Radiopharmaceutical Chemistry in Peking University (PKU)

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In the past 15 years our research activities covered the following four aspects: (1) Labeling of monoclonal antibodies (McAb) with radionuclides. Both direct and indirect labeling methods by using various radionuclides were studied. (2) Designing and synthesis of radiopharmaceuticals used for myocardial imaging. Two types of Tc compounds were intensively studied: BATOs and complexes containing $[^{99m}Tc{\equiv}N]^{2+}$ core and $[^{99m}Tc{=}NAr]^{3+}$ core. (3) Synthesis and screening of boron- and gadolinium-containing compounds for use in neutron capture therapy. (4) Investigation of the coordination chemistry of Tc.

The Department of Technical Physics, Peking University consists of two specialities: nuclear physics and applied chemistry. The applied chemistry speciality used to be radiochemistry speciality before 1983. Now its research activities cover four directions: radiopharmaceutical chemistry, material science, environmental chemistry, and radiation chemistry. The research on radiopharmaceutical chemistry began in 1983.

In the first 10 years our research was focused on labeling of monoclonal antibodies (McAbs) with radionuclides. Both direct and indirect (i.e., via bifunctional conjugating agents) labeling methods were studied. The labeled radionuclides we studied were ^{90}Y, ^{99m}Tc, ^{111}In, ^{111}Ag, ^{169}Yb, ^{188}Re and ^{199}Au. Since 1992 we extended our interest to radiopharmaceuticals used for myocardial imaging. Two types of compounds were intensively studied: boronic acid adducts of technetium tris(dioxime) (BATO) and complexes containing $[^{99m}Tc{\equiv}N]^{2+}$ core and $[^{99m}Tc{=}NAr]^{3+}$ core. In the meanwhile, we carried out the investigations on relevant coordination chemistry of Tc in aqueous solution for increasing the knowledge of chemical behavior of Tc *in vivo*.

In the recent five years we were involved in development of drugs used for neutron capture therapy (NCT). We have designed and synthesized some new boron compounds as well as gadolinium compounds which have preliminarily been proved to be concentrated in tumor cells to some extent by means of screening them in cellular and animal experiments. The investigation of some subsidiary compounds, such as radiation sensitizers and p-glucoprotein inhibitors, are also being undertaken.

1. Labeling of Monoclonal Antibodies with Radionuclides

In 1975 Kohler and Milstein[1] developed a method for preparation of McAbs by fusion of an immunized spleen cell and an osteoma cell. Their invention brought a new perspective to radioimmunodiagnosis and radioimmunotherapy. Although radioiodine can be easily labeled to intact McAbs, most radioactive metal ions can not be tightly bound to them.

Two modalities for labeling of radioactive metal ions to McAb have been developed until now. In the direct labeling modality one or more -S-S- bonds in McAb molecule are first reduced to -SH groups. The reduced McAbs are then incubated with a radionuclide in an appropriate oxidation state to form a metal-McAb complex via S-M bonds. In the indirect modality radionuclide ions are combined indirectly via a bifunctional conjugating agent (BFCA). The latter uses one of its functional group in conjugation with McAb, and uses its ligating groups to chelate metal ion. In the past 15 years we studied the chemistry of labeling of McAb with various metal radionuclides by both direct and indirect methods.

1.1. Direct Labeling of Monoclonal Antibodies

1.1.1. Reduction of McAb. There are two types of disulfide bonds in an antibody molecule, one in the same peptide chain (intrachain), and the other between two peptide chains (interchain). Unless the antibody is denaturalized, intrachain disulfide bonds are not reduced because they are deeply embedded in the tertiary structure of the protein. The interchain disulfide bonds outside the hinge may be reduced to form -SH groups. Various reductants have been proposed to be used for this purpose. Stannous chloride was first used by Dreyer and Munze and modified by Rhods,[2] and then so-called pretinning method has been very popular in the reduction process. However, the easy hydrolysis of Sn^{2+} ions makes the method inconvenient in use. 2-Mercaptoethanol (2-ME) was proposed by Bremer in 1986 and perfected by Schwarz and Steinstrasser[3] and now has come to be accredited. Other reductants, such as sulfite, ascorbic acid, dithiothreose, thiosulfate, dithionate, are also used by other researchers. We have made an evaluative study on these reductants. Based on our experiments the following conclusions were drawn:

