Optimization of Intracellular Delivery of PARACEST Contrast Agents by Combining Liposomes and Cell Penetrating Peptides

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Abstract

MRI of molecular events often requires internalization of the contrast agent. Several methods are currently being used and include unencapsulated contrast agent incubation and coupling of the contrast agent to cell penetrating peptides. Liposomes are also used to enhance delivery of contrast agents due to reduced cytotoxicity and increased circulation time. These different methods were combined to optimize intracellular delivery of PARACEST MRI contrast agents for molecular imaging applications. The liposomal encapsulation of a PARACEST agent coupled to a cell penetrating peptide showed a 61.5-fold increase in the PARACEST effect from cell-internalized contrast agents.

Introduction

MRI of molecular events often requires internalization of the contrast agent in the cells of interest. Contrast agents can enter cells in vitro during overnight incubation with agent-enriched media, but this procedure can't be reproduced in vivo due to washout and limited contact of the contrast agent with the cells of interest.[1] Cell penetrating peptides (CPPs) have been explored as a means to increase cellular internalization of contrast agents, yet problems remain with delivery of CPPs to cells of interest within in vivo conditions.[2,3] Encapsulation of contrast agents in the aqueous core of liposomes can improve delivery to in vivo cells of interest by extravasating through leaky tumor vasculature,[4,5] and by avoiding clearance through the reticuloendothelial system.[6] Therefore, encapsulating CPP-coupled MRI contrast agents in liposomes may improve cellular internalization of MRI contrast agents within in vitro and in vivo conditions.

Methods

Liposomes were prepared by dissolving 50% DMPE, 35% DMPC, and 15% Cholesterol (Avanti Polar Lipids) in ethanol, and the solvent was then evaporated to form a lipid film. This film was hydrated with solutions of each contrast agent, heated to 70°C, and extruded at 70°C. The average diameter of each liposome preparation was measured using light scattering (Brookhaven Particle Sizer). Yb-DOTAM-Gly was synthesized using previously published methods.[7] A contrast agent consisting of the Tat CPP[2] coupled to Yb-DOTA, Tat-(Yb-DOTA), was synthesized using customized methods developed in our laboratory.[8] Each contrast agent with or without encapsulation in liposomes were diluted with RMPI 1640 cell culture media. MCF7c3 breast cancer cells were subcultured into 6-well plates and grown for one day before each contrast agent was added for overnight incubation. The cells were washed 3 times with sterile PBS, trypsinized, pelleted, washed twice with 300μL sterile PBS, resuspended in 150μL sterile PBS, and pelleted in a 200μL PCR tube. The PBS was removed and 180μL of cooled 2% agarose was used to stabilize the pellet.[1] PARACEST MR images of the pellets were acquired with a Bruker 9.4T MRI scanner using a spin-echo pulse sequence [TE 4404, TE 9.4, In-plane resolution 312 x 312 μm, 1 average, and a 30 μT presaturation pulse applied for 2.25 sec].

Results and Discussion

Contrast agents that generated the detected PARACEST effect were ensured to be intracellular due to exhaustive washing. The PARACEST effects of each MRI test was normalized with respect to the concentration of the contrast agent in the incubation media, and the number of exchange sites within each contrast agent (i.e., 1 site for Tat-(Yb-DOTA) and 4 sites for Yb-DOTAM-Gly). Liposomal encapsulation increased the PARACEST effect by a factor of 2 to 10 for Tat(Yb-DOTA) and Yb-DOTAM-Gly, respectively. The Tat CPP increased the PARACEST effect by a factor of 6.8 to 30.9 for encapsulated and unencapsulated forms, respectively. Overall, the combination of conjugating a CPP to a PARACEST contrast agent, and subsequently encapsulating into liposomes, boosts the normalized PARACEST effect by a factor of 61.5.

Table 1: Comparison of PARACEST contrast agent delivery and CEST effect with liposomes and cell penetrating peptides

Contrast Agent	Encapsulation	Concentration	%CEST	% CEST/ Incubation Concentration	% CEST/ Incubation Conc./ CEST sites
Yb-DOTAM-Gly	None	25 mM	1.7%	0.068% / mM	0.017% / mM
Yb-DOTAM-Gly	Liposome	25 mM	15.4%	0.616% / mM	0.154% / mM
Tat-(Yb-DOTA)	None	15 mM	7.9%	0.526% / mM	0.526% / mM
Tat-(Yb-DOTA)	Liposome	15 mM	15.7%	1.046% / mM	1.046% / mM

Conclusion

Although intracellular delivery of contrast agents is enhanced by using CPPs or liposomal encapsulation, these methods can be combined to optimize intracellular delivery. Furthermore, this combined approach is more applicable for in vivo intracellular delivery techniques.

References

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