Sodium ascorbate protects ^{211}At-labeled antibodies from reactive oxygen species damage†

S. Manabe,[*1,*2,*3] H. Takashima,[*4] Y. Koga,[*4,*5] K. Onuki,[*6] R. Tsumura,[*4] T. Anzai,[*4] N. Iwata,[*4] M. Yasunaga,[*4] Y. Wang,[*7] T. Yokokita,[*7] Y. Komori,[*7] D. Mori,[*7] H. Haba,[*7] H. Fujii,[*6] and Y. Matsumura[*4]

Radioimmunotherapy (RIT) is synonymous with next-generation antibody medication. α-particles are particularly suited to RIT on account of their potent linear energy transfer (LET) and short path range. Among the several α-emitter nuclei, ^{211}At is preferable to the rest owing to its short half-life (7.2 h) and inability to yield cytotoxic daughter isotopes during decay. Successful delivery of α-particles to the tumor site is a key requisite for effective cancer treatment, which can be achieved by using specific antibodies against tumor antigens.

We investigated ^{211}At-labeled antibodies for cancer therapy.[1)] Briefly, ^{211}At, generated by the RIKEN AVF cyclotron, was conjugated to trastuzumab, a therapeutic antibody targeted against breast and stomach cancer. ^{211}At was immobilized onto trastuzumab carrying trimethylstannyl benzoate via an ^{211}At-Sn exchange reaction, followed by purification using size-exclusion column chromatography. However, using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses, we discovered that the ^{211}At-labeled trastuzumab thus obtained was damaged. Further, flow cytometry revealed that the binding affinity of ^{211}At-labeled trastuzumab to human epidermal growth factor receptor 2 (HER2) expressing SK-BR-3 cells was drastically compromised (Fig. 1).

We speculated that damage to the ^{211}At-conjugated antibodies may have been caused by reactive oxygen species (ROS), that were generated from water radiolysis by α-particles released from ^{211}At. Consequently, we established an ROS detection system based on the chemiluminescence luminol assay. Aqueous ^{211}At solutions tested positive in the luminol assay, thereby indicating the presence of ROS. Further, the assay revealed the quenching of ROS in the presence of 6×10^{-2} mg/mL sodium ascorbate.

^{211}At-labeled trastuzumab in the presence of more than 6×10^{-2} mg/mL sodium ascorbate was stable as seen using SDS-PAGE analyses, and its binding affinity was maintained. In addition, WST-8 assays demonstrated more potent cytocidal effects of ^{211}At-labeled trastuzumab in the presence of sodium ascorbate, against high HER2-expressing SK-BR-3 cells than those exerted by the immunoconjugate that was not under the protection (Fig. 2). These results conclusively demonstrated that quenching of ROS is essential for efficacious RIT. Moreover, the cytocidal effects seemed to be dependent on the level of HER2 on the cancer cells. Compared with free ^{211}At, ^{211}At-labeled trastuzumab more efficiently killed SK-BR-3 cells, whereas the cytotoxicity was mitigated against MCF-7 cells that express lower levels of HER2 (Fig. 2).

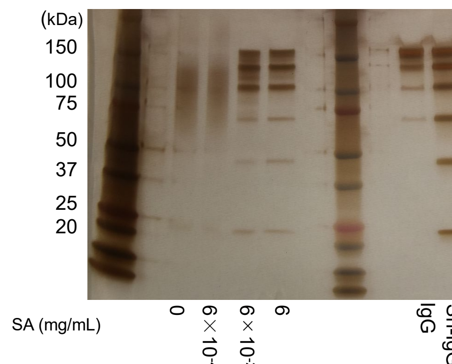

Fig. 1. SDS-PAGE showing damaged ^{211}At-labeled trastuzumab after 1 day.

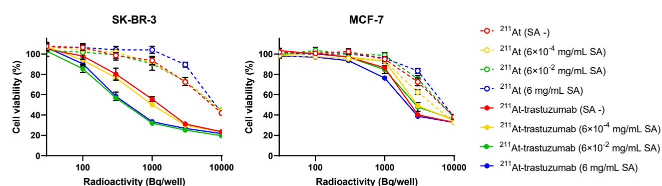

Fig. 2. Cytotoxic effects of ^{211}At-labeled trastuzumab.

While the reducing agent L-cysteine demonstrated similar ROS-quenching activity as sodium ascorbate, others such as sodium hydrosulfite and maltose, had a weaker effect.

We therefore conclude that ^{211}At-labeled antibodies are damaged by ROS that are generated by the action of ^{211}At on water. The presence of sodium ascorbate resulted in the quenching of ROS, which prevented ^{211}At-labeled antibody damage, and maintained its function. The present data warrant further studies for development of the above-described cancer therapy.

This manuscript has been condensed from the article in ACS Omega. Figures are reproduced with permission from American Chemical Society.

Reference

1) H. Takashima *et al.*, Cancer Sci. **112**, 1975 (2021).

† Condensed from the article in ACS Omega **6**, 14887 (2021)

*1 Pharmaceutical Department, Hoshi University
*2 Research Center for Pharmaceutical Development, Tohoku University
*3 Glycometabolic Biochemistry Laboratory, Cluster for Pioneering Research, RIKEN
*4 Division of Developmental Therapeutics, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center
*5 Department of Strategic Programs, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center
*6 Division of Functional Imaging, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center
*7 RIKEN Nishina Center