(1) 2-ME is a very good reducing agent for the conversion of disulfide bond to hydrosulphonyl groups. The reduction is quite mild and the labeling efficiency is usually high (≥95%). Since 2-ME is a quite strong complexing agent for $^{99m}TcO^{3+}$ and $^{188}ReO^{3+}$, any excess of 2-ME after reduction of McAbs must be removed by gel filtration to prevent its complexation with $^{99m}TcO^{3+}$ or $^{188}ReO^{3+}$ in the subsequent step. The final McAb solution is greatly diluted and has to be concentrated again before use.[4,5]

(2) Ascorbic acid (AA) is also a good reducing agent. The reduction of McAb by AA is fast and complete. The excess of AA does not interfere in the further reaction and can act as a protecting agent against the oxidation of McAb by air.[6]

(3) Sodium bisulfite ($NaHSO_3$) reduces McAb a little bit slower than ascorbic acid. A larger excess of bisulfite is needed in order to get the same reduction percentage for McAb in comparison with ascorbic acid.[7,8]

1.1.2. Direct labeling of McAb with ^{188}Re. Perrhenate ion ($^{188}ReO_4^-$) in saline was reduced with $SnCl_2$ in the presence of citrate–tartrate (molar ratio 1:3) at pH 5.2 for 40 min. Rectal carcinoma antibody CL_3 was reduced with $NaHSO_3$ in 1000 times excess at pH 5.2 for 40 min. Ascorbic acid (500 times of McAb) was then added as an anti-oxidizer. The labeling reaction was initiated by mixing of the above two solutions and lasted for 1.5 h. The labeling efficiency was higher than 95%

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with colloidal ^{99m}Tc less than 3%. The ^{188}Re-labeled CL_3 was stable *in vitro* to DTPA, but was not to cysteine. The immunoactivity of ^{188}Re-labeled CL_3 was substantially maintained.[8]

Similar results were obtained by using ascorbic acid instead of $NaHSO_3$ and citrate alone instead of citrate–tartrate mixture.[6]

We feel that the intermediate complexing agent is important. Its complexing ability towards $^{188}ReO^{3+}$ should be stronger than the weak (non-specific) binding sites of McAb, but weaker than –SH groups. It can prevent the formation of colloidal SnO_2 and Re(V). The strong complexing ability of it will slow down the reduction of $^{188}ReO_4^-$ and decrease the labeling efficiency.

1.1.3. Direct Labeling of McAb with ^{99m}Tc. Since the reduction of TcO_4^- is easier than ReO_4^- and TcO^{3+} is more labile than ReO^{3+} in respect to ligand substitution reactions, the reduction of $^{99m}TcO_4^-$ and the labeling of McAb with $^{99m}TcO^{3+}$ are usually combined to a single step. Stannous chloride ($SnCl_2$) is nearly exclusively used for the reduction of $^{99m}TcO_4^-$ to $^{99m}TcO^{3+}$. Various hydroxycarboxylic acids, such as citric acid, tartaric acid, glucoheptonic acid, etc. can be used as the intermediate complexing agent. The reduction-labeling reaction completes at pH ≈7 within 1.5 h. A labeling efficiency higher than 95% can be achieved without difficulties.[4, 5]

1.1.4. Direct Labeling of McAb with ^{199}Au. Tetrachloroaurate ion ($^{199}AuCl_4^-$) was converted to $^{199}AuClS(CH_2CH_2OH)_2$ or $^{199}Au(SO_3)_2^{3-}$ and then labeled to reduced McAbs. The ^{199}Au-McAb was quite stable towards thiourea in the absence of oxygen.[7]

1.2. Indirect Labeling of Monoclonal Antibodies. Indirect labeling of McAbs with radionuclides was comprehensively reviewed.[9,10]

1.2.1. ^{111}In. We used DTPA as a bifunctional conjugating agent for labeling of McAb with ^{111}In and obtained a labeled McAb with a very high specific activity of 1.1×10^9 Bq/mg antibody (30 mCi/mg antibody). The ^{111}In-labeled McAb was used for the diagnosis of colon carcinoma. Among 15 colon carcinoma-bearing patients whose disease had been confirmed by other diagnostic methods, 14 patients gave a positive reaction. The ^{111}In was produced by bombardment of Cd target with proton beam. A special separation and purification procedure was developed. The ^{111}In product was proved in ultra high purity. Our experiment evidenced that only ultra high pure ^{111}In could ensure a satisfactory ^{111}In-labeled McAb. The presence of any isotopic and non-isotopic impurity greatly deteriorated the labeling quality.[11–21]

