What does the future hold for chemotherapy with the use of lipid-based nanocarriers?

COMMENTARY

"What does the future hold for chemotherapy with the use of lipid-based nanocarriers?"

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Introduction

Cancer is one of the leading causes of death worldwide and, although many advances in therapy have been attained, some issues remain to be addressed. Nowadays, the most common approach is administration of chemotherapeutic agents, which are not tumor cell-specific and present several side effects both at cellular (e.g., multidrug resistance onset) and physiological levels, causing a series of adverse symptoms in already debilitated patients. In the last two decades, thanks to nanomedicine, many nanocarriers have been designed for cancer diagnosis and treatment (i.e., theranostics) [1]. Nanomaterials display many advantages with respect to conventional therapies: i) they present a very small size, with a high surface-to-volume ratio, ii) they can be functionalized with specific ligands for targeting purposes, and iii) they are known to selectively accumulate around tumor tissues because of the enhanced permeability and retention (EPR) effect [4].

Among different kinds of nanoparticles, lipid nanocarriers can encapsulate hydrophobic/hydrophilic molecules, enhancing their stability and prolonged release. Many studies have demonstrated their efficacy in association with chemotherapeutic agents. It is worth mentioning Doxil®, constituted by doxorubicin-loaded Polyethylene glycol (PEG)ylated liposomes, the first nanoparticles-based drug approved by Food and Drug Administration (FDA) for medical application [2]. Lipid nanosystems are very versatile and can be modified for avoiding immune system recognition, functionalized for targeting a specific tumor cell type, and engineered for responding to external stimuli [3].

Many kinds of lipid-based nanoformulations are currently under evaluation in basic research and clinical context, and some of them are already commercially available. Here, we report the most innovative and recent advances in the field of lipid nanocarriers and discuss their key advantages towards cancer therapy.

Lipid nanocarriers for cancer therapy

Liposomes

Significant contributions to cancer therapy have been given by liposomes, the most diffused nanovectors produced to deliver drugs to cancer cells. Liposomes are spherical vesicles formed by

the self-assembly of phospholipids. These are amphiphilic macromolecules that, in aqueous environments, tend to form lipid bilayers where the hydrophobic fatty acid tails is confined within the bilayer, whereas the polar heads, consisting of a phosphate group, are oriented toward the solvent. This cell membrane-like structure, the fact that phospholipids are the main components of cell membranes, and their biocompatibility make liposomes good candidates for applications in nanomedicine [4]. Their peculiar morphology allows them to encapsulate both hydrophilic and hydrophobic drugs. The first ones are usually enclosed in the aqueous core. Hydrophobic drugs, on the other hand, are intercalated in the lipid bilayer, limiting possible toxicity to healthy tissues and increasing their bioavailability [5]. In order to give the desired properties to liposomes, other lipids and/or cholesterol are often added to the formulation. For instance, cholesterol is used to increase the fluidity of the lipid bilayer [6]. Moreover, a layer of poly(ethylene glycol) (PEG) is usually added to impart steric stabilization, to extend blood circulation, and to reduce uptake from mononuclear phagocytic system. Some formulations have been designed to be responsive either to external (e.g., ultrasounds, light and temperature) or internal (e.g., pH and specific enzymes) stimuli [7]. Knights-Mitchell *et al.*, as an example, developed thermosensitive liposomes loaded with doxorubicin and coated with gold, which were responsive to near-infrared laser illumination. Experiments demonstrated that the drug was released rapidly and in a more controlled way with respect to conventional chemotherapy [8].

Micelles

Micelles are spherical aggregates made of amphiphilic lipids (generally with a single lipid tail), where the lipophilic portion is packed in the nanoparticles core and the hydrophilic region faces the aqueous solvent. Hydrophobic drugs are, therefore, loaded in the core of the micelle. The hydrophilic shell, usually decorated with PEG, imparts higher solubility and stability to the system [9]. Micelles have demonstrated to be able to deliver many chemotherapeutic cargoes. Hybrid polymeric micelles, instead, are formed from the self-assembly of lipids conjugated to PEG [10].

For example, PEG-phosphoethanolamine micelles were able to selectively induce apoptosis just in cancer cells, leaving healthy cells intact [11].

Solid lipid nanoparticles and nanostructured lipid carriers

Solid lipid nanoparticles (SLNs) are composed of lipids that are solid at body temperature, such as mono, di- or triglycerides, fatty acids, waxes, and PEGylated lipids [4]. SLNs ensure a higher drug stability and prolonged release compared to liposomes. Moreover, the use of organic solvents is not necessary during their fabrication procedures, decreasing their toxicity profile. The solid lipid matrix core allows for the encapsulation of hydrophobic drugs; however, its high crystallinity can often lead to a very low drug loading efficiency and/or very slow release kinetics.

More recently, nanostructured lipid carriers (NLCs) have been proposed in order to overcome these issues. NLCs are also composed of lipids, but at least one of them is liquid at room or body temperature. This gives rise to the formation of partially crystalline solid matrices, increasing the loading capacity of the nanocarrier [12]. Li *et al.* designed NLCs loaded with a combination of lapachone and doxorubicin, that were effective in overcoming multidrug resistance [13].

