Analysis of complex formation between rhenium and various hydrophilic ligands using HPLC and preparation of ^{186}Re-carrying liposomes

I. O. Umeda,[*1] Y. Hamane,[*1] M. Nagata,[*1] A. Tenzaki,[*1] H. Haba,[*2] A. Nambu,[*2] X. Yin,[*2] H. Fujii,[*3] and T. Takahashi[*1]

Theranostics is an emerging and expanding medical field based on therapeutic interventions after imaging to verify the presence of a biological target. Radioisotope-based therapeutics, *i.e.*, radiotheranostics, is perhaps the most clinically advanced application of theranostics, with many developments and emerging opportunities. A key aspect of radiotheranostics is that the selection of patients for radiotargeted treatments is based on imaging of the same target area. Therefore, imaging and therapeutic intervention are closely linked.[1)] Technetium-99m is the most widely used nuclide in diagnostic nuclear medical imaging, because the energy of the corresponding gamma-ray emission (140 keV) is ideal for imaging using gamma cameras, and it can be generated on demand at medical facilities using ^{99}Mo/^{99m}Tc generators. Rhenium, a group 7 homologous element of technetium, is assumed to have chemical properties similar to those of Tc. ^{186}Re (half-life $T_{1/2} = 3.72$ days) emits beta rays of 1.07 MeV, appropriate for radionuclide therapy. Thus, ^{186}Re and ^{99m}Tc should be an ideal "theranostics pair." To this end, ^{186}Re has been produced by neutron irradiation in nuclear reactors, which is difficult to realize in Japan. At the RIKEN RI beam factory (RIBF), we succeeded in producing no-carrier-added, high-purity ^{186}Re using an accelerator-based ion beam irradiation. We are aiming to develop new radiotheranostics using ^{186}Re.

For radiotheranostics, a common platform for diagnostic and therapeutic nuclides is important. We have been investigating the application of liposomes, well-known drug carriers, as a radiotheranostics platform. Liposomes are promising tools for radiotheranostics, because they can in principle encapsulate any radionuclides, and deliver them to the target lesion. In this study, we conducted a basic investigation for efficient encapsulation of ^{186}Re into liposomes.

We encapsulated various radionuclides in liposomes with high efficiency using the remote loading method, *i.e.*, a ligand exchange reaction across the liposomal membrane using lipophilic and hydrophilic ligands.[2)] In this method, the complex formation between the nuclide and ligand and their stability are important factors. There are few data on the complex formation of ^{186}Re. We first developed an analytical method to evaluate their formation and stability using high performance liquid chromatography (HPLC) and a stable isotope of rhenium.

Rhenium-186 was produced in the ^{186}W$(d, 2n)^{186}$Re reaction. A 24-MeV deuteron beam delivered from the AVF cyclotron was irradiated onto a $^{186}WO_3$ pellet target (isotope enrichment of ^{186}W: 99.79%; thickness: 580 mg/cm^2). After irradiation, ^{186}Re was purified by chemical separation.[3)] An aqueous solution of ^{186}Re-perrhenate in 0.01 M HCl (radioactivity concentration: 83.0 MBq/mL) was supplied. Ammonium perrhenate was used as a stable isotope of rhenium. Diethylenetriamine-$N, N, N', N,'' N''$-pentaacetic acid (DTPA), dimercaptosuccinic acid (DMSA), hydrazinonicotinamide (HYNIC), and ethylenedicysteine (EC) were examined as hydrophilic ligands. HPLC analysis was conducted using Shimazu Prominence LC-20 equipped with aerosol-based detector NQAD and gamma-ray radiodetector. Liposomes comprised DSPC and cholesterol, or PEGylated, and preloaded hydrophilic ligands.

The formation of complexes between metal ions and ligands is difficult to analyze using conventional HPLC systems, because these samples have no UV absorption, and are highly water-soluble. We realized this using unique columns, combination of cation exchange column (CapcelPak CR) and unique ODS column (Capcell Core ADME), and a unique detector, NQAD. Using this system, we were able to detect metal ions, ligands, and the metal ion-ligand complexes as separate peaks. Figure 1 shows HPLC profiles of rhenium, technetium, EC, and their complexes.

Using this system, the formation of complexes between rhenium and various hydrophilic ligands was investigated. All the ligands used formed complexes with ^{99m}Tc. Under the conditions studied, DTPA and HYNIC did not form complexes with rhenium. In case of DMSA and EC, complex formation with rhenium was observed. However, unlike ^{99m}Tc, the complexes were formed only under acidic conditions, high temperature, and required approximately ten times more reducing agent than in the case of ^{99m}Tc.

Next, we preloaded EC into liposomes and attempted to encapsulate ^{186}Re and ^{99m}Tc into liposomes using the remote loading method. Incubation at 40°C allowed only ^{99m}Tc to be encapsulated, but heating to 90°C allowed ^{186}Re to be encapsulated into liposomes. Since ^{186}Re radioactivity was present in the water-soluble fraction of liposomes, it was assumed

*1 Kavli IPMU, University of Tokyo
*2 RIKEN Nishina Center
*3 EPOC, National Cancer Center

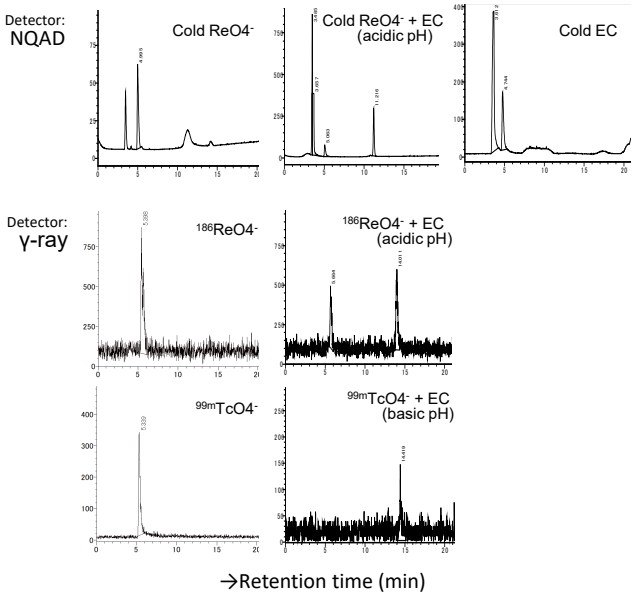

Fig. 1. HPLC separation of metal ions, ligands and metal-ligand complexes. Stable rhenium was used to determine the analytical conditions. NQAD was used for detection. ^{99m}Tc and ^{186}Re were then used to confirm the peaks.

that the ^{186}Re-EC complex was formed.

In this study, we have established a means to analyze complex formation between hydrophilic ligands and rhenium, and evaluated optimal conditions for stable rhenium complex formation. Based on the results, we have successfully prepared ^{186}Re-EC/^{99m}Tc-EC-encapsulated liposomes. These are expected to be useful radiopharmaceuticals for radiotheranostics. We are further investigating the quality control of the drugs and their pharmacokinetics and tumor accumulation.

References

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