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NANOSIZED DRUG DELIVERY SYSTEMS FOR PLATINUM-BASED ANTICANCER DRUGS

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Abstract. The current review highlights the main advances achieved in utilizing nanosized carriers for optimized drug delivery of cisplatin and other cytotoxic platinum coordination compounds. The treatise is emphasized on exemplary types of carriers (e.g. liposomes, nanoparticles, polymeric micelles, dendrimers, inclusion complexes), their generic properties, advantages, drawbacks, biopharmaceutical and pharmacological aspects as well as the major outcomes of the reported in vitro and in vivo investigations.

Key Words: antineoplastic agents; targeted drug delivery; EPR effect; PEG; liposomes; nanoparticles; polymeric micelles; dendrimers; molecular hosts

Introduction

The platinum coordination compounds are unequivocally among the most important antineoplastic agents used at present for the management of solid tumors [1-3]. The successful commercialization and clinical introduction of the prototype cisplatin in the early 1970`s has revolutionized the management of testicular cancer – which is until now among the few examples of successfully curable solid malignancies [3-5]. Furthermore cisplatin is widely used for the treatment of ovarian cancer, head and neck squamous cell carcinoma and has an important palliative role in many other solid tumors [3]. Despite the clinical success of cisplatin, however, it suffers from poor selectivity upon malignant cells and is associated with significant toxic effects upon the kidneys, the peripheral nerves and the auditory system; moreover, cisplatin is the most emetogenic antineoplastic agent [6, 7]. Apart from this unfavorable toxicological profile, there are other significant hurdles limiting the usefulness of cisplatin: the intrinsic unresponsiveness of some common neoplastic diseases e.g. colon adenocarcinoma, the development of acquired resistance in initially responsive tumors in the course of treatment [1, 8]. Thus the clinical efficacy of cisplatin, together with its major limitations have fuelled intensive research efforts focused upon the development of analogues with better tolerability, broader antineoplastic spectrum and/or superior activity in comparison to the prototype. To meet these objectives thousands of platinum coordination compounds have been synthesized and evaluated, which up to now resulted in the successful development and commercialization of five clinically utilized cisplatin analogues (Depicted in Fig. 1) [1, 4, 5]. Among these carboplatin and oxaliplatin have widespread clinical use [1], whereas the remaining drugs are available only in particular countries – nedaplatin in Japan [9], lobaplatin in China [2, 10] and heptaplatin in South Korea [11-14]. Unfortunately none of the successors of cisplatin could be considered superior to the prototype in terms of lower toxicity, superior clinical efficacy and bypassing resistance mechanisms; for instance nedaplatin and carboplatin are totally cross-resistant with cisplatin and the significant amelioration of nephrotoxicity characteristic for these drugs is achieved at the expense of reduced cytotoxicity against malignant cells [2]. Oxaliplatin, although showing low degree of cross-resistance with cisplatin does not affect a vastly different spectrum of tumors and has rather limited clinical utility so far [1]. Furthermore despite the low nephrotoxicity of the novel platinum drugs they display modified rather than reduced toxicological potential compared to cisplatin, whereby the

dose-limiting toxicity is switched to myelosuppression in carboplatin, nedaplatin and lobaplatin and to severe peripheral neuropathy in oxaliplatin [1, 2, 9].

The failure of existing platinum drugs to overwhelm the limitations of cisplatin as an anticancer drug, especially regarding the issues of resistance could be greatly ascribed to their structural resemblance to the prototype and hence their closely-related biochemical and pharmacological properties. More recently the advances of bioinorganic chemistry and the unraveling of the mode of action of cisplatin and the resistance mechanisms gave rise to more elaborate and rational approaches for designing promising anticancer platinum complexes [4, 5].

