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Lipid prodrug nanocarriers in cancer therapy

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ABSTRACT

Application of nanotechnology in the medical field (*i.e.*, nanomedicine) plays an important role in the development of novel drug delivery methods. Nanoscale drug delivery systems can indeed be customized with specific functionalities in order to improve the efficacy of the treatments. However, despite the progresses of the last decades, nanomedicines still face important obstacles related to: (*i*) the physico-chemical properties of the drug moieties which may reduce the total amount of loaded drug; (*ii*) the rapid and uncontrolled release (*i.e.*, burst release) of the encapsulated drug after administration and (*iii*) the instability of the drug in biological media where a fast transformation into inactive metabolites can occur. As an alternative strategy to alleviate these drawbacks, the prodrug approach has found wide application. The covalent modification of a drug molecule into an inactive precursor from which the drug will be freed after administration offers several benefits such as: (*i*) a sustained drug release (mediated by chemical or enzymatic hydrolysis of the linkage between the drug-moiety and its promoiety); (*ii*) an increase of the drug chemical stability and solubility and, (*iii*) a reduced toxicity before the metabolization occurs. Lipids have been widely used as building blocks for the design of various prodrugs. Interestingly enough, these lipid-derivatized drugs can be delivered through a nanoparticulate form due to their ability to self-assemble and/or to be incorporated into lipid/polymer matrices. Among the several prodrugs developed so far, this review will focus on the main achievements in the field of lipid-based prodrug nanocarriers designed to improve the efficacy of anticancer drugs.
Gemcitabine (Pubchem CID: 60750); 5-fluorouracil (Pubchem CID: 3385); Doxorubicin (Pubchem CID: 31703); Docetaxel (Pubchem CID: 148124); Methotrexate (Pubchem CID: 126941); Paclitaxel (Pubchem CID: 36314).

1. Introduction

In the field of anticancer drug delivery, there has been a growing interest in the use of drug nanocarriers in order to improve therapeutic efficacy and to reduce the risk of adverse reactions due to the inherent toxicity of these molecules [1–4]. A great deal of effort is currently been paid to the design of nanocarriers able to safely transport various kinds of drugs and to efficiently release their load at their site of action [5–7]. Among the different classes of nanoparticulate systems, polymer nanoparticles [8–10], micelles [11], liposomes [12], solid lipid nanoparticles [13] and magnetic nanoparticles [14] are the most extensively investigated.

Usually, drugs are encapsulated/physically entrapped into nanocarriers during the formulation process (*e.g.*, self-assembly for polymer nanoparticles and micelles and thin-film hydration/extrusion for liposomes). Although the use of drug-loaded nanocarriers has conducted to very promising results in the recent literature [15–20], many systems still present severe limitations that may hamper their further translation to clinical trials and therefore to the market. Among them, the '*burst release*', which consists in the quick release of a significant fraction of the drug simply adsorbed at the nanocarrier surface. As consequence a significant fraction of the drug is released before reaching the pharmacological target in the body, leading to low activity and side effects, conversely to a sustained drug release. Additionally, the difficulty to encapsulate poorly-soluble drugs that tend to crystallize, may lead to colloidal destabilization and would necessitate the use of organic co-solvents during the formulation process. Finally, poor drug-loadings are generally achieved (typically a few percent) thus requiring the administration of a high amount of carrier material, which can itself provide toxicity and side effects. For all these reasons, alternative

Abbreviations: 5-FU, 5-fluorouracil; ara-C, cytosine arabinoside; AUC, area under the curve, chol, cholesterol; CLA, conjugated linoleic acid; BLI, bioluminescence imaging; dCK, deoxycytidine kinase; dCK, deoxycytidine kinase; dFdU, difluorodeoxyuridine; DNA, deoxyribonucleic acid; DMPC, dimyristoylphosphatidylcholine; Dox, doxorubicin; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; Dtx, docetaxel; DSPE-PEG, poly(ethylene glycol)–distearoylphosphatidylethanolamine; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; eq., equivalent; FUdR, floxuridine; Gem, gemcitabine; Gem-PSqMA, gem-poly(squalene methacrylate); i.p, intraperitoneal; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; MDR, multi drug resistance; MTD, maximal tolerated dose; MTX, methotrexate; NP, nanoparticle; PEG, poly(-ethylene glycol); PLGA, poly(lactide-co-glycolide); PSMA, prostate-specific membrane antigen; PI, polyisoprene; Ptx, paclitaxel; RES, reticuloendothelial system; SiRNA, small interfering RNA; SLN, solid lipid nanoparticles; $sPLA_2$, secretory phospholipase A2; SQ, squalene; SQDox, squalene-doxorubicin; SQGem, squalene-gemcitabine; SQPEG, squalene-poly(ethylene glycol); SQPtx, squalene-paclitaxel; USPIO, ultrasmall particles iron oxide

