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Light induced cytosolic drug delivery from liposomes with gold nanoparticles

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ABSTRACT

Externally triggered drug release at defined targets allows site- and time-controlled drug treatment regimens. We have developed liposomal drug carriers with encapsulated gold nanoparticles for triggered drug release. Light energy is converted to heat in the gold nanoparticles and released to the lipid bilayers. Localized temperature increase renders liposomal bilayers to be leaky and triggers drug release. The aim of this study was to develop a drug releasing system capable of releasing its cargo to cell cytosol upon triggering with visible and near infrared light signals. The liposomes were formulated using either heat-sensitive or heat- and pH-sensitive lipid compositions with star or rod shaped gold nanoparticles. Encapsulated fluorescent probe, calcein, was released from the liposomes after exposure to the light. In addition, the pH-sensitive formulations showed a faster drug release in acidic conditions than in neutral conditions. The liposomes were internalized into human retinal pigment epithelial cells (ARPE-19) and human umbilical vein endothelial cells (HUVECs) and did not show any cellular toxicity. The light induced cytosolic delivery of calcein from the gold nanoparticle containing liposomes was shown, whereas no cytosolic release was seen without light induction or without gold nanoparticles in the liposomes. The light activated liposome formulations showed a controlled content release to the cellular cytosol at a specific location and time. Triggering with visual and near infrared light allows good tissue penetration and safety, and the pH-sensitive liposomes may enable selective drug release in the intracellular acidic compartments (endosomes, lysosomes). Thus, light activated liposomes with gold nanoparticles are an attractive option for time- and site-specific drug delivery into the target cells.

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1. Introduction

Target sites of many drugs are located intracellularly. For example, transcription factors, RNAi compounds, DNA, and modulators of intracellular proteins must be delivered into the target cells. Therefore, intracellular delivery is a prerequisite for the efficacy of many important drug classes.

Liposomes are effectively endocytosed into the target cells, but drug release from the liposomes in the cells is often sub-optimal. Different approaches have been used to facilitate intracellular drug release from the liposomes and other nanomedicines. For example, pH-sensitive [1,2], thermosensitive [3–6], photosensitive [7,8], ultrasonically [9,10] and electromagnetically triggered [11–13] formulations have been used. Biological triggers, such as enzymes [14] or microbes [15], have also been utilized in some experiments. Paasonen et al. [5,6] and later publications [16–21] show a light triggered content release from the liposomes with gold nanoparticles. The triggered release is based on the absorption of light energy by gold nanoparticles. The absorbed light energy is released as heat to the lipid bilayers causing phase transition in the lipid bilayers and content release from the liposomes [22–24].

In principle, the light triggered drug release from the liposomes can be used in the tissues that can be reached with the light irradiation. The light induced drug release from liposomes may be applicable in the treatment of the diseases affecting the eyes, gastrointestinal tract, skin, lungs, nasal cavity, ears or superficial tissues. For example, the tissues in the back of the eye (retina, choroid) are commonly treated in the clinics with lasers that could also be used to trigger drug release in the eye. Examples of the diseases affecting the posterior eye segment include glaucoma [25], retinopathies [26–28], choroidopathy [29], Coat's disease [30], retinoblastoma [31], and age-related macular degeneration [32,33].

Light penetration into the human tissues depends on the wavelength: penetration at 700–900 nm is at least 20 times more effective than at wavelengths below 400 nm [34–38]. In addition, the wavelength has important safety implications, since ultraviolet (UV) light may be harmful to the retina and other human tissues [39]. Previous proof-of-concept studies on light activated liposomes utilized small gold nanoparticles (1.5–3 nm) that are activated with UV light [5,6]. The absorption wavelength of gold nanoparticles depends on the shape and diameter of the nanoparticles. This allows the design of liposome formulations that are activated at visible and near infrared wavelengths, since larger gold nanoparticles (30–50 nm) absorb light at visible and near infrared regions [40–43].

Endocytosis and phagocytosis are the principal cellular uptake mechanisms of liposomes [44]. After cellular internalization, liposomes are entrapped within the endosomes and they may be re-circulated back to the extracellular space or trafficked to the lysosomes [45]. However, drug escape from the endosomes is needed for intracellular drug action. Otherwise, the liposomes will be trafficked to the lysosomes where drug may be degraded [45]. Distribution of liposomal drug from the endosomes to the cytosol can be facilitated with membrane active peptides [46] and pH-sensitive liposomes [1,2]. Previously, light activation of liposomes did not result in the distribution of liposomal contents to the cytosol [6], but synergistic effects of light activation and pH sensitivity might result in cytosolic delivery of the liposomal cargo.

