MRI-GUIDED ULTRASOUND-TRIGGERED DRUG DELIVERY TO OVERCOME DRUG RESISTANCE IN HUMAN OVARIAN CANCER

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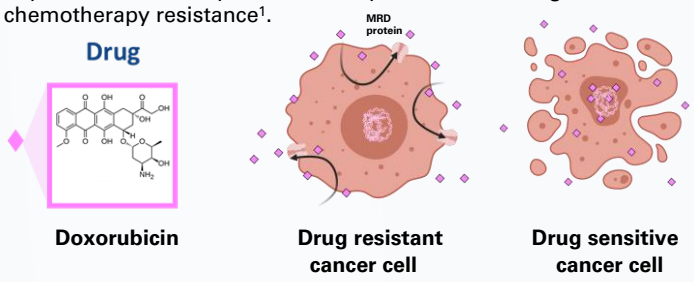
Introduction

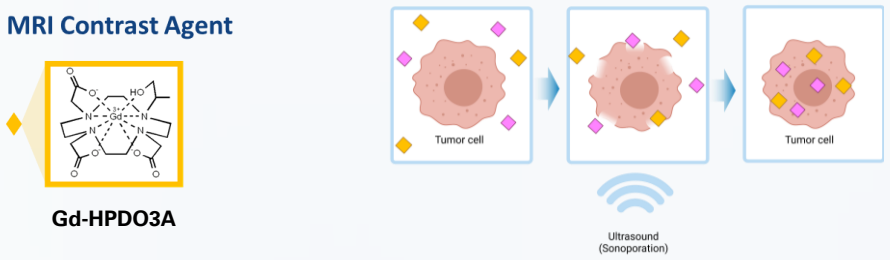
The ovarian cancer (OC) is the most fatal of all malignant tumours that affects female reproductive system. One of the most demanding challenge to improve the therapeutic efficacy is overcoming the mechanism of chemotherapy resistance[1].



The major cause of chemoresistance is the presence of MRD proteins on the cell membrane, which pump the doxorubicin out of the cell, thus preventing the reaching of its target in the nucleus.

Aim of study

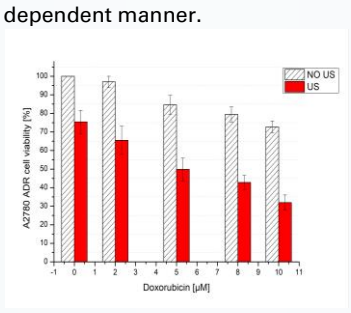
The aim of this study is to setup an MRI-guided ultrasound-based protocol to bypass OC drug resistance. A suitable process to modify temporarily the cell membrane permeability and increase the cell uptake of a given molecule is a specific ultrasound (US) application, called sonoporation (SNP). In order to monitor the treatment a clinically approved MRI contrast agent was used as MRI reporter of drug internalization.

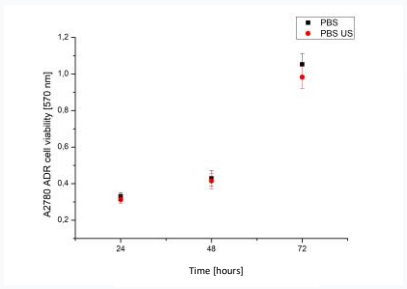


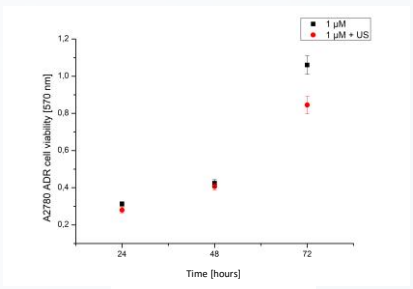
The SNP was obtained using for 1 minute low intensity unfocused pulsed US waves, produced by a 1 MHz piezoelectric transducer (Precision Acoustics, UK). The maximum efficiency was achieved varying the US parameters, as the acoustic pressure, the duty cycle and the pulsed repetition frequency.