1.2.2. ^{99m}Tc. A new bifunctional conjugating agent N, N'-bis (L-cysteinyl) -L-lysine (BCL) was synthesized. It was conjugated to McAb (or its fragment Fab) and biotin by condensation of it with an ε-NH_2 group on lysine residue. The functionalized McAb was then labeled with $^{99m}TcO^{3+}$. *In vitro* and *in vivo* experiments showed that ^{99m}Tc-BCL-McAb, ^{99m}Tc-BCL-Fab and ^{99m}Tc-BCL-biotin were quite stable.[22,23]

Metallothionein (MT) was also used as a bifunctional conjugating agent with satisfactory results. The conjugation of MT to antibody was accomplished by condensation with glutaric dialdehyde followed by the reduction of imine with $NaBH_4$.[24–26]

N, N'-ethylene-bis-L-cysteine (EC) was used as a bifunctional chelating agent for ^{99m}Tc labeling of McAbs. A new conjugating agent N-hydroxyl-1,4-epoxy-cyclohex-5-ene-2,3-di-carboxylimide (HONCE) and a water-soluble dehydrating agent 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (EDC) were used to accomplish the conjugation of EC with McAb.[27,28]

1.2.3. ^{188}Re. Four new bifuctional conjugating agents were synthesized: Bz-MA-Ala-Ala-EACA, Bz-MA-Ala-Gly-EACA, Bz-MA-Gly-Ala-EACA, and Bz-MAG_3-EACA (Bz=benzoyl, MA= mercaptoacetyl, EACA=ε-aminocaproic acid). Our strategy was simultaneous removal of protective group Bz and labeling of the de-protected bifuctional conjugating agent with ^{188}Re followed by conjugation of ^{188}Re-MAG_3-EACA to McAb. The labeling efficiency was roughly 50%. The six-carbon-atom EACA acted as a spacer between the McAb and the $^{188}ReOS_2N_2$ moiety and thus ensured a relatively high labeling efficiency and a good immunoactivity of the antibody as well. Both the *in vitro* and *in vivo* stability of ^{188}Re-MAG_3-EACA-McAb were experimentally proved very high.[29,30]

1.2.4. ^{111}Ag and ^{199}Au. We found that ^{111}Ag could be indirectly labeled to McAbs via metallothionein[24] and ^{199}Au via thiomalic acid.[31]

1.2.5. ^{90}Y and ^{169}Yb. Yttrium-90 is a very good radionuclide for therapy of tumor. 1,4,7,10-tetraaza-dodecane-1,4,7,10-tetraacetic acid (DOTA) was tentatively used as a bifunctional conjugating agent. The effort was frustrated by the slow reaction kinetics of DOTA with $^{90}Y^{3+}$. If one lets $^{90}Y^{3+}$ chelated by DOTA first, then no active group can be used for formation a peptide bond with McAb. Alternatively, if DOTA is first conjugated to McAb, the labeling with $^{90}Y^{3+}$ will proceed painfully slow at 37 °C. It was observed that the completion of the formation of Y^{3+}-DOTA complex could be achieved by boiling for 2h at pH ~8.[32,33]

Ytterbium-169 can be labeled to IgG and McAbs through TTHA.[34]

2. Myocardial Imaging Agents

2.1. Boronic Acid Adducts of Technetium Tris(dioxime) (BATO).[35–39] We synthesized several new BATOs and determined the crystal structure of one of them.[35,36] The animal experiments showed that none of these ^{99m}Tc-BATOs was superior to ^{99m}Tc-CDO-MeB in myocardial imaging.[37,38] Similar rhenium compounds (BAROs) were also synthesized. We developed a new method for preparation of BATOs by using $[^{99}Tc(thiourea)_6]Cl_3\cdot4H_2O$ as the starting material. The overall yield of ^{99}Tc was about 60%.[39]

