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Total-body PET Imaging for up to 30 Days after Injection of ^{89}Zr-labeled Antibodies

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With continued efforts towards the development of positron emission tomography (PET) scanners over the last several decades, a major milestone was achieved with the recent completion of total-body PET scanners. Both clinical and preclinical total-body PET scanners have been developed by the EXPLORER Consortium *(1-3)*. These state-of-the-art scanners hold many advantages over previously developed PET scanners. For example, PET scans can be performed much faster due to a larger field-of-view and higher sensitivity. In addition, PET scans can be performed much later after tracer injection. In many cases, whole-body dynamic PET imaging can also provide invaluable information that could not be attained before. Both the clinical and preclinical total-body PET scanners hold tremendous potential for various future biomedical applications, and the scientific community is looking forward to what can be done in the immediate future to unleash their full potential.

In this issue of *The Journal of Nuclear Medicine*, Berg et al. reported PET imaging of rhesus monkeys with the primate mini-EXPLORER scanner *(4)*. They compared four different tracers, all ^{89}Zr-labeled antibodies, and were able to acquire high quality PET images for up to 30 days after tracer injection (~10 decay half-lives of ^{89}Zr). The antibody they used was a humanized monoclonal IgG antibody against the herpes simplex viral protein gD, which was developed by Genentech, Inc.

^{89}Zr-labeled antibodies have been studied for several decades, both preclinically and clinically *(5)*. The commonly used chelator is desferrioxamine B (DFO), and various linkers have been investigated for better in vivo performance of ^{89}Zr-based tracers. Since $^{89}Zr^{4+}$ prefers the formation of an octadentate complex, and the DFO chelator only has six oxygen atoms, such a combination is not ideal in terms of in vivo stability. Because of

this, significant bone uptake has been observed at late time points in many preclinical studies. In clinical studies with ^{89}Zr-labeled antibodies, bone uptake was not as obvious and does not seem to pose a significant concern. Nonetheless, substantial effort has been devoted to the development of better chelators for ^{89}Zr. An octadentate variant of DFO, desferrioxamine* (DFO*), was developed which has an additional hydroxamate unit *(6)*. With DFO* as the chelator, lower signal of ^{89}Zr was observed in bone and liver when compared to DFO-based tracers *(7)*. As such, DFO* is a promising chelator for future clinical translation of ^{89}Zr-based PET tracers.

The principle aim of this work *(4)* was to demonstrate the feasibility of extending nonhuman primate ^{89}Zr-based PET imaging studies to up to 30 days post-injection with a primate mini-EXPLORER total-body PET system. This was possible with this imaging system because of the increased sensitivity from a 45 cm axial field-of-view and 43.5 cm bore diameter. The ability to image at such late time points could provide unprecedented biological information about the long term behavior of radiolabeled antibodies in vivo. In a secondary objective, the linker between the DFO or DFO* and the antibody, which plays an important role in the in vivo stability and behavior of ^{89}Zr-labeled antibodies *(8)*, was investigated. In this work *(4)*, two different chelators and two different linkers were used to label the anti-gD antibody. The four tracers (i.e., ^{89}Zr-DFO-Bz-NCS-anti-gD, ^{89}Zr-DFO-squaramide-anti-gD, ^{89}Zr-DFO*-Bz-NCS-anti-gD, and ^{89}Zr-DFO*-squaramide-anti-gD) were compared in rhesus monkeys over the 30 day period following tracer injection to identify the best tracer for future investigation.

The feasibility of total-body PET at 30 days post-injection of ^{89}Zr-labeled antibodies was clearly demonstrated. Even with <40 kBq of radioactivity in each rhesus monkey,

PET images were obtained with sufficient quality to identify various organs of interest. The images at early time points were of excellent quality, with the injected radioactivity amount of about 40 MBq into each rhesus monkey that weighs about 3 kg. To compare the day 0 pharmacokinetics of the four tracers, dynamic PET scans during the first hour were performed, which revealed some interesting differences between the four tracers. Both tracers with the Bz-NCS linker group showed increased activity in the bladder towards the end of the 60-minute dynamic scan in comparison to both tracers with the squaramide linker groups. These findings suggest that the squaramide linker is significantly more stable in vivo than the commonly used Bz-NCS linker. Whether the chelator was DFO or DFO* did not appear to make a significant difference within the first hour of injection.

The whole-body radioactivity of each rhesus monkey was also measured over the course of 30 days. Since all variables were quite consistent for the four PET tracers except the chelator/linker used, the dramatic difference in whole-body radioactivity retention is quite interesting, which varied drastically from ~30%ID to >90%ID at the second time point (Day 2 or 3). Based on ELISA results, all IgG concentrations are essentially the same for all four groups. Although there were some differences in the radiolabeling purities (i.e., greater impurities for the Bz-NCS groups), the authors suggested that the primary reason for this unexpected finding was that during the transportation of the radiolabeled antibodies, the high activity concentrations resulted in radiolytic degradation of the linker or chelator, which occurred to a greater extent in the Bz-NCS groups than the squaramide groups. These results suggested that in the future, greater care will need to be taken when transporting radiotracers, such as proper

temperature control. Additional quality control and purification of the tracers may also be needed at the PET facility before injection (which was not performed in this study) to ensure more accurate and meaningful PET imaging data, quantification, and result interpretation.

At late time points, the biodistribution and quantitative tracer uptake in the four groups were largely similar within each group of three primates. However, there were some notable differences between the four tracers such as whole-body retention and liver/bone uptake. It is unclear what is the reason for this. One interesting aspect of this study was that tracer injection into the left brachial artery was administered after unlabeled anti-gD antibody (10 mg/kg) was injected in the right brachial artery. Tracer injection into the artery in quite unusual, which led to some radioactivity remaining at the injection site for several animals in the ^{89}Zr-DFO-Bz-NCS-anti-gD and ^{89}Zr-DFO-squaramide-anti-gD groups. This may be partially responsible for the experimental findings, which needs to be investigated in detail in future studies.

The results of this work are an important reminder that the choice of chelator is not trivial. The use of octadentate DFO* gave rise to fewer osteophilic catabolites of ^{89}Zr-labeled antibodies and improved PET image signal at late time-points, which is likely the result of a more stable complex with ^{89}Zr when compared to the hexadentate DFO. In addition, the squaramide linker may also provide some advantages in terms of shelf-life stability when compared to Bz-NCS. Most importantly, this study *(4)* demonstrated the tremendous potential of total-body PET scanners, which enabled unprecedented late time point imaging of ^{89}Zr-labeled antibodies. Such long term serial PET imaging could help answer many biological questions about the in vivo behavior of (radiolabeled) antibodies

(2). To glean such biological insight, many additional experiments will be required to supplement the PET scans to validate the biological relevance of the long-term PET imaging data, since the radioactivity at 30 days post-injection might be largely dissociated from the antibody/drug, especially with a residualizing metal such as ^{89}Zr. We look forward to future studies in this regard. With broader availability of both preclinical and clinical total-body PET scanners in the future, intensified scientific endeavors will be needed from various research groups around the world, which will eventually lead to improved clinical translation/investigation of novel PET tracers and better patient care.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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