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EDITORIAL COMMENTARY

Cancer theranostics with ^{64}Cu/^{177}Lu-loaded liposomes

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Personalized medicine has come to the forefront of cancer research and patient management in recent years. Among the many advances, nanomaterials stand to make significant impact in both the preclinical and clinical settings. Tumor-specific nanoconstructs should ideally allow for rapid concentration of imaging and/or therapeutic agents at the target site, and clear from the body within a reasonable period, thereby minimizing normal tissue toxicities. This idealized scenario is not yet a reality. Many nanoparticles can provide fantastic imaging and therapeutic capabilities in preclinical models, but their clinical applications to date have been limited by suboptimal pharmacokinetic and excretion profiles, systemic accumulation, and potential long-term toxicity [1, 2].

Among the myriad of nanocarriers proposed, liposomes are perhaps the most extensively investigated and have found many uses in the clinic [3]. Liposomes, which are closed bilayer phospholipid systems, have long been recognized as efficient drug delivery vehicles [4], with the first liposomal constructs being described in the 1960s [5, 6]. Applications in metabolic disorders, diabetes, fungal infections, immunological diseases, cancer, etc. have been explored for liposomal drug delivery. Many liposomal drug systems have garnered regulatory approval in various countries, with a large number of others currently in various stages of clinical trials [7]. The use of liposomes as imaging agents has subsequently gained momentum as the drug delivery applications have expanded. Their uses for ultrasonic [8], fluorescence [9], magnetic resonance [10], and PET/SPECT imaging [11] have provided invaluable insights into the pharmacokinetics of these systems.

In this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, Petersen et al. evaluated the *in vivo* pharmacokinetics and dosimetry of PEGylated (i.e. polyethylene glycol-coated) liposomes for diagnostic (^{64}Cu-liposomes) and radiotherapeutic (^{177}Lu-liposomes) applications in neuroendocrine H727 tumor xenografts [12]. PEGylation of liposomes can be a double-edged sword, whereby too little PEG leads to suboptimal *in vivo* stability and too much PEG can result in poor tumor uptake and accelerated blood clearance (the ABC effect) [13]. As such, the "PEG dilemma" has been a topic of intense research. In this study, the authors attempted to determine an optimal formulation for liposomes by using two different PEG concentrations (5 mol% and 10 mol%) and tracking the *in vivo* biodistribution and tumor accumulation of ^{64}Cu-labeled liposomes via positron emission tomography (PET) imaging.

Remote loading of radioisotopes into the liposomes has been found to be more stable than surface-labeling techniques, as the radioisotopes are encapsulated within the liposomes [14]. Additionally, this technique can be easily customized for any isotope-chelator combination, thereby bypassing the need for tedious and specific conjugation chemistry. As expected, liposomes with lower PEG density (5 mol%) were found to have a shorter blood circulation half-life and a more sizable initial clearance than the 10 mol% PEG liposomes. The longer circulation time of the 10 mol% PEG liposomes

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afforded higher tumor accumulation, which were selected for further therapeutic studies.

The authors presented a dosimetric study of these 10 mol% PEG liposomes, giving estimated human radiation doses for both ^{64}Cu-liposomes and ^{177}Lu-liposomes. Using the biodistribution of ^{64}Cu-liposomes as a surrogate for the therapeutic ^{177}Lu-liposomes, absorbed doses of 22.8 Gy for a small (2 g) tumor or 2.3 Gy for a large (20 g) tumor were calculated based on a 200 MBq injection in humans. Currently, targeted radiotherapy using ^{177}Lu-DOTA-TOC or ^{177}Lu-DOTA-TATE is a last line of defense in the treatment of neuroendocrine tumors (NETs) at many treatment centers [15, 16]. With the increase in tumor accumulation of ^{177}Lu that may result from the use of liposomes, this form of therapy may find clinical use in the future. However, it should be noted that less favorable tumor-to-muscle (T/M) ratios were obtained with the 10 mol% systems when compared to 5 mol% PEG liposomes, indicating that the latter may be a more viable option from a diagnostic and safety point of view.