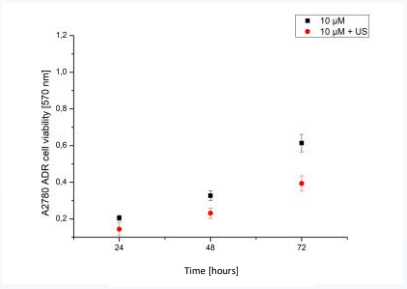
Viability and proliferation upon ultrasound treatment

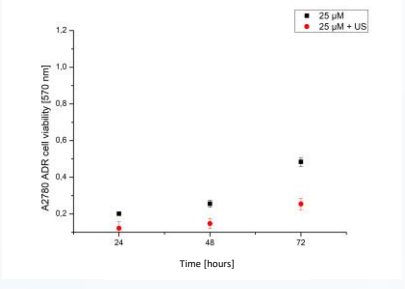
In vitro experiments were performed on human OC drug resistant to doxorubicin (A2780-ADR). They were incubated with chemotherapeutic (doxorubicin) at different concentration (2-25 μM) and the viability was assessed immediately after the ultrasound treatment by the exclusion trypan assay. The cell proliferation ability was evaluated by MTT assay at 24, 48 and 72 h. The ultrasound therapy without doxorubicin seems not affect the viability (> 75%) and the proliferation. On the other hand, the combination of doxorubicin and ultrasound seems affected the viability and proliferation in dose-dependent manner.





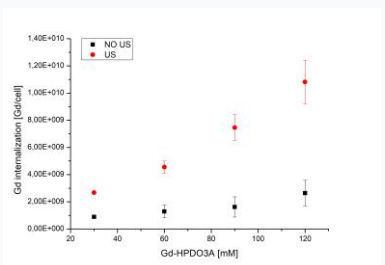


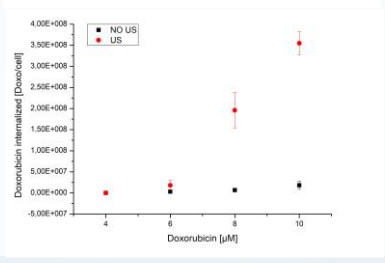




MRI contrast agent and drug internalization after ultrasound application

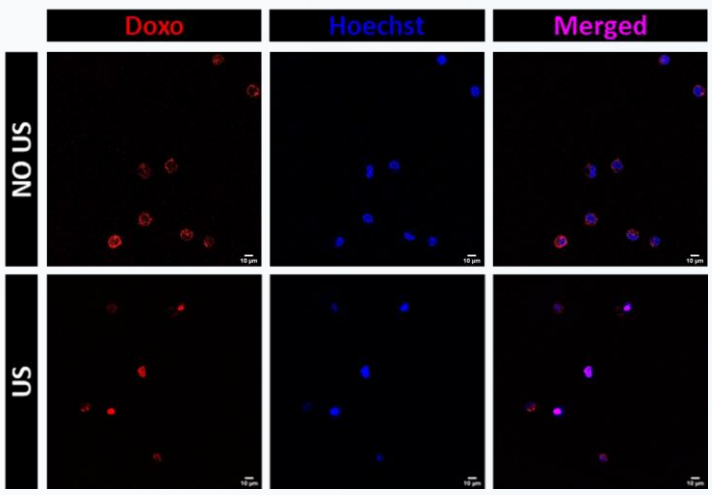
The internalization of both the molecules was linearly dependent on the concentration of incubation, these data show that the contrast agent may act as MRI reporter of the co-internalized doxorubicin.





Drug uptake imaging *in vitro*

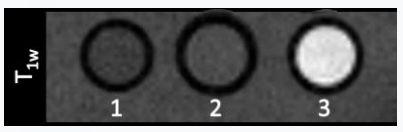
The. doxorubicin is fluorescent drug (red) widely used for the OC chemotherapy



A2780 ADR cells were incubated for 1 minute with doxorubicin (5 μM) (NO US) or incubated and immediately insonated for 1 minute (US) and washed to remove the non internalized material. The confocal microscopy images show nuclei in blue and doxorubicin in red. In NO US the drug seems to be around the cell nucleus. In US the drug is localized in the cell nucleus (pink).

