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Review article

Recent studies on the delivery of hydrophilic drugs in nanoparticulate systems

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ABSTRACT

Some approaches to enhance the entrapment efficiency within nanoparticulate systems, chiefly of hydrophilic molecules, developed mainly by the Turin University Pharmaceutical Technology group, are discussed. Several approaches have been developed: the first entails associating anticancer hydrophilic molecules to liposomes, developing lipophilic prodrugs of these molecules, and encapsulating them in liposomes. The transformation of hydrophilic drugs into lipophilic prodrugs can also overcome problems of poor entrapment efficiency and rapid release from polymer nanocarriers. Examples are nanospheres and nanocapsules produced from various PEGylated poly(alkylcyanoacrylate) copolymers. Strategies have also been developed to enhance hydrophilic drug entrapment in solid lipid nanoparticles (SLN). Hydrophobic ion pairing was designed to enable various antitumor drugs to be entrapped in SLN, produced by the coacervation method. Another technique comprises the covalent linkage of antitumoral or antiviral drugs to a squalenoyl-derived chain, affording bioconjugates that self-assemble as stable nanoparticles. A further development comprises mesoporous silica nanoparticles with immobilized hydrophilic antioxidants, for topical applications: they were complexed with hydrophilic antioxidants (Trolox® or rutin). Polymer-shelled and perfluoropentane-cored nanobubbles have also been designed, as versatile multifunctional carriers for the delivery of gases, drugs, and genes; the size range is below 500 nm, with shell thickness in the 30–50 nm range.

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1. Introduction

Colloidal systems, such as liposomes, nanoparticles, and microemulsions, have generally been reported in the literature as

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carriers of hydrophobic drugs. However, the delivery of hydrophilic molecules is also a challenging goal, and one that requires a multidisciplinary approach. This review essentially focuses on the various strategies that may be employed to deliver hydrophilic active substances.

Many drugs are hydrophilic, and of these many are low-molecular-weight molecules (less than 500 Da). The United States Pharmacopeia (USP) classifies hydrophilic drugs, in the range from very soluble to soluble in an aqueous medium, if their solubility is above 33 mg/ml. Hydrophilic drugs are often subject to low intracellular absorption, enzymatic degradation, rapid clearance, suboptimal distribution, development of resistance, poor pharmacokinetics, low therapeutic index and, in the case of antitumoral drugs, failure to accumulate and be retained within the tumor. Drug entrapment in colloidal delivery systems can, in many cases, overcome these difficulties, since it may improve pharmacokinetics, protect the drug against *in vivo* degradation, sustain drug release, increase patient comfort by avoiding repetitive injections, and reduce side effects. In the case of antitumoral drugs, nanoparticulate systems also possess the advantage of enhancing permeability and retention (the EPR effect), resulting in their higher accumulation in tumors [1,2]. The possibility of modifying the surface of nanoparticulate systems, for example by PEGylation, leads to stealth colloidal systems, which can escape rapid uptake by the mononuclear phagocyte system; the addition of specific ligands onto their surface can also provide more effective release at the target site.

This review examines the different approaches, developed mainly by the Pharmaceutical Technology Group of the University of Turin, describing the most relevant preclinical results. Some of the new technologies developed for the delivery of hydrophilic drugs are also applicable to certain very low water soluble molecules, and are thus mentioned in brief.

1.1. Liposomes

Liposomes are nanoconstructs, usually sized at the nanoscale, and consist of natural or synthetic phospholipids surrounding a water core. Since phospholipids are the major component of liposomes, they possess low toxicity, and are biodegradable and biocompatible. Liposomes form spontaneously when phospholipids are dispersed in water; when composed of natural phospholipids, they are biologically inert, weakly immunogenic, and possess low intrinsic toxicity. Depending on the number of layers and the diameter, liposomes are classified as multilamellar vesicles (MLV), large unilamellar vesicles (LUV, diameter 100–400 nm), and small unilamellar vesicles (SUV, diameter < 100 nm). They are also classified by surface charge (zeta potential), as cationic, neutral, or anionic liposomes.