Before clinical use of these liposomal constructs for internal radiotherapy applications, their potential normal tissue toxicity needs to be thoroughly studied. In this work, relatively high spleen, liver, and stomach wall doses were reported (2.54×10^{-2}, 2.14×10^{-2}, and 3.25×10^{-2} mSv/MBq, respectively, for the 10 mol% PEGylated ^{177}Lu-liposomes), providing a contraindication for direct clinical application of these constructs. While the longer circulation half-life is indeed beneficial for obtaining a higher tumor accumulation, it also allows for more radiation dose to be deposited in normal tissues. The clearance mechanisms of these liposomes also need to be considered in the determination of normal tissue doses, as with all injected agents. It is worthwhile to note that, while there are toxicity concerns with radiolabeled liposomes, they are still generally considered to be less harmful than many other nanoplatforms. In addition, the ample clinical data on the use of liposomes can significantly facilitate clinical translation of liposome-based agents [4].

A few other aspects that are related to this exciting report may merit further research. As apparent from the transmission electron micrographs and broad elution peaks in size exclusion chromatography, the product is not a single homogeneous species, which warrants further optimization of the synthetic process. In addition, full characterization of the stability of the radiolabel, as well as integrity of the nanoconstruct, is essential for *in vivo* imaging. If ^{64}Cu is trans-chelated, it will bind to serum proteins *in vivo*, which will be indistinguishable from the ^{64}Cu-liposomes in a PET image. The appreciable kidney uptake (4–8 %ID/g) at all time points in this work can perhaps be attributed to such trans-chelation phenomenon or disassembly of the liposomes. For radiotherapeutic applications, the implications are even stronger as it may lead to higher radiation doses to the kidneys and cause renal toxicity. Another interesting finding is that typically higher PEGylation levels correspond to lower liver uptake of the nanoparticles, whereas the opposite was observed in this study. The reasons behind this phenomenon should be elucidated prior to future clinical translation.

Several other factors also need to be investigated to fully determine the therapeutic efficacy of ^{177}Lu-liposomes. The residence time in the tumor and the amount of ^{177}Lu loaded into the liposomes will factor into the radiation dose delivered to the tumor. From a radiobiological point of view, this will greatly impact the success or failure of such treatments. Furthermore, the authors noted an extremely heterogeneous distribution within larger tumors, with the ^{64}Cu-liposomes mainly concentrated around the outer edge. This is not ideal for treatment scenarios, where the dose distribution within the tumor tissue needs to be uniform for a maximum therapeutic effect. The beta particle emitted from ^{177}Lu has a practical range of under 2 mm in water, making it a good choice for preclinical studies. However, radionuclides with a longer range may be more practical for clinical applications, especially if uptake heterogeneity remains an issue. Since DOTA is a universal chelator that can be used to complex many other radioisotopes such as ^{90}Y, the same system could be easily adopted for remote loading of ^{90}Y if needed.

This team of investigators has previously studied tumor-targeted liposomes, using the somatostatin peptide analog octreotate (TATE) as the targeting ligand, and concluded that "no absolute benefit in tumor accumulation over the non-targeted liposomes was found" [17]. However, the higher T/M ratios observed with TATE-conjugated liposomes indicated potential advantages of active targeting with these nanosystems. NETs lend themselves naturally to somatostatin receptor targeting; however, for nanomedicine, other strategies such as vasculature targeting may be more beneficial and deserve to be investigated in the future [18]. In addition, liposomes directed against multiple targets may allow for increased tumor accumulation and enhanced therapeutic efficacy.

Delivery systems such as liposomes hold great promise in the pursuit of personalized medicine. By encapsulating drugs or other therapeutic agents and allowing higher accumulation in targeted tissues with (ideally) reduced normal tissue toxicity, these systems can potentially lead to increased therapeutic indexes and make a real clinical impact in diseases such as NETs and other cancer types. Pre-treatment screening with agents such as ^{64}Cu-liposomes will enable physicians to determine whether a patient is a suitable candidate for ^{177}Lu-based therapy, via non-invasive PET imaging to evaluate whole-body distribution of the less harmful formulation (i.e., ^{64}Cu-liposomes) before administering tumoricidal doses of ^{177}Lu-liposomes. The investigators in this team have a long and successful history of clinical translation of novel radiopharmaceuticals in various cancer types such as NETs [19, 20]. Their expertise, combined with the many other advances

in this field, will allow liposome-based theranostic agents to make a significantly greater impact in the clinic. We look forward to exciting future clinical results from this multidisciplinary team.

Compliance with ethical standards

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