AnnexinV-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. AnnexinV and synaptotagminI are proteins that bind specifically to phosphatidylserine (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 annexinV. Several superparamagnetic iron oxide (SPIO) particles have already shown to generate a relatively large signal decrease in T_2-weighted MR images of apoptotic cells using either annexinV [3] or the C2A domain of synaptotagminI [4] to target the contrast agent. Recently a SPIO particle was presented that was bound to both annexinV for targeting and Cy5.5 for optical applications [5]. SPIO 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 synaptotagminI. This showed a relatively modest increase of the signal intensity in T_1-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 annexinV-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 T_1-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. AnnexinV was covalently bound to the liposomes for targeting. Liposomes were sized to 100nm, which was verified by dynamic light scattering. Consequently the paramagnetic liposomes will contain 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 annexinV-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 annexinV-functionalized liposomes respectively at a dose of approximately 0.9 µmol lipid/ml binding buffer (2.5 mM $CaCl_2$). Following incubation the mean fluorescent intensity/cell was measured with flow cytometry. Subsequently $\approx 5 \cdot 10^6$ cells were fixed in 4% paraformaldehyde and sedimented for each sample. T_1 values of the pellets were acquired at 6.3T and the cell samples were analysed with confocal laser scanning microscopy.

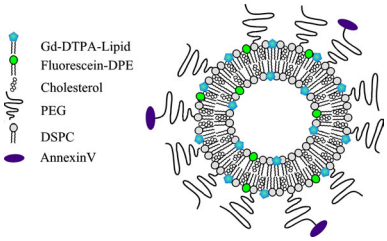

Figure1: Liposome system as a multimodal contrast agent. Covalent binding of annexinV makes the contrast agent specific for targeting apoptosis.

Results

Non-functionalized- and annexinV-functionalized liposome size was measured to be 102nm and 113nm respectively. Ellipsometry showed only binding of annexinV-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 annexinV-liposomes compared to all control samples (Table1). CLSM images (Figure2, top row) predominantly showed fluorescence on the cell surface of apoptotic cells that were incubated with annexinV-liposomes (Figure2D). The T_1-map of the corresponding cell pellet also showed a relatively large decrease in the T_1 relaxation time compared to control samples that were incubated with non-functionalized liposomes (Table1). The decrease in T_1 relaxation time of viable cells incubated with annexinV-liposomes appeared to be caused by aggregation of annexinV-liposomes, which was possibly caused by the used annexinV concentration. Optimizing this concentration should reduce the formation of aggregates, and thereby exclude unbound contrast agents from the cell pellets.

Conclusion

AnnexinV-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 mononuclear phagocyte system (MPS). Additionally annexinV conjugated liposomes have low immunogenicity compared to biotin-avidin/streptavidin systems. The in-vitro results presented here together with the low immunogenicity make this lipid based contrast agent a promising candidate for in-vivo application.

aFas	CA	MFI (AU)	T1 (s)
-	L	2	1.20 ± 0.04
-	AL	21	0.63 ± 0.08
+	L	2	1.03 ± 0.09
+	AL	293	0.35 ± 0.13

Table1: Mean fluorescent intensity/cell (MFI) and T_1 relaxation times were measured for viable cells (-aFas) and apoptotic cells (+aFas) that were incubated with non-functionalized liposomes (L) or annexinV-liposomes (AL).

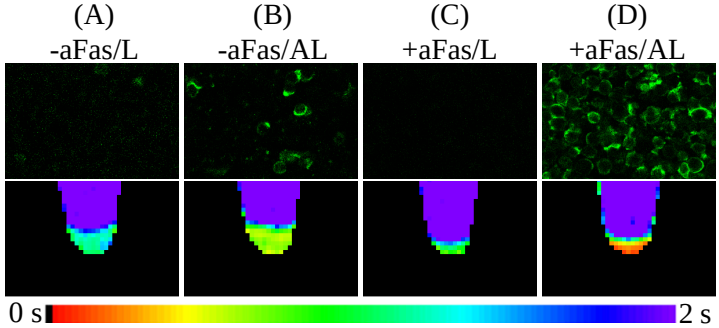

Figure2: CLSM images (top row) and its corresponding T_1-maps (bottom row) of viable cells (-aFas) or apoptotic cells (+aFas) that were incubated with either non-functionalized liposomes (L) or annexinV-liposomes (AL).

References

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