I-1. PROJECT RESEARCHES

Project 3

PR3

Production of medical RI by reactor irradiation

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INTRODUCTION: Neutron irradiation of ^{176}Yb offers Japan the most practical route to a domestic supply of the β-emitter ^{177}Lu for cancer theranostics. Clinical tracers such as ^{177}Lu-DOTA-TATE and ^{177}Lu-PSMA-617 have already demonstrated therapeutic benefit [1, 2]. The 2024 (final) year therefore integrated on-line column separation, temperature-responsive liposomal formulation, and ultrafast radiolabeling of targeted probes into one GMP-oriented workflow.

EXPERIMENTS:

Step	Key conditions	Purpose
1. On-line column separation	Extraction-chromatography with real-time γ-monitoring; 1.5 M HNO_3 (300–400 min) elutes Yb, 4 M HNO_3 (≥ 500 min) collects ^{177}Lu	Obtain n.c.a. ^{177}Lu rapidly with low operator dose
2. Thermosensitive liposome (TSL) formulation	Remote loading of [^{177}Lu]Lu-DTPA; labeling eff. > 70 %, purity > 95 %	Create heat-triggered DDS while preserving specific activity
3. Rapid probe radiolabeling	2-min chelation to DOTA derivatives at 50–90 °C; yield 75–86 %	Produce EphA2-targeted probes for pre-clinical testing

RESULTS AND DISCUSSIONS:

On-line Column Separation: A Ge-semiconductor γ-detector integrated into the extraction column enabled real-time monitoring of elution. Yb was completely removed with 1.5 M HNO_3 (300–400 min), while 4 M HNO_3 (≥ 500 min) selectively yielded n.c.a. ^{177}Lu, cutting operator dose and QC time (Fig.1). Bis-2-ethylhexyl-phosphoric acid (HDEHP)-based resins and related extractants remain the work-horse for Lu/Yb separation [3, 4].

Thermosensitive Liposomal Formulation *& Pre*-clinical Evaluation: Using the high-specific-activity ^{177}Lu fraction from Section 1, remote loading produced TSLs with labeling efficiency > 70 % and purity > 95 %. [^{177}Lu]Lu-DTPA leakage was < 5 % at 37 °C but > 70 % at 43 °C; mild heating in Colon-26 tumor mice improved the tumor-to-blood ratio, validating heat-triggered release. Radiolabeled liposomes have been widely explored for theranostics [5], and the present strategy builds on earlier urine-excretable TSL work [6].

Rapid Probe Radiolabeling *& Theranostic* Development: Purified ^{177}Lu supported **2-min** chelation to DOTA derivatives with 75 % (50 °C), 83 % (70 °C), 86 % (90 °C) yields (Fig.2), enabling assembly of EphA2-targeted probes now entering cytotoxicity and comparative studies versus commercial ^{177}Lu.

Fig.1. Extraction-chromatography set-up with on-line γ-ray detector for Yb/Lu separation.

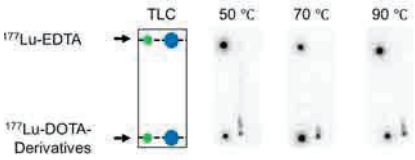

Fig.2 Radio-TLC analysis

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[1] T. Das et *al*., Applied Rad. Isotopes, **65** (2007) 301.
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[5] H.-Y. Low et *al*., Molecules **28** (2023) 115-120.
[6] M. Munekane et *al*., Molecular Pharmaceutics **21** (2024) 5728-5735.

PR3-1

Development of tumor-targeted radiotheranostics probes and its clinical application

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INTRODUCTION: Theranostics is a new medical technology that combines therapeutics and diagnostics. The key to the realization of theranostics is a drug known as theranostic probes. The characteristic of the radiotheranostics probes we are developing is that we consider a single molecule as an aggregate of target recognition units, linker units, and chelating units, and design molecular probes based on the concept of "unit-coupling molecular probes," in which independently developed units are freely combined. This drug design theory is not only effective for designing molecular probes with relatively large molecules such as antibodies and other proteins and bioactive peptides as the nucleus, but also can also be applied to organic small molecular compounds. In this study, we will utilize the theory of creation of unit-coupling molecular probes to develop drugs that can ultimately be applied clinically. First, a basic study of the production of ^{177}Lu in the KUR was conducted. Next, labeling with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) derivatives was investigated to evaluate whether ^{177}Lu produced at the KUR could be used as a radiopharmaceutical.