In this study, we investigated light activated liposomes with important new features. Firstly, we loaded the liposomes with gold nanoparticles that can be triggered with visible and near infrared light. Secondly, lipid composition of Ickenstein et al. [47–50] was optimized to allow highly effective light triggering of drug release. Thirdly, we developed pH-sensitive liposomes that showed a fast content release at the acidic endosomal pH, that may enhance the therapeutic effect without exposing the surrounding tissues to the medication. The functionality of the formulations was studied with physicochemical methods, release tests, and cell culture studies. Overall, the new light activated liposomes showed a triggered content release at safe wavelengths (near infrared) that penetrate the human tissues. Furthermore, the light induction resulted in efficient intracellular delivery of the liposomal contents to the cell cytosol.

2. Material and methods

2.1. Preparation of light activated thermosensitive liposomes

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine (Lyso PC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG) were bought from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). The lipids were dissolved in chloroform (Sigma-Aldrich, St. Louis, MO, USA). The liposomes were prepared using the reverse-phase evaporation method (REV) [5,6] with a few modifications. Briefly, the lipids in chloroform solutions were mixed in a glass tube (10 μmol of total lipids). The tube was placed in a water bath within a larger glass tube and attached to a vacuum rotary evaporation system (Büchi R-114, Büchi Labortechnik AG, Flawil, Switzerland). Chloroform was evaporated by heating the tube up to 60 °C and gradually reducing the pressure to 70 mbar under a nitrogen flow. The resulting thin lipid layer was dissolved in 1.2 ml of di-isopropylether (DIPE) (Sigma-Aldrich). A calcein solution (60 mM, 280 mOsm) was prepared by dissolving 374 mg of calcein (Sigma-Aldrich) and 17 mg of sodium chloride (Sigma-Aldrich) in 10 ml of milli-Q water, and the pH was adjusted to 7.4 with sodium hydroxide.

Hydrophilic gold nanorods (width 25 nm; length 60 nm; relative SD ± 10%; cetyltrimethylammonium bromide (CTAB) stabilized) were purchased from Sigma-Aldrich. Hydrophilic gold nanostars (diameter 50–60 nm; polyethylene glycol (PEG) coated) were prepared by the protocol described by Barbosa et al. [51] The nanorods have a light absorption maximum at 650 nm and the nanostars have a wide absorption peak at 700–900 nm. The gold nanoparticle solutions were concentrated by centrifugation and careful removal of the supernatant. The final nanoparticle concentration was 15 μg/μl.

The solutions of calcein (480 μl), gold nanoparticles (20 μl) and lipid-DIPE were heated to 60 °C and mixed in a glass tube. After brief vortexing the mixture was sonicated in a heated bath ultrasonicator (Elma Schmidbauer GmbH, Singen, Germany) at 60 °C until a homogeneous solution without separate phases was obtained. The glass tube was quickly transferred to a larger tube with 2.5 ml of milliQ water. The organic solvent was evaporated in vacuum in a 60 °C water bath under a nitrogen atmosphere. The pressure was gradually reduced to 180 mbar during 2 h while avoiding the formation of excessive bubbles and foaming in the sample. The sample was kept at 180 mbar for 10 min to ensure complete evaporation of the organic solvent and formation of liposomes with encapsulated gold nanoparticles in the aqueous core. The size of the liposomes was reduced by ultra-sonication for 10 min above the transition temperature of the lipids. The liposome samples were purified by gel filtration in a Sephadex G-50 (Sigma-Aldrich) column where samples were eluted with a buffer solution of 20 mM HEPES (Sigma-Aldrich) and 140 mM sodium chloride (pH 7.4, 280 mOsm). Gold nanoparticle concentration was analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (7100 DV-ICP-OES, Perkin Elmer, Waltham, MA, USA) and the gold concentration of the samples was adjusted to 50 μM with the buffer solution. Liposomes without gold nanoparticles were prepared as control samples. Light absorption spectra of the gold nanoparticles and liposomes were analyzed with a Varioskan Flash plate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.2. Preparation of light activated thermo- and pH-sensitive liposomes

1,3-Diolein (diolein) and cholesteryl hemisuccinate (CHEMS) were purchased from Sigma-Aldrich. 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was bought from Avanti Polar Lipids. Two

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