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Liposomally formulated phospholipid-conjugated indocyanine green for intra-operative brain tumor detection and resection

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ABSTRACT

Some tumor-specific near-infrared (NIR) fluorescent dyes such as indocyanine green (ICG), IDRye800CW, and 5-aminolevulinic acid have been used clinically for detecting tumor margins or micro-cancer lesions. In this study, we evaluated the physicochemical properties of liposomally formulated phospholipid-conjugated ICG, denoted by LP-iDOPE, as a clinically translatable NIR imaging nanoparticle for brain tumors. We also confirmed its brain-tumor-specific biodistribution and its characteristics as the intra-operative NIR imaging nanoparticles for brain tumor surgery. These properties of LP-iDOPE may enable neurosurgeons to achieve more accurate identification and more complete resection of brain tumor.

1. Introduction

Gliomas are the most common type of primary brain tumor and are classified into four categories according to the World Health Organization (WHO) grading system: low-grade (WHO Grade I and II) and high-grade (WHO Grade III and IV). (Kleihues et al., 1993) The complete removal of brain tumor tissues by extended resection is usually hard to perform without injuring surrounding normal brain tissues. (Schucht et al., 2015) It is therefore a challenge for neurosurgeons to remove brain tumor tissues completely by resection; leaving any cancer tissues behind can lower the patient's quality of life (QOL) and reduce survival times. (Talibi et al., 2014; Tate, 2015) A wide range of clinical applications have been developed in attempts to achieve more complete brain tumor resections (Keunen et al., 2014). However, accurate identification of the tumor region that needs to be resected is still of great importance in brain tumor surgery (Kircher et al., 2012; Jermyn et al., 2015). Over the past decade, intra-operative imaging using near-infrared (NIR) fluorescence techniques has entered the surgical theatre to fill the gap between pre-operative imaging and intra-operative reality (Vahrmeijer et al., 2013; de Boer et al., 2015).

Some tumor-specific NIR fluorescent dyes have been approved for clinical tumor imaging: indocyanine green (ICG) has been used as a targeted free NIR dye for micro-cancer imaging and breast cancer assisted sentinel lymph node mapping; IRDye800CW, conjugated with antibody targeting VEGF-bevacizumab (VEGF: vascular endothelial growth factor) has completed preclinical toxicity studies, and is currently undergoing clinical trials as a NIR dye conjugate for early cancer detection; 5-aminolevulinic acid, preferentially taken up by tumor cells leading to biosynthesis and accumulation of protoporphyrin IX, has been approved in Germany and Japan as an activatable NIR dye for imaging malignant gliomas (Luo et al., 2011; Keereweer et al., 2013; Swanson et al., 2015).

Abbreviations: BBB, blood brain barrier; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; EPR, enhanced permeability and retention; ICG, indocyanine green; iDOPE, ICG fluorophore covalently conjugated with 1,2-dioleoyl- sn -glycero-3-phosphoethanolamine; LED, light-emitting diode; LP-iDOPE, liposomally formulated iDOPE; MRI, magnetic resonance imaging; NIR, near-infrared; PBS, phosphate buffered saline; PEG, polyethylene glycol; PDT, photodynamic therapy; QOL, quality of life; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

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Intravenous injection of ICG was approved by the Food and Drug Administration in the 1950s for several clinical applications and has been shown to have a low negative-reaction profile (Dzurinko et al., 2004). The first application of ICG for macroscopic demarcation of brain tumor margins was investigated in 1993 (Hansen et al., 1993). However, this technique is not effective in distinguishing between tumor regions and normal regions of the brain that may incidentally uptake the injected dye (Tamura et al., 2012). Cellular visualization using ICG as a contrast agent is therefore needed to overcome these limitations. The investigation of potential imaging techniques in the field of brain tumor surgery, and the development of new imaging dyes and accommodated devices that are available during brain tumor surgery are therefore important (Zehri et al., 2014).

In this study, we attempted to improve the specificity and sensitivity of brain tumor imaging using an ICG fluorophore (Murahari and Yergeri, 2013). We previously developed a novel NIR fluorescent probe in which an ICG fluorophore is covalently conjugated with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), denoted by iDOPE, for incorporation into liposome bilayers as liposomally formulated iDOPE (LP-iDOPE) (Suganami et al., 2012). We then evaluated the characteristics of LP-iDOPE as a clinically translatable fluorescent nanoparticle for brain tumors, based on the enhanced permeability and retention (EPR) effect, and as a NIR fluorescent dye for intra-operative identification of brain tumors. We presume that LP-iDOPE is potentially of great value as a NIR fluorescence image-guidance intra-operative dye, which could make a clear demarcation at brain tumor borders.

