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Do plasma proteins distinguish between liposomes of varying charge density?

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ABSTRACT

Cationic liposomes (CLs) are one of the most employed nonviral nanovector systems in gene therapy. However, their transfection efficiency is strongly affected by interactions with plasma components, that lead to the formation of a "protein corona" onto CL surface. The interactions between nanoparticles entering the body and biomolecules have an essential role for their biodistribution. Because the knowledge of proteins adsorbed onto vector surface could be useful in the screening of new, more efficient and more biocompatible liposomal formulations, the behavior of three CLs with different membrane charge densities was investigated. The proteins of the three coronas were identified by nano-liquid chromatography–tandem mass spectrometry, and quantified with label-free spectral counting strategy. Fibrinogen displayed higher association with CLs with high membrane charge density, while apolipoproteins and C4b-binding protein with CLs with low membrane charge density. These results are discussed in terms of the different lipid compositions of CLs and may have a deep biological impact for in vivo applications. Surface charge of nanoparticles is emerging as a relevant factor determining the corona composition after interaction with plasma proteins. Remarkably, it is also shown that the charge of the protein corona formed around CLs is strongly related to their membrane charge density.

1. Introduction

Gene therapy, consisting in the introduction of new genetic materials to hosts, is attracting a growing interest to study gene function and its regulation, to establish and most of all to explore potential therapeutic applications to various acquired or inherited diseases [1] caused by the defect of the absence of one or more genes. Therefore, the development of suitable vectors for an efficient transfection is of fundamental importance in gene therapy.

Starting from the first application in 1987 [2], cationic liposome (CL)-mediated gene transfer has been the most extensively investigated and commonly used nonviral gene delivery approach [1,3–6]. Several different lipids, all sharing the common structure, i.e. positively charged hydrophilic head and hydrophobic tail connected via a linker, have been developed and tested for lipofection. The positively charged head group (generally amines or quaternary ammonium salts) serves to interact with DNA (lipoplexes) or other anionic biomolecules, while the hydrophobic part is the "helper" lipid. The structure of CL (including surface charge density and colipid) strongly affects its transfection efficiency [1].

However, one of the major problems using nanovectors for drug or gene delivery is their interaction with blood

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components, that can provoke vector rapid clearance from the blood circulation. Indeed, when nanoparticles enter into blood circulation, plasma proteins are adsorbed onto their surface to form a "protein corona" [7–12]. These interactions with blood biomolecules have been shown to have an essential role in nanoparticle biodistribution [10], and clearance behavior is strongly affected by nanocarrier architecture [11,13]. Therefore, protein binding to nanoparticles can be critical in modifying the interactive surface that is recognized by cells [14,15], and understanding how and why plasma proteins are adsorbed to lipid particles may serve as a fundamental predictive model for the in vivo efficiency of the vector, in view of future clinical applications.

Because the knowledge of plasma proteins adsorbed onto vector surface could be useful in the screening of new, more efficient and more biocompatible liposomal formulations, in a previous work [16] we investigated the behavior of three CLs with different membrane charge densities. In particular, we characterized from the qualitative point of view the three protein coronas by nano-liquid chromatography–tandem mass spectrometry (nanoLC–MS/MS). No significant differences were found in the composition of protein coronas. However, some quantitative differences could be inferred from the Exponentially Modified Protein Abundance Index (emPAI) values [17] provided by the search engine Mascot. For this reason, we decided to perform a quantitative analysis to have a deeper insight and better investigate about a possible specific absorption of plasma proteins onto the different CL surfaces.

MS-based quantitative proteomics can be performed employing both stable isotope labeling and label-free approaches [18–20]. Labeled methods allow the simultaneous quantification of several samples, however present also some drawbacks, such as incomplete labeling, sample dilution (rendering difficult the detection of low abundance proteins), and are associated with high costs. For these reasons, label-free strategies are widely used in biomarker studies [21]. Label-free MS measurement of peptides (and therefore of their parent protein) relies on peak intensity measurements (comparing the direct MS signal intensity for any given peptide on the basis of its retention time, and m/z ratio) or spectral counting (SC) [22–24]. In SC approach, the number of acquired MS/MS spectra matching to a specific peptide is considered an indicator of its abundance in a given sample [20,23]. SCs of peptides associated with a protein are then summed and compared across the samples, often after normalization.

In the present work, a label-free quantification of plasma proteins adsorbed onto the surface of the three liposome formulations previously investigated was performed. According to recent findings [25], CLs are excellent model systems of lipid nanoparticles in which an inner core made of DNA precondensed by oppositely charged macromolecules (e.g. polycations, cationic proteins etc.) is coated with a lipid envelope. After nanoLC–MS/MS analysis and Mascot database search, protein identification was statistically validated with Scaffold software. The same software was also used to evaluate quantitative differences between the three protein coronas with SC method.

Very recently, a few papers on MS-based quantification of protein coronas on different nanoparticle surfaces have been published [11,12,26], however this is the first study on quantification of the plasma proteins adsorbed onto different CLs.

2. Material and methods

2.1. Chemicals and standards

All chemicals were purchased from Sigma Aldrich (St. Luis, Mo, USA) unless otherwise stated. The sequencing grade modified trypsin was from Promega (Madison, WI, USA). All organic solvents were the highest grade available from Carlo Erba Reagents (Milan, Italy). Ultrapure water (resistivity 18.2 MΩ cm) was obtained by an Arium water purification system (Sartorius, Florence, Italy).

The cationic lipid 3β-[N-(N′, N′-dimethylaminoethane)-carbamoyl])-cholesterol (DC-Chol), and the neutral lipid dioleoylphosphatidylethanolamine (DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA).

Solid phase extraction (SPE) C18 cartridges were BOND ELUT (Varian, Palo Alto, CA, USA).

2.2. Preparation of cationic liposomes

The solutions of liposomes were prepared as already described [16] by solving suitable amounts of DC-Chol and DOPE in $CHCl_3$, keeping constant the moles of cationic lipids, to obtain three different molar ratios of neutral lipid in the bilayer Φ (neutral lipid/total lipid, mol/mol)=0.3, 0.5, and 0.7.

After solvent removal (under vacuum for 12 h), the resulting lipid film was hydrated with 10 mmol L^{-1} Tris–HCl pH 7.4, 150 mmol L^{-1} NaCl, 1 mmol L^{-1} EDTA (buffer A). Small unilamellar vesicles were prepared by sonication and allowed to stay at 30 °C for 24 h to achieve full hydration.

The characterization of the resulting vesicle dispersions and samples was made by means of size and zeta potential measurements, respectively [16].

2.3. Samples

Sample of human whole blood were obtained by venipuncture of ten healthy volunteers aged 20–40 years; K_2EDTA anticoagulant and protease inhibitors cocktail were immediately added. Human plasma (HP) samples were prepared at the Department of Experimental Medicine (Sapienza Università di Roma) in accordance with the institutional bioethics code.

HP from each donor was pooled, split into aliquots, and stored at −80 °C in Protein LoBind tubes (Eppendorf, Hamburg, Germany) until further use. For analysis, the aliquots were thawed at 4 °C and then allowed to warm at room temperature.

2.4. Human plasma-cationic liposome incubation

Incubation of HP with CLs was conducted as described in a previous work [27]. Briefly, 200 μL of HP were incubated with 200 μL of CL suspension in buffer A, at 37 °C for 1 h. After centrifugation (15,000 RCF for 10 min) the pellet, constituted by CL-protein complexes, was washed twice with 250 μL buffer A. For each of the three CL formulations, three experimental replicates were performed.