MRI guidance of Doxorubicin release from liposomes stimulated by pulsed low intensity non-focused Ultrasound in an experimental tumor model

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Introduction: Doxorubicin is one of the most effective drugs clinically used to treat solid tumors. It has a very wide spectrum of activity but is very toxic if administered as free drug. To reduce collateral effects and improve tumor accumulation, liposomal forms of the drug have been developed and are already used in clinics (Myocet®, Doxyl®). But the ability of these nanoparticles as delivery systems to deeply diffuse in the pathological site is nowadays quite debated and several methods to promote the release of the drug at the target site have been proposed. A promising approach is to induce the release of the drug just after the drug administration when the carrier is still circulating in the tumor vasculature (May JP et al., *Expert Opin Drug Deliv.*, 2013, 10:511-27). We have recently demonstrated, both *in vitro* and *in vivo*, that the local application of pulsed low intensity non-focused ultrasound (pLINFU) can trigger a mechanical (non heat-mediated) release from liposomes(Giustetto P. et al., *Journal of Medical Imaging and Health Informatics*, Vol.3, 356-366, 2013). Moreover, if a water-soluble paramagnetic Gd-complex is co-encapsulated with the drug, MRI, for its good spatio-temporal resolution and quantification potential, can offer the valuable opportunity to visualize the release process as well as to provide a very accurate tool to follow the therapeutic outcome.

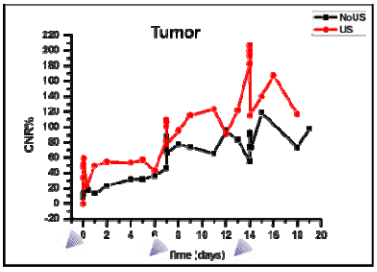
Methods: Stealth liposomes mimicking Doxyl-like formulation (DPPC, DSPC, Cholesterol and DSPE-PEG2000 methoxy 10:5:4:1 molar ratio), co-encapsulating Doxorubicin and Gadoteridol (Gd/Doxo = 1:0.092), were prepared. Liposomes were injected i.v. on a syngeneic mouse model of mammary adenocarcinoma. The theranostic drug was injected once a week for three weeks, with a drug dose of 5mg /kg bw and a Gadoteridol dose of 0.1 mmol/kg bw. The study started when tumors volume were 40-60 mm^3. Tumors were subjected to a single shot of pLINFU (frequency 3 MHz, total insonation time 2 min, duty cycle 50%), immediately after the i.v. injection, or to a sonoporation shot (frequency 1 MHz, total insonation time 1 min, duty cycle 12%) during the injection and before pLINFU exposure. MRI scans were dynamically performed in the first 90 minutes after injection, and then daily to measure both T_1 contrast-to-noise ratio (CNR) in tumor, liver, spleen, kidneys and bladder, and for monitoring tumor progression. After the treatment, mice from each group were sacrificed and the tumors were collected and fixed. 5 µm sections were stained with hematoxylin and eosin, and examined under a light microscope to analyze histological assessments. Adjacent sections were incubated with Hoescht dye and monitored at confocal microscopy.

Results: The ability of pLINFU to release both the drug and the MRI agent was tested first *in vitro*. The results revealed a very similar release profile for both the encapsulated molecules showing a Gaussian-like trend with a maximum (50 % release) reached at $t_{on=off}$ values of 0.4 s. Then, the imaging performance of the liposomal carrier was assessed *in vivo*. The CNR% values measured in the tumor for the treated and control mice groups display the presence of enhanced peaks observed for the US-group only just after the pLINFU exposure, attributed to the release of Gadoteridol (Fig.1). Moreover, a slower increase in the T_1 enhancement was observed until 4 days post-injection for both groups that can be related to the cellular fate of the MRI agent (the blood half-lifetime of Gadoteridol is ca. 3 h). The effective release of Gadoteridol was supported by the great CNR% observed in kidneys and bladder for the treated animals only (Fig.2). Importantly, the treated animals showed a significant delayed tumor growth (Fig.3). Organs were explanted at different time-points and the diffusion of the released drug was assessed using conventional histology and immunofluorescence at confocal microscopy. Ex-vivo analysis not only provided a strong support to the efficacy of the pLINFU-triggered approach, but also emphasized an active role of liposomes to induce the tumor diffusion of the drug.

With the aim of optimizing the method and improving the therapeutic efficacy, the release scheme was implemented with the addition of a sonoporation shot applied during the injection of the liposomes and before the pLINFU exposure.

Sonoporation stimulus led to an additional contrast enhancement (Fig.4) that was demonstrated to be caused by the extravasation of liposomes following the endothelium permeabilization. Interestingly, animals treated with sonoporation also showed an improved regression of the tumor growth.

Conclusions: It has been demonstrated that the local application of tumors to pLINFU can induce a drug release from liposomes encapsulating an antitumor drug and a Gd complex. MRI has a great potential for the non invasive visualization and quantification of the release process. The high clinical translatability of this image-guided therapy may pave the way to novel and more effective chemotherapeutic treatments. Furthermore, it could have a relevant role in the development of personalized medicine protocols.



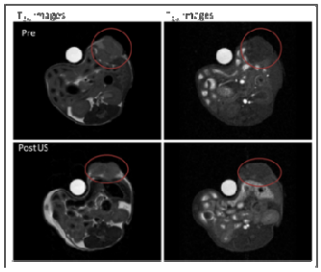
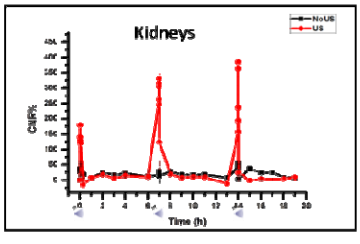


Fig.1



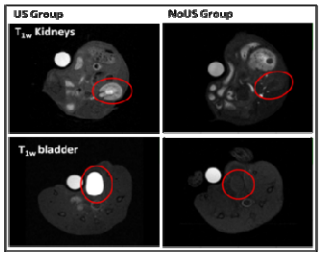


Fig.2

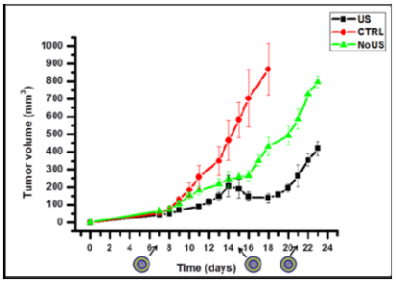


Fig.3

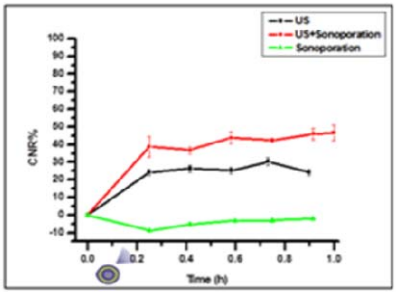


Fig.4