพื่อเป็นการหาคำตอบ งานวิจัยนี้ได้ติดฉลากแอนติบอดี 2 ชนิด คือ IOR-CEA (IgG_1) และ EMD (IgG_{2a}) ให้ได้ความแรงจำเพาะระหว่าง 549 ถึง 4414 MBq/mg พบว่าจลศาสตร์การเกิดปฏิกิริยาของ IOR-CEA ถูกเปลี่ยนจากปฏิกิริยา monovalent เป็น bivalent เมื่อความแรงจำเพาะมีค่าเพิ่มขึ้น ปรากฏการณ์ affinity enhancement นี้ ได้รับการยืนยันจากการทดลองโดยใช้ CEA ในการแย่งจับแอนติบอดีที่จับกับเซลล์มะเร็งอยู่ก่อน โดย CEA แย่ง IOR-CEA ที่ติดฉลากด้วยความแรงจำเพาะน้อยได้ดีกว่าความแรงจำเพาะมาก การเกิดปฏิกิริยาแบบ bivalent binding สามารถยืนยันได้จากการที่แอนติบอดีนำรังสีไปยังมะเร็งได้น้อยเกินคาด ทั้งๆ ที่ immunoreactivity ของแอนติบอดียังไม่เสื่อมคุณภาพอันเนื่องจากผลการติดฉลาก สำหรับ EMD การเปลี่ยนแปลงได้เกิดในทิศทางกลับกันกับ IOR-CEA คือจาก bivalent เป็น monovalent เมื่อเพิ่มค่าความแรงจำเพาะ Immunoreactivity ของ EMD จะเสื่อมคุณภาพไปบ้าง จึงสันนิษฐานว่า ปฏิกิริยาแบบ monovalent น่าจะเป็นผลสืบเนื่องจากการแตกตัวของ IgG เป็น Fab fragment เพราะที่ความแรงจำเพาะมากขึ้น EMD จะนำรังสีไปยังเซลล์ได้มากเกินคาด ดังนั้น การเพิ่มความแรงจำเพาะของการติดฉลากต้องคำนึงถึงการเปลี่ยนแปลงของจลศาสตร์การเกิดปฏิกิริยาของแอนติบอดีซึ่งอาจเป็นไปในทางบวกหรือลบได้

* หน่วยรังสีรักษาและเวชศาสตร์นิวเคลียร์, ภาควิชารังสีวิทยา, คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพฯ 10400