EXPERIMENTS: ^{177}Lu Production: To obtain ^{177}Lu, Lu_2O_3 and Yb_2O_3 were irradiated at 1 MW for 24 hours and 5 MW for 6 hours.
^{177}Lu-DOTA Labeling Study: An appropriate amount of 12M hydrochloric acid was added to the separated and purified ^{177}Lu, which was then heated at 100-140°C using a hot plate.$^{177}LuCl_3$ was reacted with DOTA derivatives in 0.1 M MES buffer. The reactions were carried out at different temperatures (50, 70, and 90°C) and reaction times of 2 min. The labeling ratios, defined as the ratios of the spot intensity of labeled material to the total spot intensity, was calculated using radio-TLC and autoradiography.

RESULTS: The HPGe energy calibration was performed using ^{133}Ba. The γ-ray spectrum from the HPGe measurement of ^{177}Lu used is shown in Figure A. The γ-ray spectrum confirms the presence of ^{177}Lu. Radio-TLC analysis in Figure B shows that the radiochemical yields were 75%, 83%, and 86% at 50°C, 70°C, and 90°C, respectively. These results suggest that ^{177}Lu produced at KUR can be used for labeling DOTA. In the future, we plan to evaluate the cell-killing effect of ^{177}Lu produced using cancer cells and compare it with commercially available ^{177}Lu.

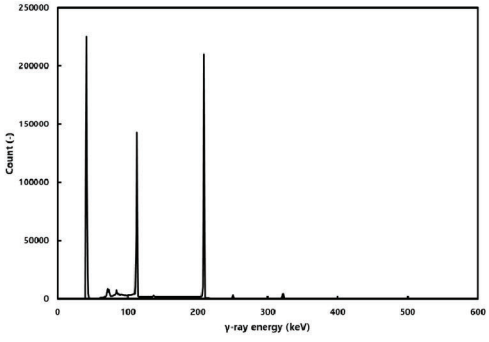

Figure A. HPGe spectrum for ^{177}Lu

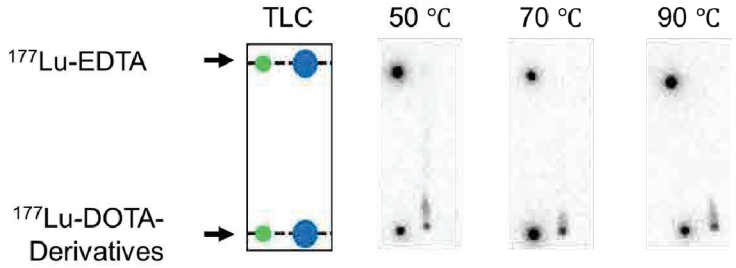

Figure B. Radio-TLC anaylsis

PR3-2

Development of thermosensitive liposomes encapsulating a ^{177}Lu-labeled urinary excretable compound for targeted radionuclide therapy

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INTRODUCTION: Liposomes are useful drug delivery carriers for cancer treatment, and many radiolabeled liposomes have also been developed for radiotheranostics[1]. However, the high retention of radiolabeled liposomes in the blood results in a high background in diagnostic imaging and the occurrence of side effects such as bone marrow suppression in targeted radionuclide therapy. Then, we have developed thermosensitive liposomes (TSLs) encapsulating radiolabeled compounds that are excreted in urine[2]. The previously reported TSLs released the urinary excretable radiolabeled compounds, In-111-diethylenetriaminepentaacetic acid (^{111}In-DTPA), by heating blood in peripheral tissue of mice, resulting in rapid clearance of blood radioactivity and improved tumor-to-blood ratio. In this study, the TSL-based method was applied to targeted radionuclide therapy with Lu-177 (^{177}Lu) replacing ^{111}In.

