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Review article

On the use of liposome controls in studies investigating the clinical potential of extracellular vesicle-based drug delivery systems – A commentary

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ABSTRACT

The field of extracellular vesicle (EV)-based drug delivery systems has evolved significantly through the recent years, and numerous studies suggest that these endogenous nanoparticles can function as efficient drug delivery vehicles in a variety of diseases. Many characteristics of these EV-based drug delivery vehicles suggest them to be superior at residing in the systemic circulation and possibly at mediating therapeutic effects compared to synthetic drug delivery vehicles, e.g. liposomes. In this Commentary, we discuss how some currently published head-to-head comparisons of EVs versus liposomes are weakened by the inadequate choice of liposomal formulation, and encourage researchers to implement better controls to show any potential superiority of EVs over other synthetic nanoparticles.

1. Introduction

The interest in using small extracellular vesicles (EVs) as drug delivery vehicles in different disease conditions has seen a steady increase in recent years [1,2]. A large body of studies have provided results that highlight a possible relevance for developing drug delivery platforms utilizing either autologous or heterologous EVs to transport drug compounds into an area of disease [1,3]. However, while the therapeutic effect obtained by EV-based drug delivery systems is well-documented in most studies published within this field, drawing conclusion on a possible superiority of EV-based drug delivery systems compared to traditional, synthetic, drug-loaded nanoparticles is currently based on much vaguer evidence (please refer to Fig. 1 for an illustrated comparison of the advantages of liposomes and EVs for drug delivery). In this Commentary, we would like to stress the importance of implementing a gold standard liposomal control (e.g. clinically approved) when evaluating the therapeutic benefits of EV-based drug delivery systems to ensure that these systems have a potential of being superior to the current best practice. We focus on the differences between liposomes and extracellular vesicles for drug delivery given their similarities with respective to the general composition and design, and thus, leave out comparisons to other self-assembling drug delivery system with great clinical potential [4–6].

2. Liposomes and extracellular vesicles

Liposomes are synthetic, enclosed phospholipid bilayer structures produced from a wide variety of phospholipids, and often stabilized by large molar fractions of cholesterol [7,8]. Using such a lipid formulation to produce liposomes facilitates stable encapsulation or loading of drugs depending on their chemical characteristics (hydrophilicity, molecular weight, pH-sensitivity etc.). Because of the production methods, liposomes can be produced as a highly homogenous population of nanoparticles with very small size polydispersity, which reduces the aspect of liposome size as a factor in any given experiment on their therapeutic efficacy [7]. While drug loading into liposomes is not trivial and has to be optimized for any new type of drug, the fact that liposomes are synthetic and produced by self-assembly makes it possible to encapsulate rather large hydrophilic drugs [9]. Liposomes can be endowed with targeting ligands in a controlled manner to produce a homogenous population of drug-loaded nanoparticles both with respect to size and surface-functionalization, hereby allowing for specific

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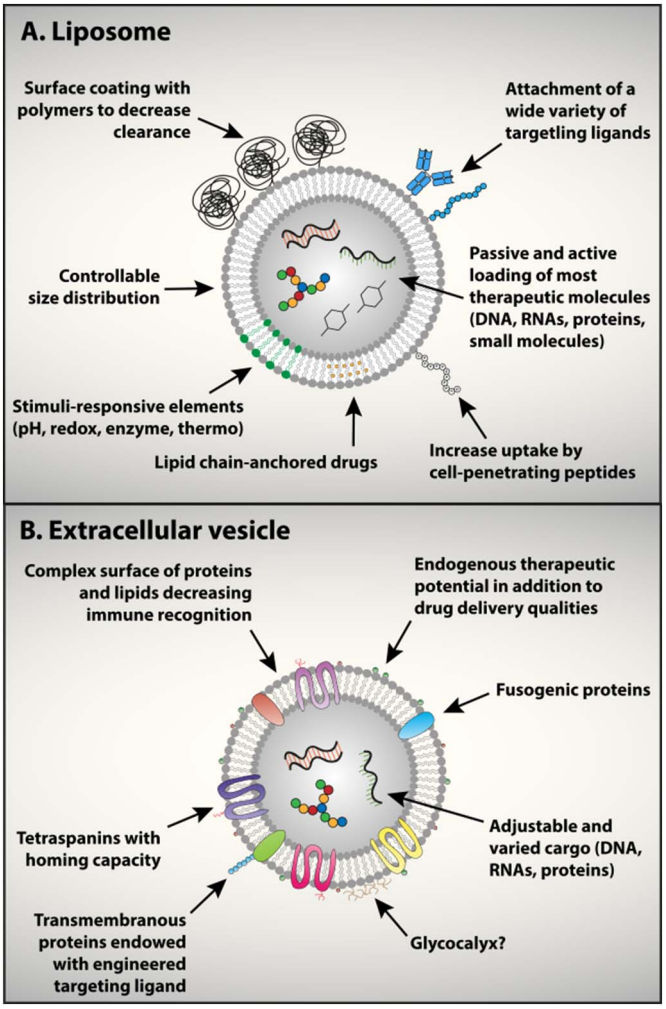