An interesting study demonstrated that triggered release mediated by hyperthermia was obtained by exploiting magnetic SLNs loaded with paclitaxel, allowing increased and controlled release of the drug to be obtained [14]. Recently, lipid-based magnetic nanovectors were synthesized and loaded with temozolomide. Upon stimulation with alternating magnetic fields, they released the chemotherapeutic agent in a controlled manner, inducing apoptosis of glioblastoma cells *in vitro* [15].

Both SLNs and NLCs have been proposed as a low-cost alternative to the other kind of lipid carriers, due to their easy preparation protocol, that can be conveniently scaled-up, and to their biocompatible and relatively cheap natural components [16].

Active targeting of lipid nanocarriers

In the attempt to reduce the side effects of chemotherapeutic drugs and to increase their efficacy, a lot of effort has been paid in making nanoparticles specific for the site of interest. Nanoparticles are

known to accumulate more in tumor tissues with respect to normal ones due to their size and to different properties of tumor vasculature. This is known as the "enhanced permeability and retention" (EPR) effect. However, active targeting offers a more precise and elegant way to target only selected tissues. In order to achieve this task, the surface of the nanoparticles is functionalized with ligands that specifically interact with receptors that are overexpressed on cancer cells. Usually, the ligands that are used for this kind of applications are antibodies, peptides and proteins, small molecules (like folic acid), and aptamers [4]. For example, antibodies that bind to transferrin receptor have been attached to several kinds of nanoparticles because transferrin receptor is overexpressed by many tumor cells. Cell penetrating peptides, instead, are used to foster the blood-brain barrier (BBB) crossing for the treatment of central nervous system diseases, such as brain cancer [17].

Notably, liposomes have been conjugated to small peptides and loaded with chemotherapeutic agents. Zhao *et al.* created a liposomal system carrying paclitaxel and functionalized with Glu6-RGD peptide that efficiently targeted metastatic bone cancer, showing superior efficacy with respect to the free drug itself [18]. Varshosaz *et al.* functionalized NLCs with trastuzumab (Herceptin) and loaded them with docetaxel, obtaining specific uptake in HER-2 positive breast cancer cells [19].

Clinical trials

Among the different kinds of lipid nanocarriers, research on liposomes is the most advanced in clinical practice for the treatment of several types of cancers. In particular, as already mentioned before, liposomes encapsulating doxorubicin (Doxil®) were the first nanoparticles to be ever accepted by the FDA in 1995 for cancer therapy [2, 4]. Since then, other five liposomal formulations were approved by the FDA (DaunoXome, Myocet, Mepact®, Marqibo®, Onivyde®/MM-398). Currently, clinical trials involving the use of liposomes in cancer therapy are 1862 [17, 20], 1155 of which directly include the word [chemotherapy] in their description. Just in 2018, 213 clinical trials using liposomes for the treatment of cancer started. Interestingly, also a

new kind of stimulus-responsive liposomal formulation is under clinical evaluation: ThermoDox®, in fact, is able to release doxorubicin in response to increased temperature.

Concerning micelles, basically all the clinical trials involve polymeric micelles, while none concerns the use of lipid-based micelles. The same applies to solid lipid nanoparticles and nanostructure lipid carriers. In fact, the only lipid nanoparticles-based system that is currently under investigation to treat patients with refractory locally advanced or metastatic solid tumor malignancies, multiple myeloma, or lymphoma is DCR-MYC, that is a lipid nanoparticle encapsulating a small inhibitory RNA (siRNA) oligonucleotide targeting the proto-oncogene MYC [21]. Strictly speaking, this nanocarrier is not applied for chemotherapy; however, it indeed represents a powerful tool in the fight against cancer. It is worth mentioning that in parallel with chemotherapy, other kinds of approach are being studied, giving extremely interesting results. For instance, stable nucleic acid lipid particles (SNALPs) and lipoplexes are an evolution of cationic liposomes for gene delivery purposes [22]. Gene therapy can be applied in cancer therapy by inducing the expression of proapoptotic and chemo-sensitizing genes, the expression of wild type tumor suppressor genes or of genes able to solicit antitumor immune responses, or to deliver small interfering RNAs (siRNAs) for targeted gene silencing [17].

Conclusions

Reported evidences show how incredibly long and complicated is the road to the clinical practice. In fact, although a lot of nanoparticles are being studied at the research level producing an incredible amount of significant data, just few of them reach the clinical trials, and even less are finally accepted and used in clinical practice [17]. Among the main limitations for entering the clinical trial phase, it is possible to highlight: i) the need for a straightforward and reproducible fabrication procedure at large scale, ii) a well-known and characterized physicochemical behavior *in vitro* and, possibly, *in vivo*, iii) the non-toxicity of the nanoparticles, iv) a good biodistribution and drug release kinetics. These are just few of the requisites that a lipid-based nanocarrier, or in general any kind of nanocarrier, should possess before clinical applications can be considered.

Being SLNs and NLCs fairly more recent compared to other kinds of systems, in particular compared to liposomes, their introduction in clinical trials appears to be still in the future [16]. However, given their attractive advantages, the authors hope for a rapid (and not so far in time) development in this sense.

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Disclosures

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