EXPERIMENTS: TSLs (1,2-dipalmitoyl-sn-glycero-3-phosphocholine/1,2-distearoyl-sn-glycerol-3-phosphocholine/cholesterol/1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] = 60:20:15:5 at molar ratio) were prepared by a thin-film hydration followed by an extrusion method. ^{177}Lu was produced at the Kyoto University Research Reactor, KURR. [^{177}Lu] Lu-DTPA loaded liposomes were prepared by a remote loading method with high labeling efficiency (>70%) and purity (>95%). The release of [^{177}Lu] Lu-DTPA from liposomes was evaluated at 37 and 43°C for 1 h by gel filtration chromatography. Biodistribution of radioactivity was evaluated in Colon-26 tumor-bearing mice at 0.5, 3, 6, and 24 h postinjection. In vivo release experiments were also performed using Colon-26 tumor-bearing mice. Biodistribution of radioactivity was analyzed with or without heating the tails of Colon 26 tumor-bearing mice for 1 h at 2 h postinjection.

RESULTS: [^{177}Lu] Lu-DTPA loaded liposomes released less than 5% and more than 70% of radioactivity at 37 and 43°C, respectively. In the biodistribution experiments using Colon-26 tumor-bearing mice, high blood retention of radioactivity and high accumulation in the spleen and liver were observed. The highest accumulation in the tumor was found at 3 h postinjection. These results were similar to the biodistribution of ^{111}In-labeled thermosensitive liposomes. In vivo release experiments showed the decreased radioactivity in blood for the heated group, resulting in the improved tumor-to-blood ratio. These results suggest that the TSL-based method can be applied to targeted radionuclide therapy with ^{177}Lu.

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[1] HY. Low *et al.,* Molecules, **28(9)** (2023) 115-120.
[2] M. Munekane *et al.,* Mol. Pharm, **21(11)** (2024) 5728-5735.

PR3-3

On-line monitoring of ^{177}Lu separation by extraction chromatography

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INTRODUCTION: ^{177}Lu (half-life: 6.64 d), produced indirectly by neutron irradiation of ^{176}Yb, is a b-emitter and one of the applicable nuclides for targeted radioisotope therapy (TRT). To date, ^{177}Lu-DOTA-TATE, ^{177}Lu labeled with DOTA-TATE for the treatment of somatosta-tin-overexpress-ing tumors [1], and ^{177}Lu-PSMA-617, ^{177}Lu labeled with PSMA-617 for the treat-ment of castration-resistant prostate cancer [2], have been investigated for TRT use.

In extraction chromatography, bis-2-ethylhexylphosphonric acid (HDEHP) [3] and 2-ethylhex-ylphosphonic acid (HEH[EHP]) [4] are well known as substrate adsorbed extractants for ^{177}Lu sepa-ration, and recently some extractants have explored. In these studies, typically the con-centrations of Yb and Lu in the eluate are determined by radiometric or elemental concentration measurements after collecting a certain volume of solution that has passed through the column. However, some technique that allows for faster analysis is desirable for the best condition survey.

In this study, we developed a real-time monitoring technique for ^{177}Lu and radioactive Yb using the on-line Ge semiconductor detector in the separation of ^{177}Lu from neutron irradiated Yb targets by extraction chromatography.

EXPERIMENTS: The experimental apparatus used in this study consists mainly of a pump, a column with a water jacket and a Ge semiconductor detector. The solu-tion is pumped up and introduced into the column, which is filled with extraction resin (LN2, Eichrom, 50–100 μm). The column temperature is kept constant by a ther-mostatic water circulation system. The solution that has passed through the column is introduced into a lead shield with a γ-ray detector installed. Then, the solution is col-lected in a sample vial as it passes through a bundled flow path in the ring shape for γ-ray measurement.

Fig. 1. Experimental apparatus used for the separation of ^{177}Lu and Yb by extraction

RESULTS: During the Lu/Yb separation experiment, the peak energies and their intensities in the γ-ray spectra change with time. Focusing on the γ-ray energy range above 100 keV, high intensities were detected at around 110, 131, 177, 198, 282, 308, and 396 keV for times between 300 and 400 min. These γ-ray peaks are attributed to ^{169}Yb (110, 131, 177, 198, and 308 keV) and ^{175}Yb (114, 282, and 396 keV), respectively. In the region around 510 min, high intensities were detected at 110 keV as well as 208 keV. Especially at the latter γ-ray energy, the intensity was confirmed only in this region. Since these can be attributed to ^{177}Lu (113 keV and 208 keV), it can be assumed that most of the Yb is leached out by passing 1.5 M HNO_3, and the remaining resin residue, composed mainly of Lu, is leached out by 4 M HNO_3 after 500 min.

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[2] M. Benesova *et al.,* J Nucl Med, **56** (2015) 914.
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