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Short Communication

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In vivo MRI visualization of release from liposomes triggered by local application of pulsed low-intensity non-focused ultrasound

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Abstract

The work aimed at developing a MRI-guided protocol for the visualization of the release of material entrapped in liposomes stimulated by the local application of pulsed low-intensity non-focused ultrasound (pLINFU). The task was achieved by formulating liposomes filled up with the clinically approved paramagnetic agent gadoteridol, because the release of the agent from the nanovesicles is accompanied by a significant MRI signal enhancement. The protocol was validated in vivo on mice-bearing subcutaneous syngeneic B16 melanoma and i.v. injected with the paramagnetic liposomes. Upon exposing tumor to pLINFU (3 MHz, insonation time 2 min, duty cycle 50%) few minutes after liposomes injection, a signal enhancement of ca. 35% was detected. The effective release of the agent was confirmed by the strong enhancement measured in kidneys calyx and bladder due to the rapid renal excretion of the agent released in the tumor.

From the Clinical Editor: In this paper, a pulsed low-intensity non-focused ultrasound-based technique was used to release a paramagnetic MRI contrast agent from liposomes, demonstrating the feasibility of this triggered release system in a mouse melanoma model for future research applications.

Key words: MRI; Drug release; Theranosis; Liposomes; Ultrasound

The use of nanocarriers in cancer chemotherapy is primarily driven by the need of optimizing the therapeutic index of a given drug. Typically, nanomedicines have extravascular targets and, therefore, the success of the pharmacological treatment is dependent on both the amount of nanocarrier that accumulates in the diseased region and the ability of the carrier to release the bioactive cargo.

Despite the clinical success of some nanomedicines (e.g. Doxyl®), the use of nanoparticles as delivery system is nowadays debated because it has been reported that many nanosystems are not able to efficiently distribute in the tumor, allowing a tissue penetration of a couple of cell layers only.[1–3] In addition, drug release is usually slow and the concentration of the therapeutic may not reach the required threshold for exerting its activity.[4]

The necessity to improve the intratumor drug availability has stimulated the search for novel delivery schemes. A smart approach is to trigger the release of the drug when the carrier is still circulating in the tumor vasculature. A representative example is ThermoDox®, currently in clinical phase II/III,[5] where the release of the anticancer drug doxorubicin from thermosensitive liposomes is induced by a local heating of the tumor.[6]

Among the external trigger stimuli, ultrasound (US) certainly plays a relevant role. In addition to the use of high-intensity focused US (HIFU) as heating source to induce a local drug release from thermosensitive carriers,[7] also low-intensity non-focused US (LINFU) has been considered in virtue of their ability to promote a mechanical, instead of thermal, release.[8,9] The therapeutic benefits of this approach have been already demonstrated at a preclinical level on some experimental tumor models.[10–12]

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We have recently demonstrated that the application of pulsed LINFU (pLINFU) induces a mechanical release that is strongly influenced even by subtle changes in the physico-chemical characteristics (bilayer composition, content, shape) of the liposomes,[13] thereby offering interesting opportunities in the field of combination therapy.

Monitoring the drug release in vivo by an imaging modality is of paramount importance because it provides a visual evidence of the efficiency of the release process, ideally allowing the quantification of the released drug. Magnetic resonance imaging (MRI) is by far the technique of election to achieve this scope, primarily for its excellent spatio-temporal resolution and quantification potential.[7]

Here, we report the first in vivo MRI study aimed at visualizing the intratumor release of the liposomal content induced by the local application of pLINFU.

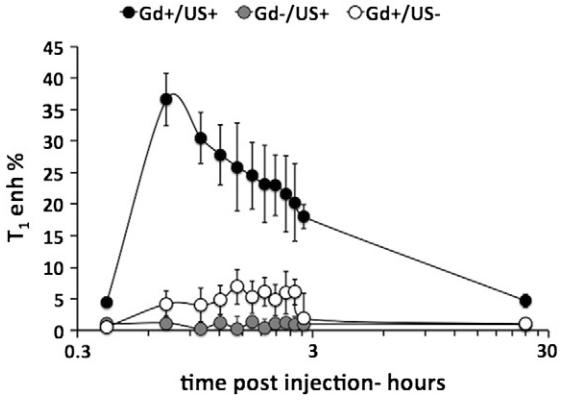

Figure 1. Temporal evolution of the T_1-contrast enhancement measured in the tumor. pLINFU was applied at time = 0.

