ENZYMATIC TRIGGERED RELEASE OF IMAGING PROBE FROM PARAMAGNETIC LIPOSOMES

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Introduction

The design of imaging probes reporting about a given enzymatic activity is a very important task in Molecular Imaging investigations. When Magnetic Resonance is the imaging modality of choice, it is necessary to design highly sensitive systems in order to overcome the relatively low sensitivity of the technique. This issue can be tackled by using aggregate systems that are able to deliver and accumulate to the biotarget a very high number of imaging agents. Among them, liposomes have attracted much attention thanks to their favorable chemical and biological properties. These self-assembling lipid-based nanovesicles are primarily used as drug-delivery carriers, and, recently, there has been a growing interest to set-up protocols for imaging guided drug delivery/release. Liposomes are also prone to destabilize themselves and release their content upon the action of specific exogenous or endogenous stimuli that, in turn, modulate the therapeutic outcome.

The aim of this work was to prepare paramagnetic liposomes encapsulating the clinically approved Gd-HPDO3A complex able to release the imaging probe in the presence of a specific enzyme characteristic of a given disease.

To do this, an amphiphilic lipopeptide was synthesized. The peptidic part (an octapeptide) was properly selected in order to act as substrate for MMP (Matrix Metallo Proteinases) family, a class of endopeptidases up-regulated in many pathologies (e.g. cancer, atherosclerotic plaques, multiple sclerosis, etc. etc.). The peptide was then linked to a stearic acid chain in order to incorporate it into a liposome bilayer and expose the enzyme substrate to the exterior medium. The potential of the resulting liposomal MRI agent was tested *in vitro* and *in vivo* on a tumor model on mice.

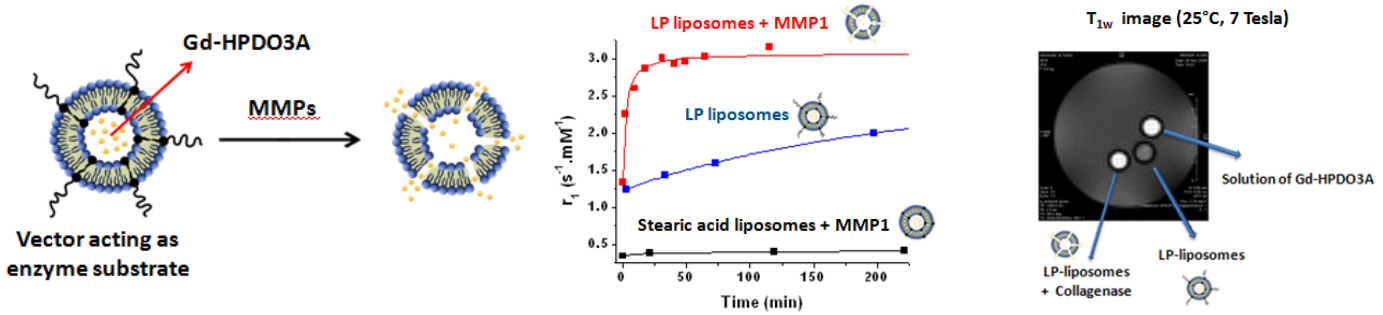
Methods

The lipopeptide C18-PLGLWAR was prepared using solid-state peptide synthesis. The choice of the PLGLWAR sequence was driven by the observation that such a sequence is currently used as test for assessing the MMPs activity.

Unilamellar paramagnetic liposomes were prepared using the thin film hydration method followed by extrusion. The liposome formulation was a mixture of dipalmitoyl- glicero-phosphocholine (DPPC), cholesterol, dipalmitoyl-glycero-succinate (DPGS), and the lipopeptide (42/10/42/10, respectively). The lipidic film was hydrated with a 200 mM solution of Gd_HPDO3A. After the hydration the non entrapped material was purified by exhaustive dialysis. Other liposomes incorporating the stearic acid only, or an inactive scrambled (LWLAPGR) lipopeptide, was prepared as controls. 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 low-resolution spectrometer, whereas 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 relaxometric properties of the liposomes loaded with the lipopeptide was tested *in vitro* measuring the longitudinal relaxivity over time of three samples: a) the suspension of liposomes incorporating the lipopeptide, b) the sample i) in the presence of MMP1, and c) liposomes incorporating the stearic acid in the presence of the proteases. The results reported below indicated that sample a) showed a slight increase in the relaxivity likely due to a partial, small instability of the liposome. In the presence of collagenase (MMP1) a very rapid and more pronounced relaxivity enhancement was observed. However, it is noteworthy that the final relaxivity is lower (3.0 vs. 4.2 $s^{-1}mM^{-1}$)than the value expected in case of a complete release of the imaging probe from the vesicle. Importantly, the liposomal sample in which the incorporated lipopeptide was replaced by stearic acid did not show any significant enhancement, thus confirming that the enhancement observed foe sample a) is related to the MMP1 activity.



This different relaxometric behavior between sample a) and b) can be well appreciated by looking at the different contrast exhibited by the two liposomes in the corresponding T_1weighted MR image (see above on the right). Preliminary *in vivo* kinetic experiment following the intratumor injection of the lipopeptide-based paramagnetic liposome indicated a rapid washout (if compared with the control liposomes) of the imaging probe from the tumor, consistent with a relevant release of the Gd(III) complex in the extracellular fluid, where MMPs accumulates.