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strategies have been developed in order to alleviate or even suppress the aforementioned drawbacks. One of these strategies takes advantage of the prodrug concept [21] and has received considerable attention in the field of nanoparticulate systems. A prodrug is formed by the covalent linkage between a drug and a (macro)molecule, and it is further metabolized *in vivo* in the active form [22]. The use of prodrugs in drug delivery provides important benefits such as: (*i*) a sustained drug release (mediated by chemical or enzymatic hydrolysis of the prodrug); (*ii*) an increase of the drug chemical stability and solubility and, (*iii*) a reduced toxicity before the metabolization occurs [23]. Overall, the prodrug strategy provides a rationale for achieving tailor-made physico-chemical, pharmacokinetic and pharmacological features.

By combining the prodrug strategy with the use of nanoparticulate systems as drug carriers, optimized formulations have been recently reported [24,25]. They helped to resolve the poor solubility of some prodrugs, to reduce adverse effects, and therefore to improve cancer therapy. Among the different classes of promoieties covalently conjugated to the drugs, lipids such as fatty acids, cholesterol derivatives, phospholipids or triglycerides, have been extensively used. They perhaps represent the materials of choice due to their biocompatibility. This explains why lipids have also been intensively used as nanocarrier materials (*e.g.*, liposomes, solid-lipid nanoparticles and nanoemulsion). Indeed, prerequisites regarding the design of nanocarriers for drug delivery are clear: the materials must be nontoxic, biocompatible and cleared from the body, for instance by biodegradation or bioerosion. Noteworthy is to mention that many of the currently available nanomedicines or in late clinical phases are made of lipids: Myocet® (liposomal doxorubicin), Caelix® (PEGylated liposomal doxorubicin) and Ambisome® (liposomal formulation of amphotericin B) [19]. Additionally, structural similarities between lipid-based nanocarriers and lipid prodrugs may facilitate prodrug loading/insertion, for instance by a facile nanocarrier membranes anchoring. In this regard, the present review will discuss main achievements in the field of lipid-based prodrug nanocarriers and will focus on cancer therapy. The readers interested in polymer prodrug nanoparticles can refer to the following recently published review [25].

2. Nanoparticulate lipid prodrugs for drug delivery

For the sake of simplicity, the structures of all anticancer drugs discussed herein and their coupling sites are reported in Fig. 1. Classification of the anticancer drugs, lipids used for their derivatization and the nanoscale drug delivery system based on these prodrugs are reported in Table 1.

2.1. Antimetabolite agents

Since their introduction in the clinical setting in the 1950s, antimetabolites (*i.e.*, drugs that interfere with essential biosynthetic processes) have gained an important role in the treatment of various tumor types, in monotherapy as well as in combination with other chemotherapeutic agents [26,27]. Despite their different mechanisms of action, which include: (*i*) reduction of purine and pyrimidine synthesis; (*ii*) incorporation into DNA as false nucleosides (*e.g.*, pyrimidine analogues) and (*iii*) interference with various enzymes involved in synthesis of nucleic acids, they all converge in the inhibition of the DNA synthesis and the induction of cell apoptosis [26,28–30].

Insurgence of cellular resistance to methotrexate (MTX) (inhibitor of the dihydrofolate reductase) together with its systemic toxicity represents a serious problem for the efficacy of the treatments and much effort has been devoted to the design of MTX derivatives with improved pharmacological profiles. For instance, methotrexate has been linked to *rac*-1,2-dioleoylglycerol through ester linkage. The resulting lipophilic prodrug, containing two aliphatic acyl chains, was formulated with phosphatidylcholine and phosphatidylinositol leading to mixed liposomes with a diameter in the 100–150 nm range (Fig. 2) [31,32] which enabled to overcome tumor cell resistance *in vitro* (114 times reduction of resistance in human leukemia cells compared to free MTX) [32]. To be noted however that safety issues were associated to these liposomes as they induced a significant complement activation (*i.e.*, release of C3a and C consumption) and reduction of plasma clotting ability in a dose-concentration manner [33].

