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SEARCH FOR NEW AND IMPROVED RADIOLABELING METHODS FOR MONOCLONAL ANTIBODIES

A review of different methods

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The radiolabeling of monoclonal antibodies for routine clinical applications have been intensively researched during the past decade. The traditional radiolabeling methods are direct radioiodination using oxidative reagents, such as chloramine T, and coupling metals by use of bifunctional chelates like diethylenetriamine-pentaacetic acid (DTPA). More recently technetium labeling has also been used. In this review the selection of different radioisotopes is discussed as well as the various traditional or newer methods to introduce the radiolabel into the antibody structure. Labeling methods for radiohalogens, for technetium and rhenium isotopes, and for 3-valent cation radiometals are reviewed. Some of the newer methods offer simplified labeling procedures, but usually the new methods are more complicated than the earlier ones. However, new labeling methods are available for almost any radioelement group and they may result in better preserved original nature of the antibody and lead to better clinical results.

A wide range of monoclonal antibodies are produced and have been shown to express high affinity to tumor antigens with variable specificity. Many of them have been suggested as potential candidates of in vivo diagnostic tools for gamma imaging, after radiolabeling. Some of the radiolabeled monoclonal antibodies are even proposed for radiotherapeutical applications. Up to now only very few radiolabeled monoclonal antibodies (MoAb) have reached a moderate spread for oncological diagnostic applications and still fewer are in experimental use for cancer radiotherapy. Today (to the author's knowledge) there is only one radiolabeled MoAb product (Oncoscint™) that has been authorized in some European countries for diagnostic use in clinical oncology. As to clinical therapeutic trials no product has even reached the phase III stage.

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The problems linked with radiolabeled antibodies are usually related to the lack of specificity of these experimental radiopharmaceuticals. This may be due to the lack of specificity for a certain tumor type of the original antibody but more often to reduced immunoreactivity of the radiolabeled MoAbs or in vivo degradation of the radioantibody complex. Harsh labeling conditions have often been the major cause of reduced immunoreactivity.

The intention of the present article is to review some of the new labeling methods for MoAb labeling with traditional and newer radionuclide labels. Only the potential in vivo use is considered here. The aim is to present some of the more feasible methods for radiolabeling, which might be applicable to research groups without extensive resources for research in radiochemistry and radiopharmaceutical chemistry.

Selection of radionuclides

Radiolabeling of proteins, such as monoclonal antibodies or their fragments, should generally be simple, reliable and give reasonably high radiochemical yield without further purification steps. The obtained specific activities

should be high enough either for diagnostic or therapeutic use and the label should be stable in vivo.

The selection of radionuclide for MoAb labeling depends firstly on the intended application. Rapid in vivo imaging and therapeutic trails will for instance require quite different radiation characteristics of the labeling radionuclide. Other selection criteria are the availability and cost of radionuclide. The chemical properties of the selected radionuclide are also crucially important, when the labeling procedure is designed.

The half-life of the radionuclide should be within a suitable range, varying from hours to several days. The half-life should match to kinetics of the protein, which may vary greatly. If the intact antibody has a slow accumulation in the target tissue and a slow clearance from blood pool, and is used for therapy, then the half-life of the radionuclide should clearly be of the order of days to allow enough time for obtaining an adequate absorbed dose in the target. On the other hand, when a fragment of antibody with much faster kinetics is used for diagnostic purpose, then acceptable results may be obtained with radionuclides with much shorter half-lives.

The radiation properties are naturally essential for selection of radionuclide. For imaging with gamma camera we need γ-radiation with suitable energy whereas therapy is usually based on β-radiation with suitable energy or α-radiation.

For practical reasons the chemical properties should be well understood and the procedures should be simple and give reproducible results. The radiation protection aspects must not be forgotten. The radionuclides which are safe to handle in large quantities will clearly be more popular when choosing between radionuclides which otherwise are comparable.

Table 1 shows some radionuclides with potential use in vivo as radiolabels of monoclonal antibodies. They have been divided into three subgroups: halogens, techetium–rhenium and other metals. The radiolabeling with elements in each group require very different methods and are connected with different problems. The availability of suitable radionuclides may be difficult without access to nuclear reactors and cyclotrons and adequate production and purification capabilities.