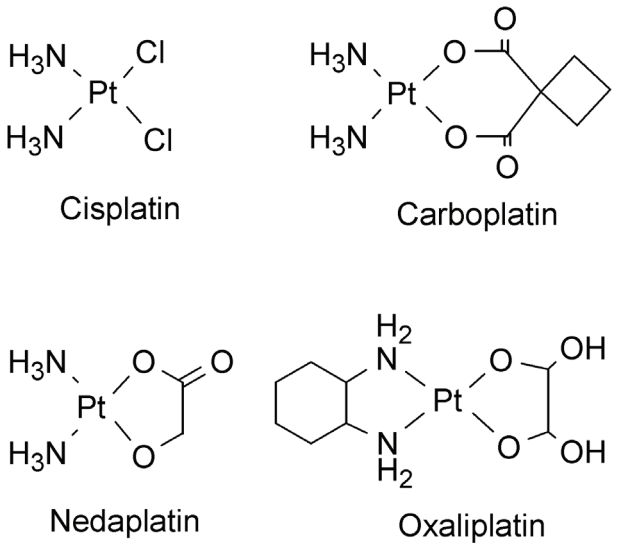

Fig.1 *Chemical structures of the most important clinically used platinum-based anticancer drugs*

Apart from the lengthily and expensive design and elaboration of novel analogues one of the most attractive alternative strategies to overcome the limitations of cytotoxic drugs, including platinum metallopharmaceuticals is their formulation into nanopharmaceutical platforms, i.e. nano-scale carriers, such as liposomes [15-17], polymeric nanoparticles (nanospheres, nanocapsules, polymeric micelles, multiarm core-shell co-polymers, protein or polysaccharide conjugates *etc*.) [18-20], and more recently into nano-containers based on host-guest interactions [21-26]. Due to their unique properties the nanopharmaceuticals offer significant advantages over classical parenteral formulations of anticancer drugs and have been well demonstrated to decrease drug binding to non-pharmacological targets, to favorably alter the systemic and intratumoral trafficking of encapsulated agents and to greatly ameliorate the debilitating dose-limiting toxicities, associated with this class of antineoplastic drugs [27, 28].

To a great extent this is due to the fact that drugs are encapsulated within nanocontainers with a controlled microenvironment, whereby the drug is protected from side interactions with body tissue components, xenobiotic efflux transporters and biotransformation systems. Thus the pharmacokinetic and tissue distribution of a drug encapsulated in a nanoplatform are no more dependent on its intrinsic properties, but are governed by the tissue disposition and elimination patterns of the carrier [29, 30]. Moreover, additional benefits of nanoparticulate systems include sustained or trigerrable release kinetics, increased bioavailability at the respective targets sites with concomitant increased efficacy, reduction of the nominal dosage required and amelioration of the severity and incidence of adverse reactions [29-31].

This review is focused on representative examples of nanopharmaceutical platforms for platinum coordination compounds with special emphasize on liposomes, globular architecture polymeric nanoparticles (micelles, dendrimers and stars) and macrocyclic molecular hosts.

Strategies for passive of active tumor targeting of platinum drugs

It is well known that the growth of solid malignant tumors is dependent on a process of *de novo* formation of blood vessels known as angiogenesis [32, 33]. The newly formed vasculature of tumors however is leaky relative to the vessels in normal tissues which makes solid tumors hyperpermeable towards colloid-sized carriers, e.g. liposomes and polymer nanoparticles [16, 34, 35]. The compromised barrier function of the vasculature, together with the inadequate lymphatic drainage of tumors conditions the accelerated accumulation of blood-borne nanoparticles, i.e. the 'enhanced permeability and retention effect' (EPR effect) has been the central paradigm that has fuelled the development of antineoplastic nanopharmaceuticals during the last three decades [32, 34-38].

One of the hallmark challenges associated with nanocarriers is that these have to circulate long enough in order to attain enough accumulation at tumor lesions *via* the EPR [30, 39, 40]. Due to the colloidal size of nanocarriers these are recognized and phagocytized by the cells of the mononuclear phagocyte system (MPS) (previously designated as reticuloendothelial system) which leads to disappointingly short

circulation half-lives [16, 41-43]. The most important approach towards bypassing MPS sequestration has been the incorporation of PEG residues on the surface of polymer particles or liposomes [39, 44-47]. PEGylation creates a hydrophilic repulsive barrier around nanocarriers which increases their colloidal stability, hinders interactions with serum components and opsonins, and eventually prevents recognition by the MPS cells [39]. This imparts MPS-avoidance or "stealth" properties to the delivery device, increasing its systemic circulation time significantly [27, 39, 46, 48]. Moreover, PEGylation of macromolecular carriers has been well documented to favorably decrease their immunogenicity [49], although some of the adverse effects associated with stealth liposomes, have been attributed to immune responses.