2. Material and methods

2.1. Particle synthesis: preparation of LP-iDOPE

iDOPE was prepared as previously reported (Suganami et al., 2012). Cholesterol (1.0×10^{-3} M, Nippon Fine Chemical Co., Ltd., Tokyo, Japan), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 7.5×10^{-3} M, NOF Corporation, Tokyo, Japan), *N*-(carbonyl-methoxypolyethyleneglycol 5000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine sodium salt (DSPE-PEG, 0.5 mM, NOF Corporation), and iDOPE (2.5×10^{-3} M, iDOPE/DOPC molar ratio of 1/3) were dissolved in a mixed organic solvent consisting of CH3OH/CHCl3 (volume ratio: 1/9). A thin lipid film was formed by removal of the solvent under reduced pressure. After addition of an aqueous buffered solution (phosphate buffered saline [PBS], pH 7.4) at room temperature, the liposome dispersion was filtered through a 0.1-mm pore polycarbonate filter attached to a LiposoFast-Stabilizer (Avestin Inc., Ottawa, Canada).

2.2. Molecular modeling

The three-dimensional structure of ICG was constructed using MOE (version 2009, CCG Inc., Montreal, Canada) according to PubChem Compound CID 11967809 (National Center for Biotechnology Information, Bethesda MD, USA). The three-dimensional structure of DOPE was constructed using VMD (http://www.ks.unic.edu/). The three-dimensional structures of ICG and DOPE were used to construct that of iDOPE using the build command in MOE. Molecular mechanics calculations were performed to obtain the local minimum structures of partial membrane region of LP-iDOPE, containing DOPC and iDOPE, using the AMBER99 force field in MOE.

2.3. Measurement of particle size

Particle size analysis of LP-iDOPE was performed using dynamic light-scattering measurements (SZ-100, HORIBA Ltd., Kyoto, Japan).

2.4. UV–vis–NIR and fluorescence spectroscopy

The absorption spectra of LP-iDOPE and ICG dissolved in an aqueous buffered solution [phosphate buffered saline (PBS), pH 7.4] at room temperature were obtained using a UV–vis–NIR spectrometer (UV-3500, Shimadzu, Kyoto, Japan). Fluorescence emissions from LP-iDOPE and ICG dissolved in an aqueous buffered solution (PBS, pH 7.4) at room temperature were observed using a fluorescence spectrometer (F-4500, Hitachi, Tokyo, Japan).

2.5. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Chiba University and the National Institute of Radiological Sciences. Male 7- to 8-week-old Fisher 344 rats were obtained from the Japan SLC, Inc. (Hamamatsu, Japan). Orthotopic glioma model of 9L-L/R cells in rats: A density of 5×10^5 9L-L/R cells with 10 μL PBS were injected into rat brains using a microinjector (Harvard Apparatus, South Natick, MA, USA). Briefly, the rats were anesthetized with 2.0% isoflurane (Abbott Japan, Tokyo, Japan) and placed in a stereotactic apparatus. A burr hole was made at an appropriate location (1 mm posterior to the bregma and 3 mm right of the midline). A 25-gauge needle was inserted at a point 3 mm ventral from the dura. These experiments were also done in accordance with the recommendations for the proper care and use of laboratory animals and according to The Law No. 105 and Notification No. 6 of the Japanese Government.

2.6. MRI

All rats were anesthetized with 2.0% isoflurane (Abbott Japan, Tokyo, Japan) and administrated with 0.4 ml of Gd-DTPA (Meglumine Gadopentetate, 0.002 ml/g, 50 mmol/l, Bayer, Leverkusen, Germany) intraperitoneally 15 min before MRI measurements. All MRI experiments were performed using a 7.0T horizontal MRI scanner (Magnet: Kobelco and JASTEC, Kobe, Japan; Console: Bruker Biospin, Ettlingen, Germany), with a volume coil for transmission (Bruker Biospin) and an eight-channel phased array surface coil for reception (Rapid Biomedical, Rimpar, Germany). Multislice T1-weighted MR images covering the entire brain (T1WI; multislice spin echo, TR/TE = 400/9.57 ms, slice thickness = 1.0 mm, slice gap = 0, number of slices = 16, matrix = 256×256, field of view = 25.6×25.6 mm^2, average = 4) were acquired. The slice orientation was transaxial for all scans. Image reconstruction and analysis were performed using ParaVision (Bruker Biospin).

2.7. NIR fluorescence imaging

The biodistribution of LP-iDOPE was studied by intravenously injecting LP-iDOPE, containing 14.0 mg/kg of LP-iDOPE, through a tail vein of Fisher 344 rats bearing 9L-L/R cells and imaged at 1 day and 7 days after injection, using an In-Vivo MS FX PRO imaging system (Carestream Health, New York, NY, USA).

3. Results and discussion

3.1. Preparation of LP-iDOPE

As part of our on-going research for the development of multi-purpose NIR fluorescent liposomes for optical imaging and nanoparticle drug carriers, we rationally designed a novel NIR fluorescence probe in which an ICG fluorophore is covalently conjugated with DOPE, i.e., iDOPE, for incorporation into liposome bilayers (Suganami et al., 2012). As shown in Fig. 1a, we speculate that iDOPE infiltrates the lipid membrane by incorporating into 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). In this study,