Of the halogens three iodine isotopes, ^{123}I, ^{125}I and ^{131}I, are readily commercially available. The vast majority of

Table 1

Some potentially useful radionuclides for radiolabeling of monoclonal antibodies (Physical data from ref. 1)

Isotope	Half-life	Decay	Application	Remarks
^{18}F	1.8 h	$\beta+$	PET D	Too short half-life
^{76}Br	16 h	$\beta+$ (54%)	PET D	
^{77}Br	2.4 d	EC, γ:s	D	
^{123}I	13 h	EC, γ	D	
^{124}I	4.2 d	$\beta+$ (23%)	PET D	
^{125}I	60 d	EC, x-rays	T	Animal experiments
^{131}I	8.0 d	$\beta-$, γ	T, D	
^{211}At	7.2 h	α	T	
^{99m}Tc	6.0 h	IT, γ	D	From generator
^{186}Re	3.8 d	$\beta-$, γ	T, D	Not carrier-free
^{188}Re	17 h	$\beta-$, γ	T, D	From generator
^{67}Cu	2.6 d	γ, $\beta-$	D, T	
^{90}Y	2.7 d	$\beta-$	T	
^{97}Ru	2.9 d	EC, γ	D	Not carrier-free
^{105}Rh	1.5 d	$\beta-$, γ	T, D	
^{109}Pd	14 h	$\beta-$, γ	T, D	Not carrier-free
^{111}In	2.8 d	EC, γ	D	
^{151}Pm	1.2 d	$\beta-$, γ	T, D	
^{153}Sm	1.9 d	$\beta-$, γ	T, D	Not carrier-free
^{159}Gd	19 h	$\beta-$, γ	T, D	Not carrier-free
^{161}Tb	6.9 d	$\beta-$, γ	T, D	
^{166}Ho	1.1 d	$\beta-$, γ	T, D	Not carrier-free
^{210}Bi	5.0 d	$\beta-$	T	

Explanations: γ: gamma radiation with energy, allowing external detection
β: beta decay as main type of decay
EC: electron capture
α: alfa decay
IT: internal transformation
T: therapeutical application
D: diagnostic application.

antibody labelings with halogens have thus been performed with these isotopes. Several groups, including our own, have produced, or are planning the production of ^{124}I and ^{211}At. Almost all groups working with PET systems have regular supply of ^{18}F and some have studied the production of bromine radionuclides (2).

^{99m}Tc is the most commonly used radionuclide in nuclear medicine and is available at all times in every major nuclear medicine department. The rhenium isotopes are at present not commonly available. ^{186}Re is a reactor product, obtained from enriched ^{185}Re target by neutron irradiation. This means that ^{186}Re is not carrier-free, but can have a specific activity up to about 100 GBq/mg depending on the reactor. When only limited amount of antibody is available, it may thus be difficult to get high enough activity without disturbing the original behavior of the antibody. On the other hand, ^{188}Re is obtained carrier-free from a generator system. The practical obstacle in this system is to get high enough specific activity of the parent nuclide ^{188}W. It is produced by double neutron capture and requires long irradiation times in a very powerful reactor. Only few such reactors are available in the world. Knapp et al. (3) have described a very convenient generator system, which essentially operates like the ordinary ^{99m}Tc-generator. We have developed the system to facilitate the use of ^{188}W, with lower specific activity which would greatly expand the use of ^{188}Re (unpublished data).

A third group of radionuclides (Table 1) consists of metals, commonly attached to antibodies indirectly by bifunctional chelation. Of these metals, yttrium and indium are routinely available from various sources. ^{111}In is also available from a few sources in ultrapurified form for MoAb labeling. Also ^{90}Y should be purified before successful MoAb labeling can be performed. The availability of ultrapurified ^{90}Y has so far been very limited. We have developed a generator system with a subsequent purification step by modifying the system designed by Chinol & Hnatowich (4). The $^{90}Sr/^{90}Y$ generator gives carrier-free ^{90}Y, which is suitable for labeling of MoAbs. This method gives ^{90}Y in chloride solution, suitable for direct attachment to chelated antibodies. The levels of impurities are typically <5 ppm for the elements Co, Cr, Cu, Ni, Pb, Sr, Zn and <10 ppm for Fe which allows effective labeling.