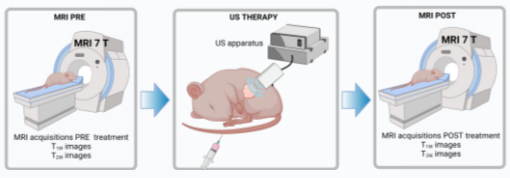
MRI agent uptake imaging *in vitro*

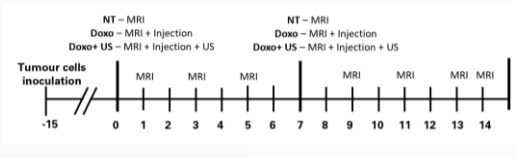
A2780 ADR were incubated with PBS (1) Gd-HPDO3A (90mM) for 1 minute (2) and incubated and immediately insonated for 1 minute (3). Then, they were washed and T_{1w} images of pellets were acquired using MRI. SNP caused the cytosolic localization of the MRI agent, thus explaining the high capability to generate T_1 contrast. The internalization of the MRI contrast agent was confirmed relaxometry.



In vivo MRI study

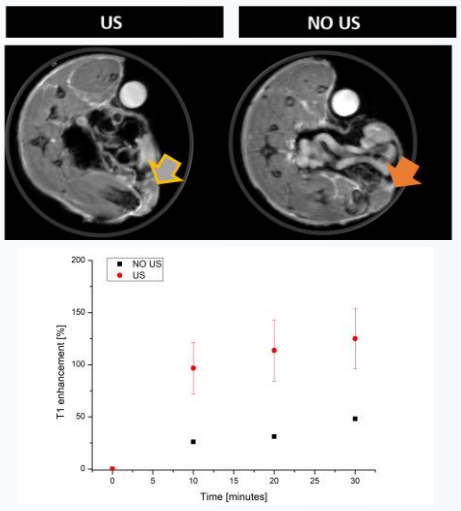
Preliminary *in vivo* experiments were carried out on xenograft mouse model of chemoresistant ovarian cancer of A2780-ADR. Three experimental In athymic nude mice groups (US n = 3, NO US n=2, NT n=3) were enrolled in the in vivo study, both NO US and US were injected with Gd-HPDO3A 0.3 mmol/Kg and doxorubicin (5mg/Kg) at day 1 and 7 and monitored for two week.





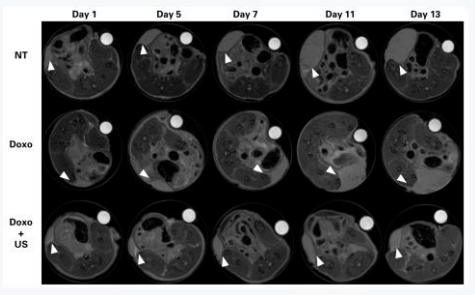
Tumor MRI contrast upon the ultrasound treatment

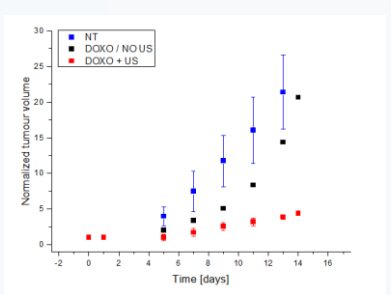
US-group was locally insonated immediately after the injection in order to induce the cell internalization of the labelling agents in the tumour. T_{1W} images were acquired before and after the insonation up to 30 minutes. A significant contrast enhancement was detected in the tumor after US stimulation



Tumor progression

The MRI-monitored tumor progression was significantly blocked by sonoporation stimulus, thus highlighting the excellent therapeutic potential of this approach, which removed the resistance of the tumor towards doxorubicin.





Conclusion

The SNP-mediated cell uptake efficiency of doxorubicin and gadoteridol was optimized by acting on the parameters involved in the insonation procedure, such as the acoustic pressure. The increased uptake of the drug was paralleled by the MRI T_1 contrast enhancement, thus demonstrating the great potential of MRI in monitoring, both in vitro and in vivo, the efficiency of the drug internalization, and consequently, of the therapeutic outcome. Thus, these preliminary results illustrate the potential of the SNP method to overcome the mechanism of resistance in OC.

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Reference

[1]. Holmes D. Ovarian cancer: beyond resistance. Nature. 2015;527(7579):S217