Different Radionuclides in DOTA-EB-TATE Effect Different Uptake in Somatostatin Receptor–Positive HEK293 Cells

TO THE EDITOR: Tian et al. developed a trifunctional ligand with an albumin-binding component (Evans blue), which features a longer-circulation half-life as compared with an unmodified DOTA conjugate. It has been shown in a preclinical model that the prolonged circulation improved the accumulation of this tracer in somatostatin receptor (SSTR)–positive tumors (*1*). In this study, bioimaging was performed by ^{86}Y-DOTA-EB-TATE and PET as a substitute for ^{90}Y-DOTA-EB-TATE for therapy. Dosimetric calculations revealed an increase of the tumor dose by a factor of 4 compared with the biodistribution without an albumin-binding functionality.

Similarly, Bandara et al. showed in a preclinical study that, in comparison to ^{177}Lu-DOTATATE, ^{177}Lu-DOTA-EB-TATE resulted in an increased tumor uptake over time, no unexpected accumulation, and efficient tumor control, despite similar activities (*2*).

Chen et al. applied ^{177}Lu-DOTA-EB-TATE in humans and derived an increase in the tumor dose by a factor of 8 whereas kidney and bone marrow dose increased by a factor 3.2 and 18, respectively (*3*).

We investigated the labeling and the in vitro binding characteristics of ^{68}Ga-, ^{64}Cu-, ^{177}Lu-, and ^{90}Y-DOTA-EB-TATE in SSTR-positive HEK293-sstr$_2$ cells (*4*) in comparison to unmodified DOTA-TATE conjugates. The cells were plated in 6-well plates 24 h before addition of the radiolabeled DOTA-EB-TATE and DOTA-TATE conjugates (200 kBq). Experiments were performed in 1 mL of standard cell culture medium at 37°C for 60 and 120 min. At each time point, the cells were washed with phosphate-buffered saline and lysed with 0.3 M NaOH. The uptake was determined in triplicate for each radionuclide and each time point and was expressed as a percentage of the total activity associated with 1 million cells. The labeling procedure was similar for all preparations, and the yields were more than 95%. We found for DOTA-EB-TATE that after 1 h the cellular uptake was 9.1% ± 0.69%, 2.0% ± 0.12%, 4.9% ± 0.26%, and 4.6% ± 0.21% and increased at 2 h to 15.6% ± 0.51%, 4.3% ± 0.18%, 9.0% ± 0.71%, and 8.1% ± 0.12%, respectively. In comparison, the bifunctional probes without the albumin-binding unit revealed a 1-h uptake of 23.7% ± 1.47%, 4.3% ± 0.18%, 8.7% ± 0.90%, and 7.2% ± 1.09%, which increased at 2 h to 27.3% ± 1.66%, 5.5% ± 0.36%, 12.6% ± 1.71%, and 10.2% ± 0.18%, respectively.

These data demonstrate that the modified radiotracer featured a lower initial uptake compared with the unmodified one, regardless which isotope was used. However, the incremental gain of the uptake within the second hour was comparable between both radiotracers. This demonstrates that the radioisotope strongly influences the uptake of the SSTR ligand. The highest diagnostic performance is expected from the radiopharmaceutical with the highest uptake, namely ^{68}Ga-DOTATATE.

As a consequence of the different uptake, only different isotopes of the same element (such as ^{86}Y/^{90}Y or ^{64}Cu/^{67}Cu) can be used for assessment of biokinetic data, whereas theranostic "pairs" of isotopes (such as ^{111}In/^{177}Lu) are not appropriate. No solely diagnostic isotope of lutetium is known. Therefore, the use of a low amount of radioactivity for qualitative and quantitative (e.g., dosimetry) imaging is an elegant approach that allows a subsequent therapeutic application (*3*).

DISCLOSURE

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

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Reply: Different Radionuclides in DOTA-EB-TATE Effect Different Uptake in Somatostatin Receptor–Positive HEK293 Cells

REPLY: We would like to thank Dr. Kotzerke and his colleagues for the important insights into the uptake of DOTA-EB-TATE, an albumin-binding octreotate developed by us

(*1–4*). The results presented by Dr. Kotzerke are of high importance, suggesting that the radiometal chelated into the DOTA moiety affects the uptake and perhaps binding of both DOTA-TATE and EB-DOTA-TATE. The authors concluded that "As a consequence of the different uptake, only different isotopes of the same element (such as ^{86}Y/^{90}Y or ^{64}Cu/^{67}Cu) can be used for assessment of biokinetic data."