The remaining radiometals in Table 1 are still not included in the routine production programs of the major suppliers. Copper-67 can be produced by neutron or proton irradiation of zinc targets (5). ^{97}Ru, ^{105}Rh and ^{109}Pd are produced in reactors by neutron irradiation. Only ^{105}Rh can be obtained in carrier-free form since it is a daughter of the original irradiation product, ^{105}Ru. None of the last mentioned nuclides are as yet readily available from any routine source.

There are several lanthanides with interesting physical properties which can be produced by neutron irradiation in nuclear reactors. We produce weekly ^{153}Sm for making ^{153}Sm-EDTMP, a radiopharmaceutical under trial for treatment of malignant bone tumors and metastases (6). ^{153}Sm is not carrier-free, but can be produced with reasonably high specific activity. ^{159}Gd and ^{166}Ho can also be produced but with lower specific activity. ^{151}Pm and ^{161}Tb are interesting reactor products, since they can be obtained as carrier-free, when purified from the target. ^{210}Bi is also produced in reactors by direct neutron irradiation but is a carrier containing product.

In addition to Table 1 there are several potentially useful radionuclides for MoAb labeling and for other related applications. The production methodology for a large number of radionuclides has been reviewed by Ruth et al. (7).

Radiolabeling of MoAbs with halogens

Radiohalogens offer some significant benefits as MoAb labels since a) they form strong covalent binding to proteins, b) have a well-known chemistry and quite simple labeling reactions, c) can obtain high specific activities and d) the labeling conditions are not as critically related to trace impurities as for metals.

The method of choice for radioiodination of MoAbs is the direct oxidative labeling utilizing chloramine T (8). Iodides are oxidized with chloramine T to form electrophilic intermediates which can label a protein. Other oxidative reagents like iodogen (9) and iodine monochloride (ICl) (10), are also commonly used. With these methods thousands of labelings have been performed with quite satisfactory clinical results. However, denaturation of protein in the oxidative conditions is an existing problem and therefore milder oxidative reagents have been suggested. Sinn et al. (11) have suggested the use of N-bromsuccinimide. Table 2 describes the labeling methods for 1 mg of MoAb. We have used these methods to study their effects on the behavior of labeled MoAb. Generally the labeling is made in aqueous MoAb solution with appropriate oxidizing agent, to which the radioactive iodide is added. Fast reaction is terminated by using a reductive agent like metabisulfide. According to our experience some differences between the results can be seen, and the suitability of the methods seems to vary depending on antibody and type of application. Our preliminary results suggest that N-bromsuccinimide generally yields a better retained original nature of the MoAb (unpublished data). The other methods of ours do not seem to give essentially different results when the behavior of labeled protein is studied in vivo (see also Table 3).

The direct oxidative labeling methods usually guide the radiolabel into tyrosine residues of the antibody. The labeled tyrosine residues are subject to in vivo dehalogenation by enzymes. Deiodination is a significant problem, causing leakage of iodine, which can be visualized in gamma images as accumulation in thyroid, stomach and

Table 2

Direct methods for radioiodination (^{131}I) of MoAbs

Method	Protein Iodine	Description
Chloramine T	1 mg MoAb 150 MBq $Na^{131}I$	250 µl 0.5 M pH 7.2 phosphate buffered saline (PBS), MoAb, $Na^{131}I$, add 25 µg chloram T in 0.1 ml PBS, incub. 5 min. Stopped by 70 µg Na-thiosulf. and NaI.
Iodogen	1 mg MoAb 150 MBq $Na^{131}I$	Use pre-iodogen coated test tubes (200 µg/tube), add 50 µl PBS, 50 µl/150 MBq $Na^{131}I$, add MoAb, 10 min incubation, add 1 ml PBS, remove to another vial.
ICl	1 mg MoAb 150 MBq $Na^{131}I$	MoAb in saline 100 µl, 1 ml pH 7.8 borate buffer, 150 MBq $Na^{131}I$ are mixed, 0.5 ml ICl in glycine buffered sol added dropwise. Reaction stopped by adding thiosulfate and KI.
N-bromsuccin-imide (NBS)	1 mg MoAb 150 MBq $Na^{131}I$	MoAb in PBS 100 µl, 150 MBq $Na^{131}I$, 5–10 µg NBS in fresh NBS (1 mg/ml)-PBS solution. Incubation of 2 min. Stopped by adding 1–2 ml PBS.

