Bifunctional Solid Lipid Nanoparticle System as a Potential Tool for Tumor Detection Using Optical and MR Imaging

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Abstract Summary

Multifunctional nanoparticles have potential applications for cancer diagnosis by overcoming limitations associated with conventional cancer diagnosis techniques. The objective of the present study was to develop a biocompatible multifunctional nanoparticle system able to reach the tumor site where it can be used as contrast agent for magnetic resonance imaging (MRI), and as near infrared optical probe to detect the tumor. Such nanoparticle system was synthesized by encapsulating near infrared (NIR) dye Indocyanine green (ICG) in the core of solid lipid nanoparticles and conjugating the surface with gadolinium (Gd\textsuperscript{3+}) to enhance the MRI signal. The prepared bifunctional nanoparticles, 150 nm in diameter, showed enhanced T\textsubscript{1}-weighted MR signal as well as strong fluorescence intensity. The nanoparticles were taken by cancer cells effectively enabling the detection of cancer cells by imaging.

Introduction

The mortality rate of cancer, which is a leading cause of death worldwide\textsuperscript{1}, can be reduced by early stage diagnosis and treatment. Currently used diagnostic modalities, such as magnetic resonance imaging (MRI), optical imaging (OI), positron emission tomography, computed tomography, are unable to provide sufficient information due to individual limitations of a stand-alone system\textsuperscript{2}. As a result, multifunctional systems that can generate contrast by combined different imaging techniques are being developed. However, many of these systems are known to be toxic or display photobleaching of the fluorescent probes\textsuperscript{3}. Nanoparticles, for their potential as contrast agents, offer the advantages of greater biocompatibility compared to more conventional chemical agents and the potential to reduce photobleaching.

In this study, we have developed a multifunctional biocompatible solid lipid nanoparticle system for combination of MRI and near infrared (NIR) OI of tumor. These nanoparticles were prepared with clinically relevant MRI contrast agent gadolinium (Gd\textsuperscript{3+}), and FDA approved NIR dye Indocyanine Green (ICG). This multimodal (MR/NIR) nanoparticle system integrates the advantage of high-resolution 3D anatomical imaging with high-sensitivity deep-tissue in-vivo fluorescent imaging\textsuperscript{4}. Previously, our lab has developed lipid nanoparticles for therapeutic applications in breast cancer tumor model in mice\textsuperscript{5,6}. These particles were shown to be biocompatible and passively accumulate in the tumor with minimum liver uptake\textsuperscript{7}.

Experimental Methods

The nanoparticles were prepared in two steps. At first step, Gd\textsuperscript{3+} was attached to the one end of polyoxyethylene 100-stearate (Myrj59). For this purpose one end of Myrj59 was covalently attached to the Gd\textsuperscript{3+} chelator diethylene triamine pentaacetic acid dianhydride (DTPA) giving a final product Myrj59-DTPA, prior to Gd\textsuperscript{3+} addition. In the second step, synthesis of nanoparticles was performed using oil-in-water emulsion technique by mixing myristic acid, Myrj59-DTPA-Gd and ICG. The emulsion was sonicated for 5 min at 65 °C. Finally particles were filtered to remove free molecules. The obtained particles were further characterized using transmission electron microscope (TEM), zetasizer and MRI. Cellular uptake by human breast cancer cells was examined under a fluorescence microscope.

Results and Discussions

As shown in Fig. 1 (DLS and TEM), the nanoparticles were spherical in shape with size distribution of 150 nm±20 nm. The zeta potential of the particles was found to be -5.7 mV. The optical properties of the nanoparticles were evaluated by recording the absorbance spectra and fluorescence image of nanoparticle with or without ICG. The absorbance spectra of nanoparticles containing ICG shows an absorbance peak at 780 nm (Fig 2A) confirming the encapsulation of the dye in the cavity of the nanoparticles. The fluorescence was confirmed using the IVIS Xenogen imager with an excitation filter of 780 nm and emission filter of 850 nm (Fig. 2B).

To evaluate the effectiveness of the nanoparticle system as MRI contrast agents, magnetic relaxivities were measured using 7T clinical MRI instrument. T\textsubscript{1} and T\textsubscript{2} relaxation time were measured for
nanoparticles by varying the Gd concentration. Finally relaxivities ($r_1$, $r_2$) of nanoparticles were determined from the slopes of the relaxation rates ($1/T_1$ and $1/T_2$), against Gd concentration. The Gd-ICG co-loaded nanoparticles generated MR contrast in both $T_1$ and $T_2$ weighted MRI signal, with higher contrast enhancement for $T_1$ weighted MRI signal (Fig. 3). The Gd-ICG nanoparticles had $r_1$ and $r_2$ value of 6.1 mM$^{-1}$Sec$^{-1}$ and 21.6 mM$^{-1}$Sec$^{-1}$, respectively. These results suggest that such nanoparticles system can be used as $T_1$ weighted MRI contrast agent, due to their higher $r_1$ values compare to the commercially available Gd-based contrast agent Omniscan® with a low $r_1$ value of 3.9 mM$^{-1}$Sec$^{-1}$.

Conclusions

Bifunctional solid lipid nanoparticles have been synthesized demonstrating excellent optical and MRI properties for cancer diagnosis.

Fig. 1 In vitro characterization of nanoparticles containing Gd on surface and NIR dye in the core. A) Mean diameter of nanoparticle obtained using DLS, B) TEM image of nanoparticles. Inset image shows the high resolution TEM image of single particle. Scale bar correspond to 500 nm and 100 nm for main and zoom in images respectively.

Fig. 2 Optical characterization of nanoparticles, A) absorption spectra of Gd-chelated nanoparticle with and without NIR dye. B) Fluorescent image of Gd-chelated nanoparticle with and without NIR dye.

Fig. 3 Magnetic resonance data for Gd-ICG nanoparticles. Linear plots of Gd concentration versus relaxation rates A) $1/T_1$ and B) $1/T_2$, respectively, to obtain ionic relaxivities, $r_1$ and $r_2$.

References

1. World Health Organization, February 2012.