PARAMAGNETIC PH SENSITIVE LIPOSOMES WITH IMPROVED MRI PROPERTIES

E. Torres[1], E. Terreno[1], R. Cavalli[2], F. Fedeli[3], F. Mainini[1], R. Napolitano[1], and S. Aime[1]
[1]Department of Chemistry and Molecular Imaging Center, University of Torino, Torino, Italy, [2]Department of Drug Science and Technology, University of Torino, Italy, [3]Bioindustry Park of Canavese, Italy

Introduction

The development of nanometric drug carriers able to release their bioactive content upon the action of specific triggering stimuli is an hot topic in medical field. Nanosystems offer unique advantages like the improvement of the overall pharmacokinetic properties of the drug, the reduction of side effects and systemic toxicity, and the enhancement of the therapeutic efficacy.

In addition to their use in therapy, nanoparticles have been also widely investigated in diagnosis as carriers of imaging probes. The ability of these nanocarriers to act as cargo for a wide array of chemicals has prompted their use "theranostic" agents, where the imaging probe can serve to visualize the biodistribution of a given drug loaded in the same nanocarriers.

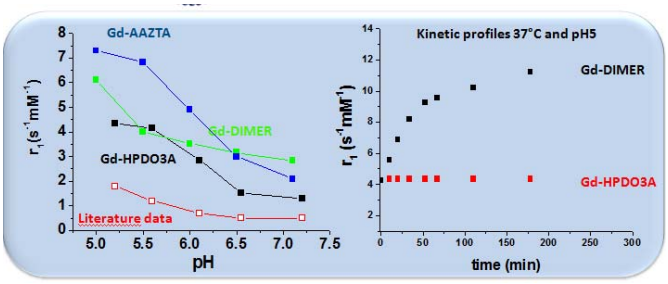
Among the nanoparticles that have been considered so far, liposomes occupy certainly a leading role being successfully used since a long time in pharmaceutical field as drug-delivery systems. These nanovesicles can be properly formulated in order to release the entrapped material as a consequence of a specific endogenous stimulus (e.g. acidification, change in redox potential or concentration of specific enzymes) that characterizes the early asymptomatic stage of several diseases. In addition to report about the drug delivery, there is a growing interest to design systems able to visualize the release of the drug from the carrier. Ideally, such a probe should provide a good image contrast when the drug is released and this task can be pursued co-encapsulating a drug and an hydrophilic MRI agent in the aqueous cavity of the liposome. As the T_1 contrast of this system can be strongly reduced owing to the compartmentalization of the paramagnetic agent, the release of the probe from the nanocarriers leads to a detectable contrast enhancement. As far as paramagnetic pH sensitive liposomes is concerned, the systems reported in the literature so far suffered from a limited contrast enhancement at acidic pHs basically related to: i) a non complete probe release, and ii) the use of imaging probe with a limited efficacy. In this contribution, a novel class of paramagnetic pH sensitive liposomes with improved formulations are presented and their basic MRI properties are evaluated both *in vitro* and *in vivo*.

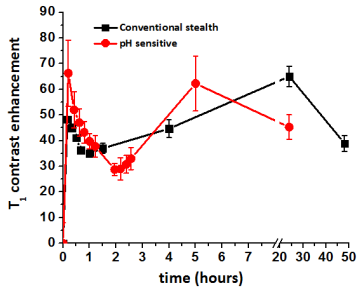
Methods

Unilamellar liposomes were prepared using the thin film hydration method followed by extrusion. The liposome formulation was a mixture of palmitoyl-oleyl-glicero-phoshphatidyl-ethanolamine (POPE), cholesterol, and α-tocopherol-hemisuccinate (THS) (44/44/11 molar fraction, respectively). The lipidic film was hydrated with solutions of Gd(III) complexes (concentration range 0.1-0.2 M) of different ligands (HPDO3A, AAZTA, a neutral DOTA-dimer, and a neutral HPDO3A-tetramer). The mean hydrodynamic size of the vesicles was determined by dynamic light scattering measurements. The T_1 measurements in vitro was carried out at 0.5 T on a Stelar Spinmaster. The *in vivo* measurements were acquired at 7 T on a Bruker Avance 300 spectrometer equipped with a Micro2.5 microimaging probe. The temporal evolution of T_1 contrast was determined *in vivo* after intratumor injection of a small volume of the liposome suspension to mice bearing xenografted B16 melanoma.

Results and Discussion

The liposome formulation tested demonstrated good release properties, with a complete release of the imaging probes Gd-HPDO3A and Gd-AAZTA in few minutes at pH 5.5. This process is accompanied with a good T_1 contrast enhancement that was significantly improved with respect literature data obtained for a different formulation. The enhancement observed *in vitro* for the system encapsulating the more efficient Gd-AAZTA complex was even higher. Interestingly, the encapsulation of neutral multimeric Gd-complexes with relaxivities higher than the two mononuclear chelates did not lead to a better performance. Kinetic measurements suggested a slow release of the bulky polynuclear agents. The *in vivo* potential of these nanoprobes was tested by injecting them directly in a xenograft tumor model on mice. The T_1 contrast was monitored over time after the injection (figure on the right).





A contrast enhancement was observed approximately 5 hours post-injection. It has been suggested that such an enhancement indicates the release of the imaging probe from the liposomes after their fast uptake by tumor cells. Interestingly, the release kinetic of pH sensitive liposomes appears, as expected, much faster than conventional stealth nanovesicles, where the enhancement reached the maximum around 1 day post-injection. Fluorescence-based experiments (spectroscopic, microscopic, FACS) allowed to get more insight about the intracellular fate of the vesicles as well as to assess the cell distribution of the liposomes (stroma vs. macrophages) within the tumor.