Table 3

The relative activity in thyroid and in tumor (ovarian carcinoma xenograft in nude mice) at various times after injection of radioiodinated IgG. Two animals were used for the time points 1 day and 3 days and one animal 7 days

Method		Time		
		1 d	3 d	7 d
CHLT	thyroid	2.5	2.5	2.0
	tumor	1.0	1.2	1.0
ICL	thyroid	3.5	3.0	1.5
	tumor	1.2	0.8	1.0
NBS	thyroid	1.0	2.0	1.5
	tumor	0.9	0.7	0.9
IPM	thyroid	1.0	1.2	1.1
	tumor	0.7	1.0	0.9

bladder. The rate and extent of the leakage varies from method to method, from antibody to antibody and from patient to patient. To circumvent this problem and the potential denaturation of the MoAb due to harsh conditions during the direct labeling, several indirect labeling methods have been developed. The idea is to use a conjugate which is radiohalogenated prior to attachment to the MoAb. The potential benefits are: a) the site of the radiolabel can be selected so that the compound is resistant to in vivo dehalogenation and b) the labeling conditions during the attachment steps can be very mild. Naturally an indirect approach is a more complicated labeling procedure and it often gives a lower overall yield. There is very little of experience from conjugated radiolabels concerning therapeutic dose levels.

Zalutsky et al. (13) and Garg et al. (14) have used N-succinimidyl iodobenzoate as iodinated conjugate. They have also successfully used the same approach for labeling of MoAbs (15) with ^{211}At. The laborious part of the labeling procedure is to synthesize the precursor, N-succinimidyl-3-(trimethylstannyl)-benzoate (ATE). This intermediate can be stored for further labelings. The iodination of ATE is performed in acidic chloroform. The radioiodinated conjugate is then attached to antibody (to any of the free amines in the MoAb) by incubating reagent and antibody in buffer pH 8.5 for 15 min.

Srivastava et al. (16) have developed an iodophenyl maleimide conjugate. Again a precursor, N-(p-iodophenyl)maleimide (IPM), is first synthetized. Radioiodination is then performed by exchange reaction. IPM has a natural affinity to protein-thiol functional groups such as cysteine residues, which can be utilized as labeling sites during mild conditions. We have compared this method with the previously described direct labeling methods. Unspecific human polyclonal immunoglobulin was used for labeling in each case and biodistribution studies were made in nude mice at various times up to 7 days. Our very preliminary results seem to verify a better in vivo stability of the IPM-labeled antibody with an obvious reduction of thyroid uptake compared to ICl or chloramine T as seen in Table 3.

Wilbur (17) has recently published an extensive review of radiohalogenation of proteins. This review describes a great number of labeling methods not only for radioiodine but also for other radiohalogens. Another comprehensive review on radioiodination has been published by Dewanjee (18).

Radiolabeling of MoAbs with Tc and Re isotopes

Technetium and rhenium labeling can also be performed by direct or indirect attachment to MoAb. Tc and Re radionuclides are usually obtained as pertechnetate (TcO_4^-) or as perrhenate (RhO_4^-) solutions. Direct labelling means that the radionuclide is linked to MoAb without using any linker molecules. Usually, indirect methods mean that a chelating complex is used for binding the radionuclide. Tc and Re labeling of MoAb has been reviewed by Griffiths et al. (19).

There are several different direct labeling methods. Wong et al. (20) published already in 1978 a method, which utilizes trisodium citrate and reduced ^{99m}Tc. The attachment to unspecific polyclonal IgG was over 95% within 30 min. In this method, the IgG was not pretreated by any activating compound and there are few, if any, successful clinical results reported using this procedure.

Prereduction of protein disulfide bridges opens thiol functional groups that are favored sites for direct labeling. Schwarz & Steinsträsser (21) pretreated IgG with mercaptan as a reducing agent and then lyophilized the partially reduced protein. The pertechnetate was reduced with stannous chloride and phosphonates prior to incorporation into antibodies. Many clinical studies have been performed using this method with quite satisfactory results. Rhodes et al. (22) pretreated protein with stannous chloride, lyophilized it, and incubated ^{99m}Tc. Unbound and low-affinity sites were removed separately. With this method or its modifications, a large number of successful clinical studies have been reported (23). Griffiths et al. (19) have also reported successful use of a direct labeling procedure.