2.2. Complexes Containing $[Tc\equiv N]^{2+}$ or $[Tc=NAr]^{3+}$. We synthesized new complexes containing $[Tc\equiv N]^{2+}$ core. The ligands that we designed and synthesized include derivatives of xanthate,[40] dithiocarbamate, dialkyl dithiophosphate, and dialkyl dithiophosphonates. Few of these complexes displayed a good myocardial uptake (>1% ID) or cerebral uptake (>0.6% ID), and are worthy of further study. Complexes of $[Tc=NAr]^{3+}$ showed a poor biodistribution, probably due to their *in vivo* instability.[41]

3. Production of $^{186/188}$Re, ^{111}Ag and ^{199}Au

3.1. New Process for Separation of Radiorhenium from Proton-bombarded Tungsten Target. An $Al_2(WO_4)_3$ target bombarded with proton beam was dissolved in aqueous NaOH solution, and then acidified to precipitate the bulk of $Al_2(WO_4)_3$ and leave the radiorhenium as $*ReO_4^-$ in the solution. After filtration the $*ReO_4^-$ was further purified by alumina gel chromatography. The process is simple and fast.[42,43]

3.2. Preparation of $^{186/188}$Re with High Specific Activity by Szilard–Chalmers Effect. Rhenium-186 and -188 are regarded as the best radionuclides used for radiotherapy and radioimmunotherapy. We prepared ^{186}Re and ^{188}Re with high specific activity by the Szilard–Chalmers effect. A series of rhenium complexes was synthesized and then irradiated with thermal neutrons (neutron flux: 10^{12} n/sec/cm^2) for 1 h. The recoiled radioactive rhenium atoms were separated by stripping them from the dichloromethane solution of irradiated rhenium compound with an aqueous solution. Various aqueous stripping agents were tested. In the case of $ReN(S_2CNEt_2)_2$, an enrichment factor of 210 and a chemical yield of 36% were achieved. The product was in pure perrhenate form with specific activity of 0.72 GBq/mg Re (19.5 mCi /mg Re). Radiorhenium with

higher specific activity (>37 GBq/mg Re) could be obtained by irradiation with 1×10^{14} n/sec/cm^2 neutron flux for 1 h.[44]

3.3. Preparation of Carrier-free ^{199}Au. Gold-199 ($E_{\beta max}$ = 0.30, 0.46 MeV, E_γ=0.158, 0.208 MeV, $T_{1/2}$=3.15 d) is a potential radionuclide for medical use. It was produced by reaction ^{198}Pt (n,γ) ^{199}Pt (β^-) and separated by ion exchange.[45]

3.4. Preparation of Carrier-free ^{111}Ag. Silver-111 ($E_{\beta max}$ =1.035 MeV (92%), 0.69 MeV (6.80%), E_γ =0.245 MeV (1.35%), 0.342 MeV(6.55%), $T_{1/2}$ =7.45 d) was produced by ^{110}Pd(n, γ)^{111}Pd(β^-) and separated by anionic exchange resin.[46] This radionuclide may be used for radiotherapy or radioimmunotherapy.

4. Neutron Capture Therapy Drugs[47]

4.1. Liposome-Encapsulated Gadolinium Complexes.[48] Liposome is an effective drug delivery agent for neutron capture therapy. Most of the publications are dedicated to the delivery of boron compounds. We explored its feasibility for gadolinium complexes. Liposome encapsulating gadolinium complex (LGd) was prepared and characterized. The influence of formulation factors, such as pH, ionic strength, buffer composition, and storage temperature and medium, upon the stability of the LGd was investigated. The rate constant of cellular uptake and its dependence upon the concentration were comparatively studied *in vitro* for LGd and free gadolinium complex that was not encapsulated into liposome micelle. The results indicate that transmembrane delivery of LGd is more efficient than that of the free gadolinium complex. The uptake in both cases follows a first-order kinetics. The kinetics of effluxion after cellular uptake was also studied for both LGd and free gadolinium complex.

4.2. Boronated Glucosides.[49,50] Two boronated methylglucosides, cesium bis(6-deoxo-2,3,4-tri-O-acetyl-α-D-methylglucoside-6-)sulfido-undecahydro-*closo*-dodecaborate(1-) and tetrabutyl ammonium 6-deoxo-2,3,4-tri-O-acetyl-α-D-methylglucoside-6-oxo-undecahydro-*closo*-dodecaborate(2-), were synthesized and characterized by IR, ^{1}HNMR and elemental analysis. After removal of the acetyl groups by basic hydrolysis, their biodistribution was studied with mice bearing hepatoma H22. The results show that they can be concentrated in tumor to some degree (ca. 10 μgB/g tumor for an injection dose of 45mg B/kg body weight). The concentration of boron in blood is relatively low (T/B≈2, where T and B denote the concentration of boron in tumor and in blood, respectively). Its clearance is quite fast.