Thanks to their favorable characteristics, liposomes have been widely used as carriers for different kinds of drugs. A number of different approaches and preparation methods are available to encapsulate hydrophilic and hydrophobic antitumoral drugs [3]. Anticancer drugs with different physico-chemical features have been encapsulated in liposomes, with the goals of improving their cytotoxic activity, extending their plasma half-life, and reducing their side effects. The encapsulation of low-molecular-weight water-soluble drugs is, in most cases, characterized by poor entrapping efficiency and rapid leakage *in vivo* [4], limiting the liposomes' shelf life and clinical utility. To overcome these problems, lipophilic prodrugs of hydrophilic drugs were developed and encapsulated in liposomes.

This approach was first used to prepare liposomes and immunoliposomes containing 5-fluorouridine (5-FUR) lipophilic prodrugs [5]. The parent drug, 5-FUR, and the prodrugs 5′-succinyl-5-FUR, and 5′-palmitoyl-5-FUR, were encapsulated in liposomes, and encapsulation efficiency, drug leakage, stability, and size were evaluated. The results showed that 5′-palmitoyl-5-FUR was the most suitable for incorporation in liposomes, in terms of minimum leakage and high encapsulation efficiency. Moreover, differential scanning calorimetry (DSC) analysis showed that the compound interacted strongly with the liposomal bilayer. To increase the efficiency of drug delivery, these liposomes were then further conjugated with an AR-3 monoclonal antibody, targeted to human colon carcinoma. The immunoliposomes were prepared from phospholipidic vesicles, incorporating a maleimido reactive derivative of phosphatidylethanolamine, able to react with the thiolated monoclonal antibody, as shown in Fig. 1.

The *in vitro* antitumor activity of these liposomes and immunoliposomes was determined on the HT-29 human colon carcinoma cell line; the results showed that the targeted liposomes were more cytotoxic than their conventional counterparts. Both plain and targeted liposomes were then injected i.p. into athymic mice grafted with the HT-29 cell line; again, the immunoliposomes were more effective as antitumor agents than the plain variety. Only 5% of residual tumor mass was present at the end of treatment, in mice treated with the immunoliposomes.

Gemcitabine (GEM) is an anticancer drug indicated for pancreatic cancer, which is rapidly deaminated to an inactive metabolite; it must therefore be administered at very high doses. A series of prodrugs of GEM were synthesized, with the aim of improving its metabolic stability and facilitating its encapsulation in liposomes [6]. The GEM 4-amino group was derivatized with C5, C12, and C18 linear-chain acyl derivatives (Fig. 2). Stability of the prodrugs during storage, in buffers, in plasma, and with the lysosomal intracellular enzyme cathepsins were evaluated.

Stability tests in buffers confirmed the high stability of the amide bond. In plasma, likewise, the prodrugs showed high stability and marked resistance to deamination. On incubation with cathepsins B and D, to evaluate the selective hydrolysis of the amide bond by liposomal enzymes inside the cell, after 24 h, 60% of the prodrug had undergone hydrolysis of the amide bond, without any degradation of free GEM.

The characteristics of the liposomes containing these prodrugs were also studied, and it emerged that GEM lipophilic derivatives containing C12 and C18 acyl chains showed the best results, in terms of incorporation into liposomes; the corresponding drug-loaded carriers showed greater cytotoxic activity than the free drug, against both HT-29 colon and KB nasopharyngeal human carcinoma cell lines [6]. The pharmacokinetic behavior and the *in vivo* antitumor activity of the parent drug, of the gemcitabine prodrug alone, and of the prodrug encapsulated in liposomes, were evaluated. Liposomes containing the prodrug showed longer plasma half-life and lead to greater tumor regression than either control or gemcitabine [7].

Through DSC, the GEM lipophilic prodrugs were studied in interaction with biomembranes, so as to any correlation between acyl chain length and the ability to interact with a model biomembrane in an aqueous compartment: the goal was to obtain biomimetic information on the prodrugs' lipophilic character and solubility [8]. Liposomal MLV and LUV were used as synthetic biomembrane models; they were composed of dimyristoylphosphatidylcholine (DMPC) and of distearoylphosphatidylcholine (DSPC). These studies entailed inserting the GEM lipophilic prodrugs into the biomembrane models, transferring these compounds through an aqueous medium, and observing their migration to empty liposomes from liposomes containing a known quantity of GEM or of its 4-(*N*)-acyl derivatives. The results showed that free drug alone does not interact with the biomembrane models used; conversely, the 4-(*N*)-acylgemcitabine derivatives interacted with the biomembrane models, this interaction