Thakur & De Fulvio (24) have reported successful direct labeling by ascorbic acid reduction of protein and subsequent addition of reduced ^{99m}Tc. Ascorbic acid is used in 3 500 molar excess for partial reduction of MoAb. We have modified this method for labeling of several proteins. In our hands the method gives excellent labeling of LDL with stable behavior in vivo which has not been achieved by other described methods (Leppälä et al. unpublished study). This method can easily be modified for Re labeling (25) and has also been applied to labeling of peptides. The benefits of the method are easy preparation and the use of generally available non-toxic chemicals. Rhenium has a chemistry very similar to that of technetium. There are, however, some differences; the reactions are usually slower with rhenium and more of the reductants should be used. Griffiths et al. (26) reported good attachment of ^{188}Re to antibody in 3–4 h in conditions when ^{99m}Tc was quantitatively incorporated within 5 min.

Theoretically it should be elegant to bind ^{99m}Tc, ^{186}Re or ^{188}Re to a bifunctional ligand which binds the radionuclide according to well-known chemistry. This radiolabeled ligand could then be attached to antibody and so there would be no unspecific binding of the radiolabel directly to antibody. However, the ligand procedure is not trivial. ^{99m}Tc-DTPA approach for labeling has not been successful, although a similar approach works fairly well for ^{111}In (27).

Fritzberg (28) has extensively studied technetium chelates and has developed so-called S_2N_2-chelates, which very effectively bind ^{99m}Tc. However, when these chelators are precoupled to antibody prior to labeling with technetium, there still occurs direct unspecific binding of reduced technetium to antibody. To circumvent this phenomenon they used a more laborious approach: they labeled the chelate with the radionuclide, purified it and bound the activated chelate covalently to antibody (29). This technique was not simple but gave very promising clinical results. However, high uptake in liver and hepatobiliary excretion of the radionuclide disturbed the interpretation of the images. Other types of chelates have also been described; Linder et al. (30) thus reported the use of boron containing BATO-compounds as bifunctional linkers. Brown et al. (31) have reported a different type of procedure for indirect coupling of ^{99m}Tc. They used a linker to attach a metallothionenin to protein. The radiolabeling was performed by exchange labeling with ^{99m}Tc-glucoheptonate. There are so far only few publications on the use of indirect labeling methods with Re-isotopes. Goldrosen et al. (32) have reported animal pharmacokinetic studies with ^{186}Re-labeled antibody.

Table 4 presents three different ^{99m}Tc labeling methods in concise form. The chelate procedures are not presented due to the complexity of the chelate synthesis.

Radiolabeling of MoAbs with metal(III) cations

There are several publications on radiolabeling of MoAbs with metal cations for diagnostic purposes. ^{111}In has been most commonly used. For therapeutical purposes a few groups have used ^{90}Y.

Direct metal incorporation into antibody is not feasible and so a chelate approach is used. Almost all commercial production, particularly with ^{111}In as label utilizes a DTPA-chelation method developed by Hnatowich et al. (34). DTPA is there firstly attached to the amino groups of lysine residues of the antibody or its fragments by using the cyclic anhydride of DTPA; the unbound DTPA is then removed. This DTPA coupled antibody can be stored for later use. Radiometal is introduced by using appropriate buffer, like citrate, to buffer the metal which is supplied in acidic salt solution. Usually the incubation does not take more than 15 min for almost quantitative incorporation.

The method is not as simple as it may look, however. The coupling of DTPA is critical; it is quite difficult to estimate in advance the ratio of DTPA-coupled antibodies to non-coupled ones, and it is also almost impossible to estimate how many DTPA-molecules there are in one antibody. The immunoreactivity decreases rapidly when