Although the data presented by the authors are intriguing, we would like to argue that: (1) These results are not specific to EB-DOTA-TATE but are seen with DOTA-TATE as well. It is common practice to use ^{68}Ga-DOTA-TATE to detect tumor somatostatin receptor 2 expression before radionuclide therapy with ^{177}Lu-DOTA-TATE, and so far this practice seems to prove itself. Moreover, ^{68}Ga-DOTA-TATE scanning has significantly lower radiation exposure to the patient than other longer-lived isotopes labeled with the same ligand. It would be unreasonable in our opinion to use ^{86}Y for imaging when a much safer option is available (*2*). The authors derive their conclusion from in vitro cell uptake and extrapolated the result to predict the in vivo pharmacokinetics. It would be more appropriate to draw a conclusion from actual in vivo studies.

We look forward to seeing data from more in-depth in vivo studies done, perhaps, by Dr. Kotzerke and colleagues.

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Reply: Radiation Dose Does Matter: Mechanistic Insights into DNA Damage and Repair Support the Linear No-Threshold Model of Low-Dose Radiation Health Risks

TO THE EDITOR: We wish to respond to Siegel et al.'s most recent letter (*1*). In the interest of brevity, we confine our remarks to the evidence that refutes their first 2 points.

The vast majority of DNA double-strand breaks (DSBs) caused by ionizing radiation are repaired by nonhomologous end joining (NHEJ), which is an error-prone process (*2–4*). Ionizing radiation causes complex DSBs due to associated damage of the adjacent base pairs or clustering of multiple break points in the DNA backbone (*5*). Siegel et al. now suggest that "the repair fidelity of the damage produced by low-dose, low-LET (linear energy transfer) radiation associated with medical imaging ***may*** be no less than that by homologous recombination for endogenously induced damage" (emphasis added). The evidence regarding the different error rates for the various DNA repair mechanisms is critical to this discussion. DNA damage repair via homologous recombination (HR) is a high-fidelity, ***template-dependent*** repair pathway for complex DNA damage including DNA gaps, DNA DSBs, and DNA interstrand crosslinks (*6*). HR achieves this accuracy using homologous sequences found elsewhere in the genome to guide the repair process. Homologous sequences occur in sister chromatids, homologous chromosomes, or repeated regions of the same or different chromosomes.

In contrast to HR, **nonhomologous** end joining (NHEJ) leads to alterations in the underlying DNA sequence precisely because it is not template-dependent (*2*). NHEJ occurs throughout the entire cell cycle whereas HR primarily occurs during the late S and G2 phases. As a result, the vast majority of DSBs induced by ionizing radiation are repaired by NHEJ while HR is best suited to repairing DSBs that arise during DNA replication.

The importance of fidelity during in vivo DNA repair is highlighted by Behjati et al.'s analysis of DNA sequences obtained from radiation-associated second malignancies (*7*). They performed whole-genome sequencing of the tumors and compared that data with DNA sequences obtained from the same patient's normal tissues. That comparison revealed 2 mutational signatures in the radiation-associated cancers that transcended tumor type: small deletions and balanced inversions. The structural features of the small deletions and their random distribution throughout the tumor's genome indicated that radiation-induced DSBs and the subsequent error-prone repair by NHEJ were causal factors in these clinically relevant cancers.

When considering the evidence about whether mutations caused by ionizing radiation can cause clinically relevant cancers, Siegel et al. argue that "***only*** epidemiologic studies . . . can decide the issue" (emphasis added). We disagree with this complete reliance on epidemiologic studies. Instead we suggest that data from both epidemiologic and mechanistic studies must be considered together if one wishes to elucidate the responsible causal chain.

We agree with Siegel et al. that readers are faced with a choice between 2 divergent viewpoints. Some readers might be comforted by the argument that exposure to the ionizing radiation used for medical imaging not only is harmless but also actually prevents cancer. However, the available evidence indicates that medical imaging is a double-edged sword. When properly used, medical imaging provides immense benefits. But like any tool, it can be overused and overuse of medical imaging carries risks.

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