Methods

Phospholipids were purchased from Avanti Polar Inc. Gadoteridol was kindly provided by Bracco Imaging. All the other chemicals were purchased from Sigma.

Liposomes were prepared as reported in the Supplementary Material. The mean hydrodynamic diameter of the liposomes was 150 nm (PDI 0.1). The concentration of gadoteridol in the liposomal suspension was 38 mM.

pLINFU apparatus and insonation setup are described in the Supplementary Material.

Animal studies were performed according to the national regulations and were approved by the local ethical committee. The preparation of the animal model is described in the Supplementary Material.

In vivo MRI experiments were carried out at 7 T (Bruker Avance 300). Two hundred microliters of the liposome suspension (gadoteridol dose 0.3 mmol/kg bw) was injected in the tail vein. Mice were divided in three groups (three mice each): group A (mice receiving Gd-liposomes and subjected to pLINFU, Gd+/US+), group B (mice receiving Gd-liposomes and not insonated, Gd+/US−), and group C (mice receiving control liposomes and subjected to US, Gd−/US+). 20 minutes post-injection, mice of groups A and C were insonated for 2 minutes (duty cycle 50%, ON period 0.5 sec). MRI T_{2w} and T_{1w} images were acquired before the liposomes injection (pre-contrast), after the insonation (for 2 hours) and after 24 hours. T_1 contrast enhancement was measured over the whole tumor (rim and core) and not only in the enhanced area (details of MRI acquisition and processing in the Supplementary Material).

Results and discussion

The MRI visualization of the release of material from nanovesicles can be performed entrapping in the carrier a high amount of a hydrophilic MRI agent (e.g. the clinically approved agent gadoteridol). In fact, when the agent is encapsulated in the liposome, the associated T_1 contrast is limited by the "quenching" effect caused by the low water diffusivity across the liposome bilayer.[14,15] Hence, when the material is released, the "quenching" is removed and the contrast lights on.[7]

Figure 1 shows the temporal evolution of T_1 contrast enhancement in the tumor for the three animal groups enrolled in the study. The local application of pLINFU generated a high contrast for the mice group A, thereby supporting the successful intratumor release of the agent.

Evidence about the effective release of the agent was gained by the detection of the T_1 contrast enhancement in kidneys (calyx) and bladder (Figure 2). As gadoteridol has a rapid renal excretion ($t_{1/2}$ of ca. 3 hours in mice),[16] the higher contrast observed in both organs for the Gd+/US+ group supports the remote release of the agent.

The not negligible contrast observed in such organs for the Gd+/US− group may be likely accounted for: i) a small release of Gadoteridol due to the interaction of the injected liposomes with the circulating immune cells (early contrast), and ii) a long-term instability of the liposomes due to their degradation in macrophages-rich organs (late contrast).[17]

The image reported on the right of Figure 3 clearly highlights the image guidance of the release process. Furthermore, the very bright signal arising from the bladder of the insonated mouse is a clear indirect demonstration of the successful pLINFU-triggered release.

Contrarily to what observed in kidneys and bladder, the enhancement measured in the liver and spleen was higher for the non-insonated Gd+/US− group (Figure 4). Likely, this is the consequence of the diminished amount of circulating liposomes in the animals exposed to pLINFU. Interestingly, the early contrast detected in the spleen was negative. As liposomes encapsulating a high amount of paramagnetic chelates can act as T_2 agents,[18,19] the negative T_1 contrast can be the result of a signal loss caused by the T_2 shortening.

In conclusion, the results presented in this communication highlight, for the first time, the role of MRI to provide an in vivo image guidance of the release of the liposomal content induced by the local application of pLINFU. However, it is worth noting that this imaging technology is not intended for following the biodistribution of the released drug (unless it has the same or very similar physico-chemical properties of the imaging agent), but, instead, it offers a valuable in vivo support to monitor the