Table 4

Three different technetium (and rhenium) labeling methods

Method	Protein ^{99m}Tc	Description and remarks
Direct labeling (Thakur)	1 mg MoAb 1 GBq ^{99m}Tc	Incub. 1 mg/ml with 3 500 molar access of ascorb. acid (pH 6.5, 1 h, 22°C). Add 4 mg $SnCl_2$ and 40 mg Citr. acid in 1 ml to ^{99m}Tc (or ^{188}Re) solution. Mix solutions and incub. for 1 h (37°C). Check labeling yields by ITLC. Usually purif. not required, SepPak purif. possible.
Direct labelling (Schwartz)	1 mg MoAb 1 GBq ^{99m}Tc	Dissolve 0.1 mg $SnCl_2$ and 1.5 mg propan-tetraphosphon acid (or other phophonate) in 2 ml saline, add ^{99m}Tc, incubate 1 min, add it into solution of 1 mg MoAb pH 7.4 phosphate buffer. Check with ITLC.
Transchelation (Burchiel) (33)	1 mg MoAb 1–4 GBq ^{99m}Tc	MoAb preconjug. with metallothionein (MT) with sulfo-succinimidyl-maleimidomethyl-cyclohexane-carboxylate (see ref). Purified by gel permeation HPLC. ^{99m}Tc labeled with glucoheptonate kit (vol 0.25 ml). Add ^{99m}Tc-glucoheptonate MT-MoAb in 0.1 ml in phosph. buffer pH 6.7, incub. for 2 h. Purified with above HPLC system.

more DTPA-molecules are coupled to the same antibody. Furthermore, DTPA is a very powerful chelator for practically any metal and thus every step of the procedure requires extremely metal-free reagents, solutions and glassware. If the required conditions are not carefully maintained, the DTPA-coupled antibody will be occupied by a metal prior to introduction of the radiometal. A very strict purity is also required for the radiometals, and they must be thoroughly purified from any metallic impurity. This is easy to understand if we realize that 75 MBq of ^{111}In is less than 5 ng. In usual solutions there are, as a rule, more than ten-fold larger amounts of several metal impurities. We have studied radiolabeling of a commercial antibody with several different ^{111}In preparations (35) and have found that the incubation time required for good quality ^{111}In-labeling is only a few minutes, but with a product of lower quality several hours' incubation is needed to get even close to quantitative incorporation of ^{111}In into antibody.

Many metals besides ^{111}In are suitable for DTPA-linkage to antibodies. We have showed that ^{57}Co and lanthanides (^{153}Sm and ^{165}Dy) form in vivo stable complexes at least when studied in a nude mice model (36, 37). On the other hand, it has been clearly demonstrated that DTPA-coupling is not strong enough for in vivo use of ^{90}Y, and that stronger chelates have to be used (38). Copper too needs a different chelate for stable binding as Deshpande et al. have shown (39).

High chemical purity is required for all metal-chelate-antibody procedures. In addition, high uptake in liver and other normal organs has disturbed the interpretation of images. Several attempts have been made to overcome this problem.

To improve stability of the DTPA-metal complex, several groups have modified the DTPA molecule. DTPA consists of five carboxylate groups, one of which is generally used for binding to antibody whereas the four remaining ones chelate the metal. This weakens the binding compared to that of unlinked DTPA. An additional linker chain for binding the antibody has been suggested to increase the stability. Brechbiel et al. (40) developed an isothio-cyanato-benzyl backbone for DTPA-antibody linking. This backbone leaves all carboxylates for chelation of the metal. It has been shown to retain the metal better in the structure and also to reduce the liver uptake to some extent. Also several other modifications for use of EDTA and DTPA have been designed (41).

In spite of the fact that better chelates have been developed, high accumulation in normal organs, such as liver and kidneys, is still a drawback for radiometal labeled antibodies. Paik et al. (42) suggested the use of diester linkage between antibody and DTPA. Diester linkage is a readily metabolized chemical linkage between chelate and antibody. They reported significantly lower uptake in normal organs and faster clearance from blood via renal excretion since the radioactive metabolites consist of small polar compounds. They stated that the tumor-to-background ratios were clearly higher after diester linkage than after ordinary peptide-DTPA linkage. However, there is as yet no clear clinical evidence of benefits of this method for human applications.

Table 5

Two metal (III) cation methods for antibody labeling

Method	Protein metal	Description and remarks
DTPA-coupling (Mather (44))	1 mg MoAb 80 MBq ^{111}In or similar	MoAb dissolved in 0.1 ml 0.05 M hepes buffer pH 8–8.5. React with cyclic anhydride of DTPA in DMSO (molar ratio 3.5:1 DTPA:MoAb). Purify by gel filtration (Sepharyl-S3000HR) 0.1 M acetate pH 6 as mobile phase. Adjust volume to 2–5 mg/ml protein. Add ultrapure radiometal as chloride in low pH in less than 0.1 ml. Check with ITLC.
Diester-DTPA (Paik)	1 mg MoAb 80 MBq ^{111}In	Aminoethyl-anilidine (70 mg) in 5 ml of 0.1 M Citr. buffer pH 5 added dropwise into 20 ml of same buffer and simultaneously cDTPA anhydride (700 mg) is added in small portions over a period of 30 min. Purif. from DTPA by repeated precipitation at pH 2. The product in 0.1 M phosph. buffer at pH 7 is rapidly mixed with ethylene glycolbis-succinimidyl succinate (EGS) for 5 min. Molar ratio of components DTPA-anilidine:EGS is less than 1. 0.2 ml MoAb solution (10 mg/ml) in phosph. buffer is mixed with DTPA-diester for 2 h. ^{111}In is added and final purification by Sephadex G-50 chromatography.