While the EPR-driven passively targeted systems have been extensively explored and dominate the commercialized and clinically tested nanopharmaceutical medicinal products these are by no means ideal substitutes for the conventional dosage forms of anticancer drugs. Albeit EPR effect results in selective delivery progressively over time, it has been shown that PEGylation while beneficial for the circulation time is detrimental for the cellular uptake of nanocarriers [16, 40]. Thus although anticancer drugs are selectively accumulated inside tumors they are retained within the carrier, which could result in subtherapeutic bioavailability inside cells, reduced efficacy and emergence of drug-resistance [16]. Hence there is a need for attaining augmented intracellular and localized, on-demand drug release in order to beneficially modify the efficacy of nanopharmaceuticals [27, 28]. On these grounds state-of-the art research has been shifted from EPR-driven systems towards more sophisticated nano-vehicles for actively targeted drug delivery of antineoplastic agents [27-29, 50]. This is achieved by surface decoration of the nanoplatforms with homing ligands ensuring site specific delivery or by use of "smart" technologies attaining triggered, on-demand release of encapsulated cargo in response to environmental stimuli or remote triggers, e.g. ultrasonication, hyperthermia, and magnetic fields [18, 20, 27, 28, 51].

Surface decoration of nanocarriers with ligands able to bind with high affinity membrane receptors over-expressed in tumor cells is among the most widely explored approaches towards tumor targeting [18, 27, 28]. If such modification is concomitant with long circulating properties and ability to passively accumulate inside solid tumors *via* the EPR effect, the active targeting is expected to further increase the specific interactions with cancer cells. Moreover, if the targeting ligand is binding to internalizing surface epitopes of cancer cells is could also aid to increased intracellular uptake of the carrier and its cargo *via* receptor-mediated endocytosis [18].

If the receptor is highly specific for the tumor cell usually the ligands are coupled to the PEG coating of the nano-carrier (i.e. pendant type ligands) [40, 44]. In a dissimilar fashion if the pendant-type ligand is expected to bind to receptors outside tumors, or to evoke immune recognition, both leading to decreased tumor accumulation of the carrier, the homing moiety could be grafted on the surface of the nanoparticle and hence its interactions with the receptor would be hampered by the protective PEG coating [44]. The most widely used homing moieties to ensure active targeting of nanoparticles and liposomes are monoclonal antibodies (mABs) [45]; small molecule cofactors and vitamins such as folate [52], riboflavin [53], biotin [54-56]; hormones and hormonal analogues [57-60]; transferrin [61, 62], carbohydrates [63, 64], ligands for adhesion molecule receptors [65-68], and aptamers [69-73], among others. The mechanistic rationale for active targeting is briefly outlined in the following section, whereas recent examples of targeted liposomal or polymeric nanocarriers are summarized in the respective sections of the review.

One of the feasible approaches for triggered drug release at the tumor site explores the lower pH of the intratumoral and other microenvironments, relative to the dominant physiological pH of 7.4 characteristic for the majority of body fluids [74-78]. Such mildly acidic conditions exist in tumor and inflammatory tissues (pH 6.8) and in endosomes (pH 5-6) [78]. It is well established that one of the most important mechanisms for cellular uptake of nanopharmaceuticals is endocytosis. Once the particle is internalized inside cells by endocytosis, the endocytic vesicles eventually evolve to late endosomes and then to lysosomes, whereby the acidity (pH 5.0) is significantly higher as compared to the general physiological condition (pH 7.4) . Unfortunately, if the drug is polar and chemically unstable (e.g. the active diaqua-metabolites of platinum agents) it could be retained inside these intracellular compartments, unable to reach its ultimate pharmacological targets, and eventually degraded by *via* to hydrolysis enzymatic cleavage. By achieving endosomal escape of encapsulated cargo pH-responsive vehicles increase the cytosolic bioavailability of free drug and hence are expected to augment its cytotoxic effects [79, 80].

Thus pH-sensitive systems, depending on their architecture, properties and acidity triggered destabilization kinetics have the advantage of site-specific drug release either within the intratumoral microenvironment or within the cytosole of tumor cells [18, 79-82]. The acidity-responsive nano-carriers that have been most widely explored are pH-sensitive liposomes and polymeric micelles. The pH-triggered release from liposomes is usually attained *via* destabilization and increased fusogenicity of the liposome membrane under acidic conditions [77, 80, 83].

