Development of ^{211}At-labeled antibody for targeted alpha therapy

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Radiotherapeutic agents labeled with various α-emitting nuclides have been developed for targeted alpha therapy. ^{211}At has a simple decay scheme and can avoid the risk of side effects caused by daughter nuclides; it is thus considered a useful α-emitter for this therapy. Given the requirements for ^{211}At-labeled pharmaceuticals in the future, RIKEN Nishina Center for Accelerator-Based Science aims to increase the ^{211}At production scale a global maximum; this prompted us to develop ^{211}At-labeled drugs and labeling methods.

For the attachment of ^{211}At, organotin compounds are the most widely used precursor owing to the weakness of the carbon-tin bond, which enables the use of a tin group as a leaving group.[1)] In this study, we used an organotin compound, *N*-succinimidyl-3-(trimethylstannyl)benzoate (m-MeATE), for ^{211}At labeling. According to the direct astatination procedure reported by Lindegren *et al.*,[2)] m-MeATE was first conjugated with a monoclonal IgG. Then, m-MeATE and IgG were mixed at a molar ratio of 20:1 and incubated for 30 min at 25°C in a pH-8.5 NaCO3 buffer. The attachment of ^{211}At was carried out by a modified protocol of the method of Li *et al.*[3)] An oxidizing agent N-chlorosuccinimide (NCS) (0.2 mg/ml) was added to the ^{211}At solution in 1% acetic acid MeOH. Then an IgG/m-MeATE conjugate in a 0.2 M sodium acetate buffer was mixed with the activated ^{211}At solution, with the final MeOH and NCS concentrations being 11% and 3.6 μg/ml, respectively. The incubation was performed for 3 min. *N*-iodosuccinimide (0.2 mg/ml) was then added at a final concentration of 5.4 μg/ml, and the reaction mixture was incubated for another 1 min. For purification, an Amicon Ultra 50 kDa column (Merck) was used in ultrafiltration.

Finally, 5.6 MBq of ^{211}At-labeled IgG was obtained using 40 MBq of ^{211}At-MeOH (shown in Table 1). In this experiment, we did not reach the labeling yield that Li *et al.* reported previously (40.2 ± 6.9%).[3)] Further, we examined the use of a PD Spintrap G-25 gel filtration column for purification, only to reduce the yield.

To demonstrate the usefulness of the ^{211}At-labeled IgG, we performed a biodistribution study using the mice with xenografted tumors specific to IgG. Three and 24 h after the ^{211}At-labeled IgG i.v. injections, the mice were dissected to measure tissue radioactivity. As a control group, free ^{211}At was also administered. The results are shown in Fig. 1. Unexpectedly, the distribution of ^{211}At-labeled IgG was almost the same as that of free ^{211}At. The high accumulation rates in the stomach and thyroid indicated the physiological accumulation of ^{211}At detached from IgG.

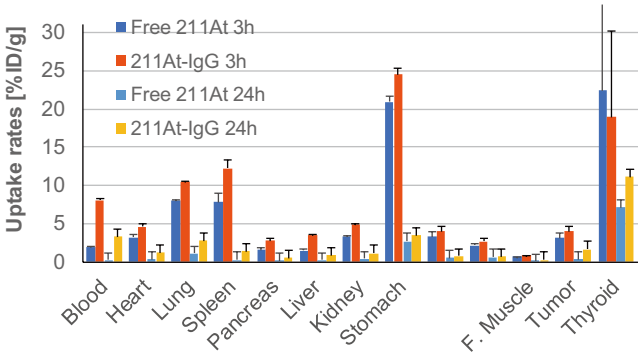

Fig. 1. Biodistribution of free ^{211}At and ^{211}At-labeled antibody 3 and 24 h after i.v. injection in the tumor-bearing mice.

Table 1. Labeling results of ^{211}At-labeled IgG purified by ultrafiltration or gel filtration.

Purification method	Radioactivity yield [%]	Protein yield [%]	Specific activity [kBq/ug]
Ultrafiltration	**13.9**	**75.0**	**12.9**
Gel filtration	**5.0**	**69.5**	**14.9**

Table 2. Labeling results of ^{211}At-labeled IgG or IgG/m-MeATE purified by reduced nonspecific binding gel.

Precursor	Radioactivity yield [%]	Protein yield [%]	Specific Activity [kBq/ug]
IgG/m-MeATE	**43.9**	**85.6**	**31.6**
Intact IgG	**12.9**	**79.3**	**12.5**

From these results, we speculated that the increased hydrophobicity by astatination may have caused the nonspecific binding of ^{211}At-labeled IgG to column filters or gels in the purification step, resulting in a low radioactivity yield despite a high protein yield. To overcome this problem, we used a specialized gel to alleviate nonspecific binding. As shown in Table 2, the radioactivity yields were significantly improved.

In the next step, we will examine a labeling method using decaborane, which is expected to have a higher stability in bonding with ^{211}At in the body.

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