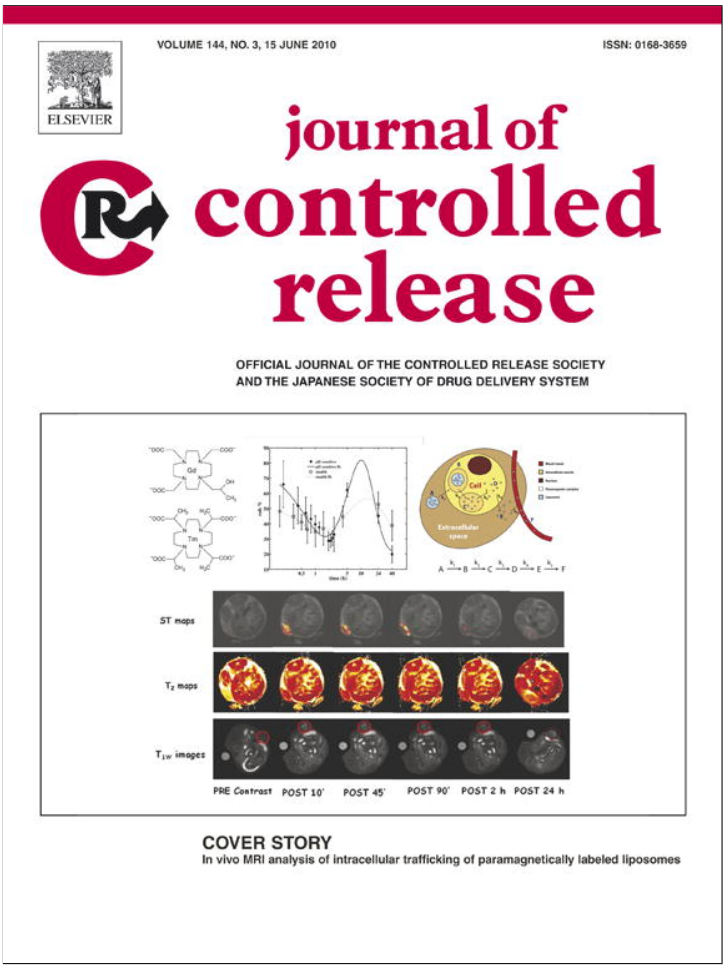

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Cover Story

In vivo MRI analysis of intracellular trafficking of paramagnetically labeled liposomes

Many nanocarriers have been explored in drug delivery, and the liposome formulation has been used frequently in part due to its ability to load both hydrophilic and lipophilic molecules. In spite of the long history, extensive development, and proven therapeutic efficacy of liposomes, their *in vivo* behavior is still poorly understood. Improved knowledge on the *in vivo* behavior of liposomes is expected to result in better formulations not only for drug delivery but also for imaging-guided therapy, which is a new emerging field of theragnosis (therapy and diagnosis). Such an understanding will undoubtedly provide useful insights for designing more efficient carriers endowed with high specificity and a better control of undesired side effects.

Liposomes have recently received a great attention in magnetic resonance imaging (MRI). Loading the vesicles with paramagnetic metal complexes resulted in new classes of contrast agents that are under intense development, because they can overcome the intrinsic low sensitivity of MRI [1]. Even if *in vivo* MRI has distinctive advantages over the competitors in terms of attainable anatomical resolution, the resolution has not reached the microscopic visualization of cellular and subcellular events. Furthermore, the translation from *in vitro*/*ex vivo* results to modeling the *in vivo* behavior of liposomal nanocarriers is not fully reliable because of the high complexity of the biological system. In this issue, Professor Silvio Aime and his group report a "smart" approach to obtain indirect evidence of *in vivo* cellular trafficking through monitoring the time evolution of the different contrast modalities (T_1, T_2 and chemical exchange saturation transfer (CEST)) of paramagnetically labeled liposomes [2].

The study utilized two types of liposomes loaded with either Gd-HPDO3A complex acting as T_1 and T_2 MRI contrast agents or $[Tm\text{-}DOTMA]^-$ simultaneously acting as T_2 and CEST agents. The bases for the approach taken by the authors are: (i) the maximum of T_1 contrast enhancement occurs when the nanovesicles lose their integrity to release their content; (ii) the maximum T_2 contrast enhancement occurs when the nanovesicles are intact; and (iii) the maximum CEST contrast is observed when intact liposomes are free in the extracellular fluids. In addition to qualitative description of the events that are taking place at the cellular level, the authors provide an attempt to describe quantitatively the kinetics of the entire process of cellular trafficking. The simultaneous kinetic analysis of the MRI responses (T_1 and T_2 and CEST generated contrasts) allowed elucidation of the *in vivo* cellular uptake and the intracellular behavior of the liposomes. In their kinetic model, the overall process was modeled using five consecutive kinetic steps corresponding to recruiting and cellular uptake, degradation or disassembling of liposomes, cytosolic release of imaging probes, cellular efflux of the probes, and washout of the probe from the tumor region to the blood stream.

This work demonstrates that, even though the MRI technique does not achieve the necessary resolution for visualizing the intratumor trafficking of these nanovesicles at the subcellular level, a kinetic model that simultaneously fits all the multicontrast dataset provides important insights into cellular trafficking and degradation of nanovesicles. When the liposomal membrane breaks down, the liposome payload is freed and the observed T_1 contrast increases. On the other hand, the CEST contrast generated from lipoCEST agents can be detected only if the nanovesicles retain their integrity. Once the paramagnetic payload is released, there is no more intraliposomal water resonance to irradiate and, consequently, the CEST contrast disappears. The ability of paramagnetic liposomes to generate a specific T_2 contribution mainly arises from the intravesicular compartmentalization of paramagnetic complexes. Hence, the T_2 shortening basically is directly correlated to the intraliposomal concentration of the paramagnetic complex, to the size of the vesicle, and to the effective magnetic moment of the metal ion in the complex. This kinetic analysis approach has made it possible to compare different evolution of the MRI responses for a pH-sensitive liposome in comparison with a control system. The release of the imaging probe from the liposomes to the endosomes and then to cytosol resulted markedly faster in the case of the pH-sensitive systems. This result fully agrees with the expectations as these nanocarriers were designed to release their contents in a pH range (5.5–6.0) that is typical of endosomes, whereas (pH- or enzyme-mediated) degradation of standard liposomes occurs mainly in lysosomes. Gd-HPDO3A complex was also used to examine the drug release from a temperature-sensitive liposome [3], and it will be interesting to test its intracellular trafficking using the kinetic analysis approach.

This study by Professor Aime is elegant in many ways. Most of all, the authors overcame the limitations in cellular/subcellular resolutions of MRI imaging by using simultaneous kinetic analysis of the MRI responses using two paramagnetic probes. The biggest advantage of this approach is that the cellular and subcellular trafficking of liposomes can be analyzed *in vivo* without isolation of the cells, and this is a great advantage over other invasive techniques. In addition, the liposomal formulation allows delivery of a drug at the same time, and thus this is another ideal approach for imaging-guided therapy. The method described by Professor Aime in this issue is expected to provide a new approach of improving the drug delivery/release processes, as the effect of formulation on *in vivo* behavior can be easily studied using the kinetic analysis model.

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