Liposomal platinum drugs

Liposomes are spherical vesicles comprising either a single phospholipid bilayer or alternating tightly packed aqueous compartments and lipid bilayers which enclose a central aqueous reservoir. Liposomes are nano- to micro-sized vesicles (50–5000 nm) and according to mean diameter and lamellarity fall into two categories, namely multilamellar vesicles (MLVs) with a size of 500–5000 nm and unilamellar vesicles (ULVs) with a size range of 50–250 nm. ULVs are further separated into small unilamellar vesicles (SUVs) sized 50-100 nm and large unilamellar vesicles (LUVs) with diameters exceeding 100 nm [84].

Liposomes are composed of naturally derived or synthetic phospholipids which conditions their excellent biocompatibility, biodegradability, non-immunogenicity and generally low toxicological and safety pharmacological potential [29, 30, 50, 85]. Due to the abundance of lipid and aqueous compartments liposomes are capable of accommodating both polar and non-polar compounds based on their solubility and partitioning characteristics [84, 86]. Lipophilic agents are typically encapsulated within the lipid bilayer of liposomal membranes, whereas polar agents are confined to the aqueous central cavity. Due to the possibility of spontaneous leakage and hydrolysis in the aqueous phase the liposomes the entrapped polar compounds face more challenges in terms of chemical and biopharmaceutical stability [84].

Several milestone advances in liposome technology have been made to meet some of the pharmaceutical and pharmacokinetics challenges associated with the drug loading efficiency, biodistribution and targeting potential of liposomes that allowed the evolution of these systems from membrane models to elaborated drug delivery platforms. These involve the advent of: techniques for size reduction and homogeneity of liposomal populations, the remote drug loading maneuvers allowing significant entrapment efficiency for weakly acidic or basic drugs [84], the realization of the steric stabilization concept [86], and the development of on-demand triggerable or fusogenic liposomes [82, 85, 87]. Thus over the last three decades the liposomes platforms have evolved from plain or conventional liposomes to long circulating "stealth" liposomes and ultimately to targeted and stimuli-responsive liposomes [44]. Moreover, the possibilities for complex surface modification, conjugation of permeation enhancing or homing moieties, incorporation of pH-responsive components etc. condition the possibilities for designing sophisticated drug delivery platforms with hybrid tumor- targeted and triggerable release properties [82].

Conventional Liposomes

The non-modified conventional liposomes are mainly composed of natural or synthetic phospholipids such as 1,2-distearoryl-sn-glycero-3-phosphatidyl choline (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidyl choline (DPPC), egg phosphatidylcholine, *etc.*, and cholesterol, without polymer coating or other steric stabilizing moieties [15, 16, 84]. Despite their favorable biphasic nature and low toxicological potential these are associated with inherited obstacles including lack of specific targeting and propensity to promptly accumulate in MPS organs with consequent fast disappearance from the circulation and disappointingly low bioavailability except in the liver and spleen [16, 40, 84]. This behavior is due to side interactions between liposomes and serum components, such as LDL and HDL leading to membrane destabilization, favoring eventual opsonization by a2-macroglobulin, fibronectin, blood clotting factors, complement components, among others [88]. Thereafter the opsonin-tagged liposomes are recognized and phagocyted by macrophages, Kuppfer cells and other MPS cells (Fig. 2) [40, 88].

To address these issues different approaches have evolved including manipulations of particle size, surface charge, phospholipid bilayer content *etc.* [16, 86, 88]. Thus addition of cholesterol has proved to increase the packing density and integrity of bilayers, hampering serum protein interactions and opsonization and increasing the ability of liposomes to retain their aqueous cargo within the circulation [88]. Moreover, it has been shown that reduction of the particle size is a highly successful strategy to evolve MPS sequestration as multilamellar liposomes with sizes ranging 500–5000 nm are far more promptly eliminated from the circulation as compared to the more recently developed large unilamellar liposomes or