Among the nucleoside analogues, gemcitabine (Gem) (deoxycytidine analogue) has been extensively used in clinical practice for the treatment of various solid tumors (*e.g.*, colon, lung, pancreatic, breast, bladder and ovarian cancers) [34]. Unfortunately, its therapeutic efficacy is restricted by some serious limitations such as: (*i*) short biological half-life due to rapid metabolization *via* deamination, (*ii*) poor diffusion into cells due to its hydrophilic nature and (*iii*) induction of resistance at several levels. Various strategies have been adopted to improve its metabolic stability and enhance its cytotoxic activity and Gem is by far the most explored antimetabolite in the lipid prodrug field. Lipophilic 4-(*N*)-acyl-derivatives protected Gem against deamination to the inactive difluorodeoxyuridine (dFdU) metabolite and facilitated the loading of the drug with high efficiency into the bilayer of liposomes, whereas free Gem easily escaped from the aqueous compartment. Conjugation to saturated and unsaturated C18 and C20 long chains was patented by Eli Lilly in 1998 (US6384019 B1) [35]. Then, several Gem derivatives have been prepared by acylation of the amino group with various linear chain lipids (*e.g.*, C5 (valeroyl); C7 (heptanoyl); C12 (lauroyl), C18 (stearoyl) in chain length) and the resulting conjugates were incorporated into liposomes [36–39]. The entrapment efficiency was strictly correlated to the liposome composition with highest values achieved with the stearoyl derivatives. Noteworthy is that in the presence of unsaturated phospholipids such as the egg phosphatidylcoline, efficient entrapment required the addition of cholesterol to the liposome formulation, while in the case of liposomes made of saturated phospholipids the prodrugs were easily encapsulated thanks to the structural similarities between linear acyl chains [39].

Composite liposomes containing phospholipids and lipid-based Gem prodrugs were shown to protect the parent molecule thus ensuring enhanced plasma half-time and intracellular release of the drug [39]. Compared to free Gem, the prodrugs exhibited improved affinity with lipid vesicles employed as both model biomembranes and carriers in the transport of antitumor drugs [37]. This study suggested that the prodrug lipophilic tail might modulate the transport and the release of Gem inside the cellular compartments. *In vivo* pharmacokinetic and efficacy studies on two different tumor models (*i.e.*, HT-29 colorectal and KB 396p nasofaringeal carcinoma) clearly revealed that stearoyl-gemcitabine (C18Gem) prodrug-containing liposomes increased the plasma half-life of Gem which, associated to a slow release of the drug from the formulation, resulted in an elevated antitumor activity (higher reduction of the tumor mass) compared to the free drug [36].

The possibility of the incorporation of the stearoyl gemcitabine derivative (C18Gem) into other nanocarriers such as micelles [40,41], polymer nanoparticles [42], solid lipid nanoparticles (SLN) [43–46] or, self-assembled nanoparticles [47] has been also investigated.

Thus, C18Gem was incorporated in SLN, prepared by lecithin/glyceryl monostearate in water-emulsion, both naked and PEGylated by means of DSPE-PEG_{2000} [46]. PEGylation conferred long circulating abilities to the resulting nanoparticles, decreased the uptake in the organs of the reticuloendothelial systems (RES) and significantly increased the accumulation into tumor after intravenous injection (6.3 folds) (Fig. 3), but the anticancer activity was not improved. Both formulations were more effective than the free drug (Fig. 3) [46]. These SLN were further modified by conjugation of the PEG chains to the epidermal growth factor (EGF) [45] in order to target the EGF receptor (EGFR), whose overexpression is observed in several cancer cells [48]. Ligand-mediated targeting was confirmed *in vitro* on three breast cancer cell lines (MCF7, MDA-MB-468 and MDA-MB-215) which revealed that uptake and cytotoxicity were dependent on the cell surface density of the receptor. Functionalization also enhanced tumor accumulation in MDA-