

MRI visualization of intratumor release/distribution of liposomal drugs triggered by sonication/sonoporation

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Introduction

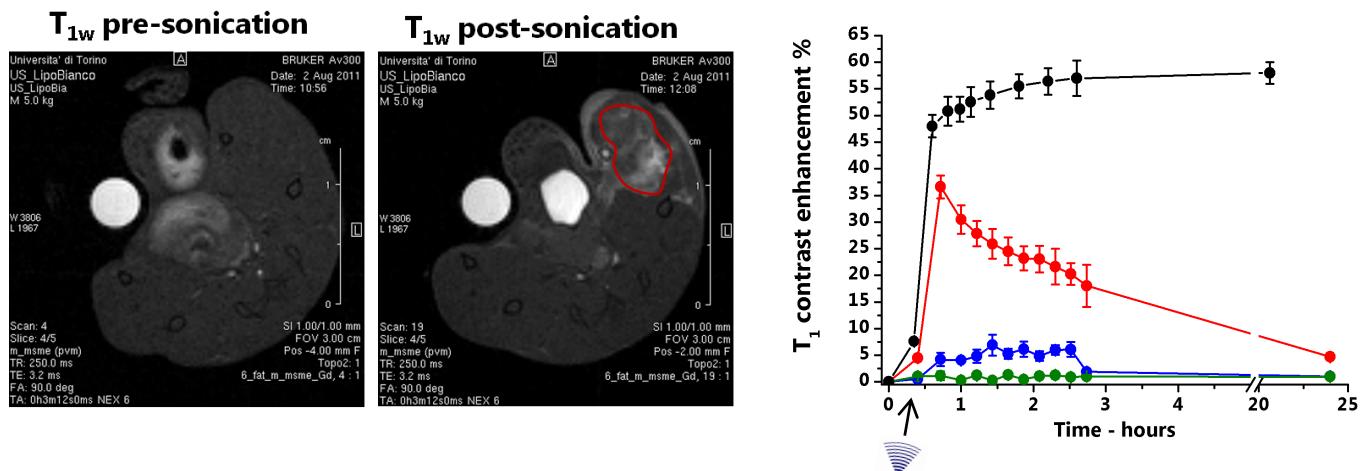
The successful use of liposomal drugs as chemotherapeutics primarily resides in the positive combination between the drastic reduction of the side-effects of the drug and the passive accumulation of the nanomedicine in the tumor *via* the EPR effect. However, there is a general consensus about the view that liposomes, once extravasated in the lesion, are rapidly taken up by tumor-associated macrophages, and, furthermore, their ability to diffuse in the tumor mass is severely compromised by both their large size and the high interstitial pressure in the tumor. Therefore, the development of innovative therapeutic strategies where the liposomal drug can be released in the extracellular portion of the lesion could have a beneficial effect on the therapeutic outcome. A possible approach for achieving this aim is to exploit the ability of low intensity US to induce a non-thermal release of the drug from the nanodrug. In addition, a different US exposure (at different frequency and intensity) may be used in combination with the previous one for inducing a local permeabilization of the cell membranes in the lesion (sonoporation) with the objective to facilitate the intratumor diffusion of the drug and allow its localization in most of the cell phenotypes that characterize the pathology (tumor cells, immuno-cells, cancer stem cells,...). We used MRI to provide the necessary non-invasive guide for assessing the drug distribution (as well as the tumor growth) through the use of liposomes encapsulating the clinically approved paramagnetic complex Gadoteridol.

Methods

The US apparatus consisted of two non-focused US transducers operating at 1 MHz (for sonoporation; pulsed application, time 1 s, variable duty cycle 5-15%, 18 V, intensity 1.6 W/cm², acoustic pressure 0.16 MPa) and 3 MHz (for drug release; pulsed application, time 2 s, duty cycle 50%, 30 V, intensity 3.3 W/cm², acoustic pressure 0.24 MPa). Conventional stealth liposomes (DPPC/HSPC/Cholesterol/DSPE-PEG2000 55/25/15/5 molar ratio) encapsulating 300 mM of Gadoteridol were prepared using the thin lipidic film method. MRI experiments were carried out at 7 T on a Bruker Avance 300 spectrometer equipped with a microimaging 2.5 Micro probe. *In vivo* experiments were carried out on a syngeneic tumor model in mice prepared through the subcutaneous inoculation of 1 million of TSA (mammary adenocarcinoma) cells in the mouse flank. The animals entered in the experiment 1 week after the inoculation when the tumor average size was ca. 100 mm³. Sonoporation was performed just before the i.v. injection of the liposomes. Sonication was applied 15' after the liposome injection.

Results and Discussion

The local sonication of the tumor applied between 5 and 30 minutes after the i.v. injection of the paramagnetic liposomes led to a local T₁ contrast enhancement of ca. 30-35 % due to the release of the contrast agent from the liposomes that are circulating in the tumor vasculature (red trace in the graph below). Such a release-mediated enhancement is caused by the removal of the relaxivity quenching generated by the low water permeability of the liposome membrane. Control experiments conducted without sonication (blue trace) did not show any significant contrast enhancement in the tumor as well as analogous experiments performed with sonication, but injecting liposomes without the encapsulated MRI agent (green trace). In addition, a clear contrast enhancement was detected in the kidneys few minutes after sonication, as expected in case of intratumor release of the hydrophilic contrast agent. Very interestingly, if a short sonoporation pulse was applied just before the liposomes injection, followed by the same sonication exposure used previously, the contrast enhancement increased, but more important, it lasted for a much longer time and remained almost constant until 24 h post-injection (black trace). Experiments carried out combining sonoporation and sonication after injecting a mixture of non-liposomal Gadoteridol and non-paramagnetic liposomes showed again a quite high contrast enhancement in the tumor 6 h post-injection, whereas the contrast vanished rapidly in the absence of the sonoporation exposure. Likely, the long-term contrast observed when sonoporation was applied can be justified by the intracellular (probably cytosolic) entrapment of the contrast agent in the tumor cells.



Conclusion

The data presented indicate that the application of pulsed low-intensity non-focused US can trigger the release of the contrast agent from the liposomes that is signaled by a T₁ contrast enhancement. The application of an acoustic radiation able to induce sonoporation can facilitate the diffusion of the released agent in the tumor mass and, furthermore, it promotes an intracellular entrapment of the agent caused by the cell membrane permeabilization. These results demonstrate the high therapeutic potential of this approach.