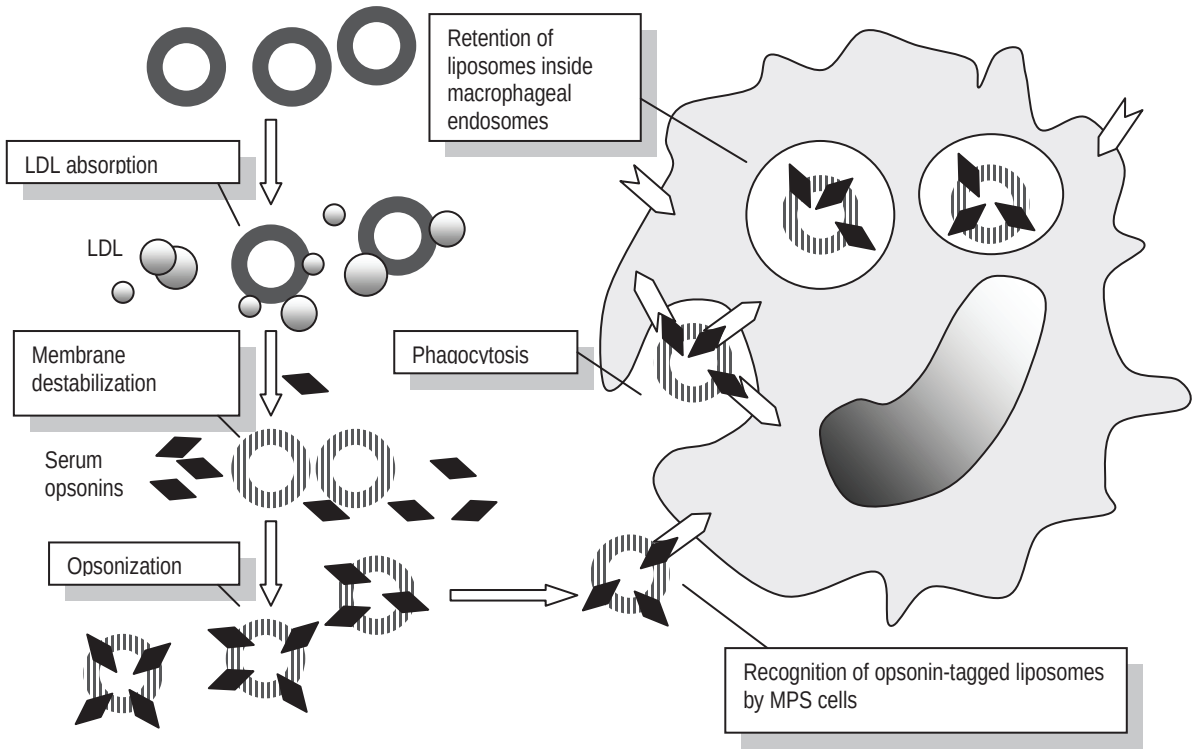

Fig.2 *Opsonization, recognition and phagocytosis of conventional liposomes by the cells of the mononuclear phagocyte system (MPS)*

the low nano-scale sized small unilamellar liposomes [16]. Despite the decreased propensity to accumulate in MPS organs the small unilamellar liposomes share the serious disadvantage of having relatively lower aqueous volumes. Hence they are characterized with generally lower drug entrapment capacity. In general, elimination or reduction of the surface charge is another favorable approach as both anionic and cationic liposomes appear to have shorter half-lives and increased intrinsic toxicity [16, 88].

Intensive research has been focused at liposomal delivery systems for platinum agents [5, 8, 89]. A representative advance in this field is cis-(bis-neodecanoato)-trans-R,R-1,2-cyclohexanediamine platinum(II) (NDDP), characterized *via* high liposomal encapsulation efficacy which is currently developed by Aronex Pharm. Inc. Aroplatin® demonstrated promising activity in diverse experimental tumors and received FDA-orphan drug designation for the treatment of malignant mesothelioma [5, 8, 89]. This formulation has been subject to clinical trials with patients with advanced solid tumors or B-cell lymphoma, and is currently undergoing phase II clinical trials in colorectal carcinoma [27].

Another water insoluble agent 2-(4-(tetrahydro-2H-pyran-2-yloxy)-undecyl)-propane-1,3-diaminedichloroplatinum(II) (THP-C11) was incorporated in LUVs. The liposomal formulation (LipoTHP-C11) showed excellent stability at 4°C for more than two months. It proved to exert cytotoxic effects in a panel of cell lines: H12.1, 1411HP, 518A2, A549, HT-29, MCF-7 and SW1736, with concomitant lower activity towards normal human fibroblasts [90].