Fig. 1. Comparison of liposomes and extracellular vesicles (EVs) as drug delivery vehicles. (A) Liposomes are characterized by high homogeneity and low polydispersity, which is mostly due to the easily controlled production (which also facilitates loading of many types of drug cargoes). They can be modified to have polymers attached to their surface that increase their circulatory properties (curled black lines). This is important for therapeutic efficacy. To target certain cell populations in a different tissues or secure specific uptake in such tissues, the liposomes can be endowed with targeting ligands (e.g. antibodies). Furthermore, liposomes can be designed to possess specific controlled release mechanisms (due to inclusion of sensitive elements like lipids or peptide linkers in the formulation) or improved cellular uptake capabilities (by endowing the liposomes with cationic lipids or cell-penetrating peptides). (B) EVs generally vary in their size distribution, and the surface protein and RNA cargo composition is also very heterogeneous. This illustrates how these drug delivery vehicles are produced from cells. They can carry different types of cargo, including DNA, RNA, proteins, and maybe even exogenously loaded small molecule drugs. Due to their endogenous origin, the production of EVs for drug delivery purposes can be difficult in terms of controlling the design, drug loading, and yield. Still, because EVs are so advanced in their lipid and surface protein composition, they may be better fit for being present in different body compartments including the systemic circulation. The complex surface of the EVs also suggests improved capabilities of targeting and cellular uptake (due to the expression of tetraspanins and fusogenic proteins, respectively), together with a theoretical reduction in immune recognition.

accumulation in areas of disease and/or improved uptake into cells expressing the receptor for the attached ligand [7,10,11]. Like most types of nanoparticles, liposomes are readily taken up by the mononuclear phagocyte system (MPS) in the liver and spleen after opsonisation, which reduces their circulatory properties substantially [12–16]. However, years of development has discovered that surface-functionalization with polymers like polyethylene glycol (PEG) improves the circulatory properties significantly, paving the way for the so-called long-circulating liposomes. These long-circulating liposomes have proven important for efficient accumulation of liposome-encapsulated drug in areas of disease [7].

EVs are produced by most cell types in the human body and have been proven to be important mediators of paracrine signalling, and hence, they are crucial for the continuous communication between cells in different tissues [17]. They are composed of a complex mixture of lipids and proteins that differs with respect to the pathway of biogenesis and cell type of origin [17,18]. While some of these differences may be explained by differences in isolation methodology, lipid analyses even point towards a large variation in the composition of EVs with a similar size distribution [18]. As for the protein composition, the same kind of variation can be observed [18,19]. Importantly, the protein pattern on the EV surface depicts the homing capacity of the individual EV, i.e. the surface proteins function as targeting ligands that ensures preferential accumulation of the EVs at specific sites in the body (Fig. 1) [20]. The variety of EV types and their corresponding compositions are therefore of great interest as so-called liquid biopsies to search for biomarkers of normo- and pathophysiological conditions and as drug delivery vehicles [2,21]. Compared to liposomes, EVs are much more diverse in their lipid composition (Fig. 1), and hence, together with the large number of different proteins, some suggest that the complex EV surface may make them more suitable for being in the body (e.g. in the systemic circulation) [1,18]. Membrane proteins on the EV surface can also be modified to express exogenous targeting ligands for controlled accumulation or uptake into cells [22], although the process leading to successful expression of peptides on the EV surface is not well understood and difficult to control [23]. Drug loading remains an issue for the use of EVs as drug delivery vehicles [24]. Because EV-based drug delivery vehicles are isolated as completed bilayer spheres, the types of drug that can be passively loaded into the EV core is also very limited. Loading of an exogenous cargo, e.g. via electroporation, has been attempted in several cases of EV-based drug delivery, but these methods likely also lead to adverse effects on both the cargo and the EVs [1,3,25,26]. Instead, nucleic acids (like siRNA, miRNA etc.) can be overexpressed in the EV-producing cell, which in principle will lead to a high concentration of a given RNA-fragment as the EV cargo [24,27]. A circumvention of the loading issue has been to produce EV-mimetics from the plasma membrane fractions of cells, hereby obtaining the favourable, advanced surface composition of the drug delivery vehicle, while still allowing for encapsulation of water-soluble drugs [28].

3. Are extracellular vesicles better than liposomes for drug delivery?

The main argument for utilizing EVs as drug delivery vehicles is the fact that they are suggested to be stable in the systemic circulation (possibly due to their low immunogenicity), have an endogenous homing capacity (although not yet fully elucidated), and the possibility of modulating the surface protein composition and cargo (e.g. by modulating the producing cell, Fig. 1) [1]. The endogenous homing capacity may make the EVs able to cross biological membranes that are not easily traversable by synthetic nanoparticles, especially if these are not functionalized with a targeting ligand [29–31]. These characteristics of EVs may in total infer a better pharmacokinetic profile compared to that of synthetic nanoparticles such as liposomes, however, this difference may also be severely affected by the heterogeneity of the EVs obtained from a biological sample and the process by which the EVs are isolated [32,33].

All of these positive aspects would in theory suggest an EV-based drug delivery system to be superior in therapeutic efficacy compared to liposomes [1]. Indeed, several studies have been published within the last few years presenting interesting results on differences between EVs and liposomes in relation to their uptake profile or therapeutic efficacy in vitro and in vivo [28,34–38]. Overall these papers suggest that EVs associate better with target cells in vitro and in vivo, which may be a testament to their special surface composition. For example, tumor