Mechanical release from paramagnetic liposomes triggered by low frequency ultrasound

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Introduction

Non, or minimally, invasive *in vivo* visualization of drug release triggered by externally-applied physical stimuli, like heating, light or ultrasound (US) is an emerging topic in molecular medicine. As far as US is concerned, most of the published investigations were performed using nano- or micro-bubbles in which the presence of the gas-filled core makes cavitation (and US detection) possible. In addition to the intrinsic potential of US as diagnostic tool, there is a great interest to combine the peculiar properties of acoustic waves with other imaging modalities, especially MRI, as witnessed by the development of the high intensity focused ultrasound (HIFU)-MRI technology.

On the other side, vesicular nanosystems like liposomes are successfully used in clinic as drug delivery nanocarriers and they are also under intense scrutiny in the emerging field of theranostic MRI agents where they represent one of the most promising nanoplatform. On this basis, it is of great relevance to assess the feasibility of using ultrasound as triggering tool to induce release from water-filled liposomes. Instead of using the heat delivered by high intensity US as releasing stimulus, the motivation of this study was to exploit the mechanical forces associated with the acoustic pressure. As the mechanical index of US is inversely related to the sonofrequency, low frequency low intense US were considered.

Methods

The US apparatus, external to the MRI scanner, consisted of a single non-focused US transducer operating, in continuous or in pulsed modes, at 27.6 kHz or 3 MHz (acoustic intensity about 2.5 W/cm^2). Liposomes with different bilayer formulations encapsulating 300 mM solution of the clinically approved MRI agent ProHance™ were prepared using the conventional thin lipidic film method. A given volume of the liposomes suspension was put in a latex spherical container soaked in a water bath endowed with phono-absorbent walls. The release of the paramagnetic agent was monitored by relaxometric measurements performed at 0.47 T on a Stelar Spinmaster instrument. MRI experiments were carried out at 7 T on a Bruker Avance300 equipped with a microimaging 2.5 Micro probe. In vivo experiments were performed by injecting liposomes directly in a xenografted melanoma B16 tumor on mice. The animals were insonated using the same apparatus described above.

Results and Discussion

A release of the encapsulated paramagnetic probe was observed after 3 min continuous US exposure either at 27.6 kHz or at 3 MHz, with a four-fold increase at the lower frequency. The release was not induced by heating as demonstrated by the very small temperature increase of the sample. As expected, the release was directly correlated with the insonation time. Interestingly, passing to the pulsed mode, the release was extremely sensitive to the duration of the 'on' phase of the US cycle (fixing the duty cycle to 50 %), and, even more important, to the bilayer composition of the nanovesicles (Figure 1). In addition, the release profiles were also affected by the composition of the liposomal cavity, vesicle shape and size. Furthermore, it has been demonstrated that the release occurred only during the US exposure, thus allowing a real control of the process. After insonation, the size of vesicles was not significantly affected, thus suggesting that the release mechanism was likely due to the formation of transient pores in the bilayer generated by the oscillating vesicles exposed to the acoustic pressure.

As preliminary proof-of-concept of the *in vivo* potential of these findings, paramagnetic liposomes were injected into a melanoma B16 tumor xenografted on mice and MRI scans of the lesion were acquired before and after the pulsed US exposure. Figure 2 clearly shows the remarkable brightening of the tumor region consequent to the US-triggered release of the paramagnetic agent.

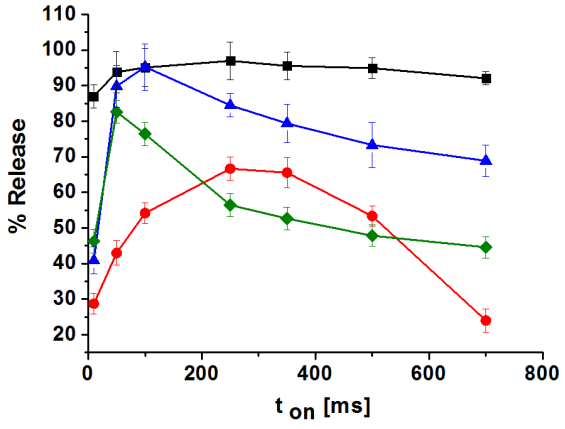

Figure 1. Release profiles as a function of the pulse time (ton) for different stealth (DSPE-PEG2000) liposomes: **DSPE** entrapping 300 mM ProHance™ (black), **DPPC** entrapping 300 mM ProHance™ (blue), **DPPC** entrapping 50 mM ProHance™ (*spherical*: olive, *non-spherical*: red). Total insonation time 3 min, duty cycle 50 %)

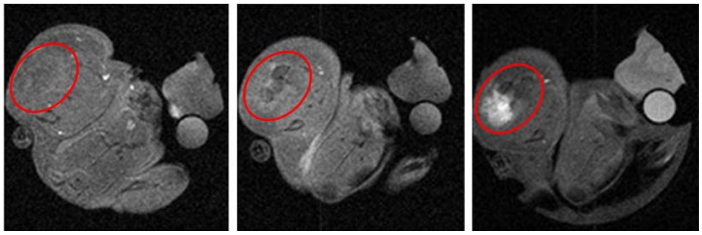

Figure 2. 7 T MRI T_{1w} images of a mouse bearing a melanoma B16 tumor (red circle) before the local injection of 10 mL of DSPC-based stealth liposomes entrapping 300 mM ProHance (left), after the injection and before insonation (middle), and after insonation (right, total insonation time 3 min, duty cycle 50 %, t_{on} 100 ms)

The opportunity to selectively trigger the release of imaging reporters, as well as drugs, from a mixture of different nanocarriers could open new and intriguing therapeutic schemes to improve the overall efficacy of the pharmacological treatment.