Annexin-V-functionalized multimodal liposomes as contrast agents for apoptotic cells.

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Introduction

Apoptosis plays an important role in the etiology of a variety of cardiovascular diseases, including myocardial infarction, heart failure and atherosclerosis [1]. Early detection of apoptosis in-vivo would allow for evaluation of disease treatment at an early stage. The use of targeted multimodal liposomes that serve both as fluorescent- and MR contrast agents allows both high-resolution MRI and optical imaging techniques to detect and study apoptosis in-vivo. Additionally these multimodal contrast agents could be used to study apoptosis in-vitro or ex-vivo at a cellular level by flow cytometry or fluorescence microscopy. Annexin-V and synaptotagmin are proteins that bind specifically to phosphatidyserine (PS). In viable cells the phospholipid PS is found only on the inner layer of the cell membrane. During apoptosis PS is exposed to the outer layer of the cell membrane [2], and hence becomes accessible for binding to extra-cellular annexin-V. Several superparamagnetic iron oxide (SPIO) particles have already shown to generate a relatively large signal decrease in T2-weighted MR images of apoptotic cells using either annexin-V [3] or the C2A domain of synaptotagmin [4] to target the contrast agent. Recently a SPION particle was presented that was bound to both annexin-V for targeting and Cy5.5 for optical applications [5]. SPION particles require relatively low concentrations for MRI detection. However, they provide the images with locally decreased signal intensity where an increase in signal intensity would be preferable. Therefore avidin-GdDTPA was conjugated to the biotinylated C2A domain of synaptotagmin. This showed a relatively modest increase of the signal intensity in T2-weighted MR images of apoptotic cells, probably due to the relatively small amount of GdDTPA that was bound to avidin [6].

In this study we describe the use of a novel annexin-V-functionalized multimodal lipid based contrast agent for detection of apoptotic jurkat cells in-vitro with MRI and optical modalities.

Materials and methods

Paramagnetic liposomes were prepared containing 25mol% GdDTPA-lipid for T1-weighted MRI and 0.1mol% fluorescein-DPE for optical applications (Figure1). PEG-PE was included in order to increase the circulation time of the contrast agent in-vivo. Annexin-V was covalently bound to the liposomes for targeting. Liposomes were sized to approximately 12500 GdDTPA molecules on the outer layer of one liposome, which is about a 100-fold increase in GdDTPA concentration per contrast agent compared to the system presented by Jung et al. [6]. However, its size may restrict the liposomal density at the cell surface. The affinity of the annexin-V-functionalized liposomes for PS/PC bilayers (20/80) was verified with ellipsometry. Jurkat cells were cultured in RPMI 1640 medium. Cells were treated with CD95 Fas human antibody (aFas) for 4.5 hours to induce apoptosis, which was verified by flow cytometry. Both apoptotic and viable cells were incubated for 30 minutes with non-functionalized or annexin-V-functionalized liposomes respectively at a dose of approximately 0.9 µmol lipid/ml binding buffer (2.5 mM CaCl2). Following incubation the mean fluorescent intensity/cell was measured with flow cytometry. Subsequently ~ 5 x 10⁶ cells were fixed in 4% paraformaldehyde and sedimented for each sample. T1 values of the pellets were acquired at 6.3T and the cell samples were analysed with confocal laser scanning microscopy.

Results

Non-functionalized and annexin-V-functionalized liposome size was measured to be 102nm and 113nm respectively. Ellipsometry showed only binding of annexin-V-functionalized paramagnetic liposomes to PS/PC bilayers (not shown). Flow cytometry showed a large increase of the mean fluorescent intensity per cell for the apoptotic cells that were treated with the annexin-V-liposomes compared to all control samples (Table1). CLSM images (Figure2, top row) predominantly showed fluorescence on the surface of apoptotic cells that were incubated with annexin-V-liposomes (Figure2D). The T1-map of the corresponding cell pellet showed a relatively large decrease in the T1 relaxation time compared to control samples that were incubated with non-functionalized liposomes (Table1). The decrease in T1 relaxation time of viable cells incubated with annexin-V-liposomes appeared to be caused by aggregation of annexin-V-liposomes, which was possibly caused by the used annexin-V concentration. Optimizing this concentration should reduce the formation of aggregates, an

Conclusion

Annexin-V-functionalized liposomes at a dose of 0.9µmol lipid/ml were shown to serve as a MR/optical contrast agent for apoptotic jurkat cells in-vitro. Coating the liposome surface with PEG increases its circulation half-life in-vivo due to reduced recognition by cells of the mononuclea
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Table1: Mean fluorescent intensity/cell (MFI) and T1 relaxation times were measured for viable cells (+aFas) and apoptotic cells (+aFas) that were incubated with non-functionalized liposomes (L) or annexin-V-liposomes (AL).

![Table1](image1)

![Figure1](image2)

![Figure2](image3)