Studer & Meares (44) have very recently reported a convenient synthetic method for making bifunctional chelates. Their system involves modification of EDTA or DTPA. As an example, they started from nitrobenzyl-EDTA which was esterized with a readily metabolized ala-leu-ala-leu peptide chain. Besides producing strong bifunctional chelates this method offers a practical advantage: only the very last step of the synthesis is critical to trace metal impurities since the reactions are carried out in suitable organic solvents.

In vitro and animal studies suggest obvious progress in the development of metal-chelate-MoAb systems. Real benefits of these improved coupling methods can be expected in the future compared to the routinely used metal (^{111}In)-DTPA-MoAb system.

Radiolabeling of MoAbs with other methods

Theoretically, one of the most promising methods for application of radiolabeled monoclonal antibodies in clinical routine is the use of two- or three-step methods, such as the biotin-avidin system. The idea is to utilize the strong affinity of biotin to (strept)avidin. This method can overcome one of the major drawbacks, slow kinetics of radiolabeled MoAbs, which causes unnecessary high absorbed radiation dose in normal organs. One way is to first inject an antibody-avidin complex as suggested by Hnatowich et al. (45), which is allowed to circulate until the accumulation is completed and then inject the radiolabeled biotin. Biotin is a fairly small molecule with fast kinetical behavior and renal excretion of unbound biotin. Another option is to biotinylate an antibody which is pretargeted before radiolabeled avidin is injected. The biotin-avidin system can be further developed: biotinylated antibody can thus be pretargeted into the tumor, whereupon avidin can find the antibody and finally a radiolabeled biotin to locate and bind itself to avidin. One potential drawback of this two- or three-step method relates to the use of (strept)avidin, since that molecule is in itself immunogenic and thus may cause some difficulties in the clinical situation. There is some preliminary clinical evidence of success with this approach. Kalofonos et al. (46) reported very encouraging results in patients. The background radioactivities were greatly reduced when ^{111}In-biotin was injected 2–3 days after initial injection of antibody-avidin. Paganelli et al. (47) have reported some preliminary results of radioimmunotherapy of pretargeting with biotinylated antibody, avidin and biotinylated ^{90}Y-DOTA chelate.

Site-specific modification of an antibody for binding of the radionuclide is an interesting option that has been suggested to overcome some of the difficulties discussed above. The oligosaccharides of the antibody can be oxidized and subsequently coupled to DTPA as reported by Rodwell et al. (48). This modification is suitable for metal (III) isotopes, but also other methods can be used to make the binding of ^{99m}Tc and other nuclides more stable. This

method has a benefit compared to routine DTPA coupling, which usually occurs randomly to any of the lysine residues, since the attachment of the label is guided to a specific site, which is not crucial for antigen recognition. There are almost unlimited possibilities to modify antibodies for site-specific attachment of radionuclides. This engineering of antibodies for optimal binding of each radiolabel can potentially yield undisturbed radioisotope-antibody complex.

Conclusion

The use of radiolabeled antibodies has increased remarkably during the last decade. New antibodies for diagnostic and therapeutic use are continuously produced. The radiolabeling methods have been developed to give better radiopharmaceuticals, which are safer to use and give more accurate diagnostic results and possibly also better response to therapy.

The ideal radiolabel and radiolabeling method varies from antibody to antibody and from application to application. Thus, in each individual case the optimal method has to be tested. The selection of radionuclide depends naturally also on costs and availability. For a given radiolabel there are usually several labeling methods, and the selection of method depends on the resources available. Fortunately there exist simple methods for any group of radiolabels which might yield satisfactory results. However, optimal radiolabeling may require considerable amounts of experimental and research work before the ideal radiopharmaceutical is achieved.

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