4.3. Monovalent Organoboron Cations.[51] Monovalent cations such as ^{201}Tl$^+$, ^{99m}Tc-MIBI, and ^{99m}Tc-tetrofosmin are not only the most popular myocardial imaging agents but also the tumor imaging agents because they are accumulated in tumors to various degrees. Following this fact we synthesized a new boron compound 1-(4-methylphenyl)-4-methyl-4-dimethylamino-1-boron-2,6,dioxo-cyclohexane, which could be protonated to an ammonium cation $MeC_6H_4B(OCH_2)_2C(Me)NMe_2H^+$ (compound a) at a biological pH, and three bis(ligand)dihydroborane (1+) cations $[BH_2D^1D^2]^+$: D^1=NEt_3, D^2=4-methylpyridine (compound b); D^1=D^2=4-methylpyridine (compound c); D^1=D^2=2,6-dimethylpyridine (compound d).

We studied their biodistribution in Kunming mice bearing sarcoma S180 and found that these compounds could be concentrated in tumor to some extent. The T/N (the ratio of the concentration of boron in tumor tissue to that in normal tissue) and T/B were 1.3–1.8 and 1.0–1.5, respectively. The maximum boron concentration was 2.1–3.2 μg/g (injected dose: 5 mg B/kg body weight). We found these compounds were highly toxic, probably due to their *in vivo* instabilities. Therefore, only very low dose could be used.

4.4. Boronated Porphyrins.[52] We synthesized a simple boronated porphyrin, 5,10,15,20-tetrakis(4-(4,4-dimethyl-1,5-dioxo-1-boro-cyclohexyl) phenyl) porphyrin (TBPP). Since the removal of the four protective groups (2,2-dimethyl-1,3-propanediol) is extremely difficult, we directly used it for cellular and animal experiments. The ingestion of TBPP by osteoma cell SP20 from a 144 μmol/L TBPP culture solution was 45 μgB/g cell. Approximately a half of the ingested TBPP was excreted in 3 h in a TBPP-free culture solution. The biodistribution of TBPP was studied with mice bearing Hepatocarcinoma BALB/C. When the injection dose was 2.5 mg/kg body weight, the concentration of TBPP in tumor, blood, liver, and brain were 0.51, 0.34, 2.9 and 0.80 μg/g, respectively, at 6 h after injection. Since this compound is sparely soluble in water, 2-hydroxypropyl-β-cyclodextrin was added.

4.5. Subsidiary Compounds

4.5.1. p-Glucoprotein Inhibitors. In some tumor cells the multidrug resistance gene (MDR) may be overexpressed, resulting in high level p-glucoprotein on the cellular surface. We are trying to develop an assay procedure based on ^{99m}Tc-MIBI or ^{99m}Tc-tetrofosmin radionuclide imaging. We expect to use this method to screen p-glucoprotein inhibitors. The aim of this study is to predict the drug action in chemotherapy for a given cancer and a given patient, and to improve the uptake of anti-tumor drugs.

4.5.2. Radiation Sensitizers. In 1989 Prof. J. L. Sessler (University of Texas at Austin) discovered "Texas-sized" expanded porphyrins (Texaphyrins). The Gd^{3+}-Texaphyrin complex revealed very good tumor-seeking and radiation-sensitizing properties. It may remarkably improve the effect of X- or γ-ray radiotherapy for cancer. We expect that such compounds will also magnify the relative biological effectiveness (RBE) of NCT. We therefore tried to design compounds other than Texaphyrins but possessing similar properties by using quantum chemical and molecular mechanical calculations. The synthesis is in progress.

5. Conclusion

In the past 15 years we tried to follow the new trend in radiopharmaceutical chemistry. Unfortunately, we have not been engaged in PET pharmaceuticals study due to the lack of both a PET machine and a cyclotron suitable for production of β^+-emitters. Our unremitting efforts have led to the publication of some 50 papers, and growing up a number of M.Sc. and Ph.D. students.

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