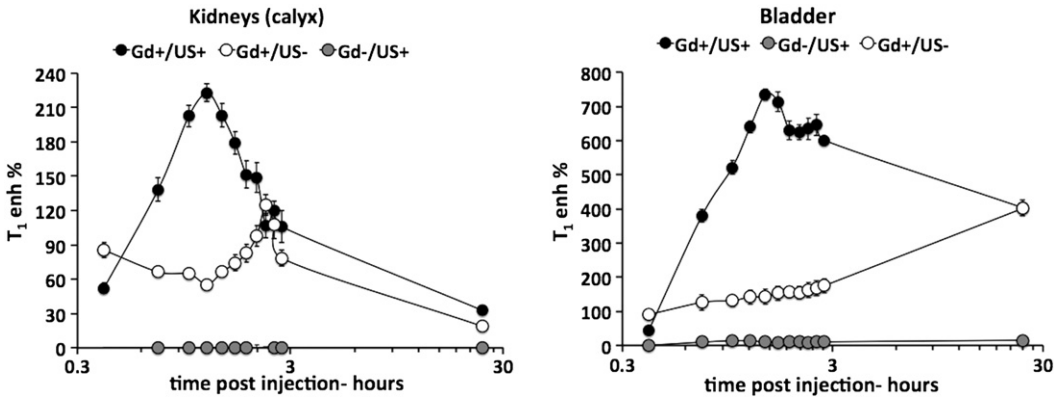

Figure 2. Temporal evolution of the T_1-contrast enhancement in kidneys calyx (left) and bladder (right). pLINFU was applied at time = 0.

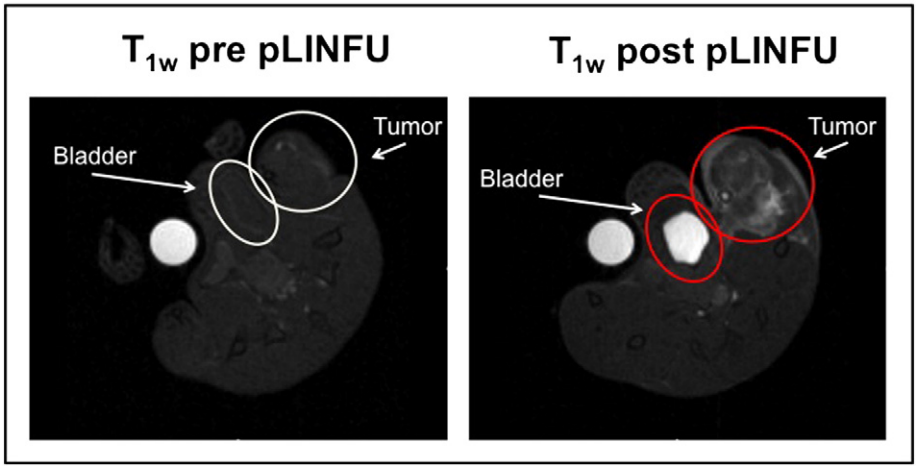

Figure 3. T_{1w}-MR images acquired before (left) and 40 min. after pLINFU application (right). The bright circled area is the reference tube used for the normalization of the contrast enhancement.

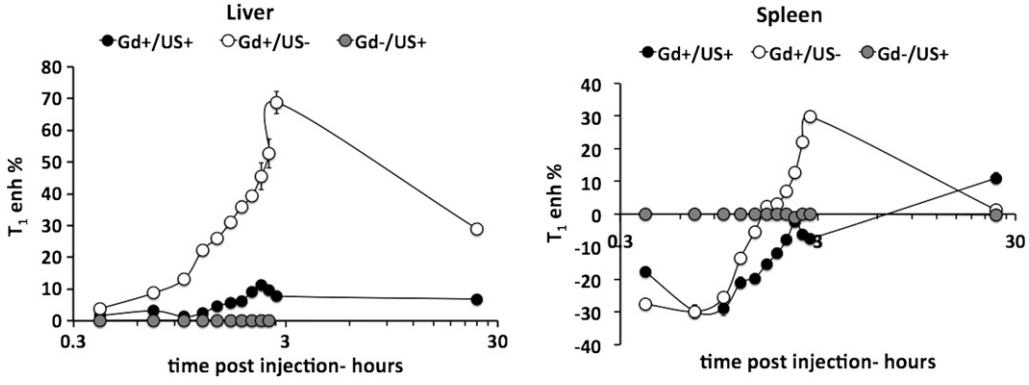

Figure 4. Temporal evolution of the T_1-contrast enhancement measured in liver (left) and spleen (right). pLINFU was applied at time = 0.

effective release of the drug. Interestingly, preliminary results indicated that gadoteridol and the anticancer drug doxorubicin displayed the same release extent in vitro after exposure to pLINFU (see Supplementary Material).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nano.2014.03.012.

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