"Stealth" Liposomes

Owing to the circumvention of the most important problems peculiar with conventional liposomes, namely the short circulation half life and the prompt sequestration in MPS organs the long-circulating or "stealth" liposomes have earned their place as dominant drug delivery platforms for anticancer drugs to be subject of both preclinical and clinical studies [16, 40, 91, 92]. Stealth liposome strategy is based on the process of steric stabilization, i.e. creating a repulsive polar coating around vesicles by grafting liposomal membranes with lipid-anchored hydrophilic polymers [39]. To meet this objective a number of different natural and synthetic polymers have been employed as steric stabilizing agents. These include, but are not limited to polyethylene glycol (PEG), chitosan, polysialic acids, polyvinyl alcohol (PVA), poloxamers, poly(acrylamide), poly(vinyl

pyrrolidon), poly(acryloyl morpholine), poly(2-methyl-2-oxazoline), poly((2-ethyl-2-oxazoline), poly(vinyl alcohol), hydroxypropylmethylcellulose, *etc*. [16, 40, 88]. In line with its biocompatibility, pharmaco-toxicological inertia, acceptably low immunogenicity as well as because of its extensively validated efficacy as steric stabilizing agent PEG has remained the golden standard for engineering long circulating liposomal nanoplatforms [27, 39]. PEGylated liposomes are most often prepared by introduction of distearoylphosphatidylethanolamine-PEG-2000 (DSPE-PEG-2000) (Fig. 3) which hampers their interactions with HDL and serum opsonins, and conversely decreases the capacity of MPS cells to recognize and phagocyte liposomes (Fig. 3), leading ultimately to significantly increased plasma half-lives [93].

The efficacy of the stealth liposome concept for attaining optimized tumor-site bioavailability and its comprehensive experimental and clinical justification have fuelled immense and enduring efforts for elaboration of PEGylated-liposomal antineoplastic agents, such as platinum drugs [94-99]. Exemplary stealth liposomal formulations evaluated in clinical trials are summarized in Table 1, and reviewed elsewhere [100, 101].

An important example of clinically validated liposomal formulations is SPI-077 – a stealth-liposomal cisplatin. This platform is featured by a high encapsulation efficiency, favorably tailored biodisposition and pharmacokinetics and conversely ameliorated toxicity relative to the free drug. Despite these beneficial characteristics however SPI-077 has failed to demonstrate prominent efficacy advantages compared to cisplatin presumably due to the very slow release of encapsulated cargo [8].

A more recent liposomal drug delivery system of cisplatin under clinical development is Lipoplatin™, elaborated by Regulon Inc. [102, 104]. This stealth liposomal system has shown significant advantages compared to free cisplatin in both preclinical and clinical settings, in terms of improved intratumoral accumulation, avoidance of the hallmark toxicity, concomitant with significant antineoplastic efficacy equal or even superior to that of the non-encapsulated drug [102, 104, 106]. Lipoplatin™ is currently undergoing several phase II and phase III trials in combinations with other antineoplastic agents, such as gemcitabine, 5-fluorouracil, and vinorelbine [2, 102-104, 106-109]. The same company has developed a stealth liposomal formulation of oxaliplatin (Lipoxal™) whose preclinical development has shown promising effects in resistant tumor models [94] and potent radiosensitizing activity in F98 glioma [95, 96]. Lipoxal™ is currently subject to clinical evaluation. A Phase I trial in patients with progressive and recurrent gastrointestinal cancers has shown that the product is well-tolerated and greatly ameliorates the non-neurological toxicities of oxaliplatin [105].

Table 1. *Stealth liposomal or non-PEG polymer coated liposomal formulations of platinum anticancer drugs in clinical trials*

API	Name	Formulation	Indications
Cisplatin	SPI-077	Stealth liposomes (i.v.); HSPC, CHOL and mPEG-DSPE	Head and neck cancer, lung cancer (Phase I-II) [8]
Cisplatin	Lipoplatin™	Stealth liposomes (i.v.); SPC, DPPG, CHOL , mPEG-DSPE	Several cancer types; (Phase II-III) [102-104]
Oxaliplatin	Lipoxal™	Stealth liposomes (i.v.)	A completed Phase I trial in advanced GIT cancer [105]

Abbreviations: API: Active pharmaceutical ingredient; HSPC: Hydrogenated Soy Phosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; mPEG-DSPE-poly(ethyleneglycol)-distearoylphosphatidylethanolamine.

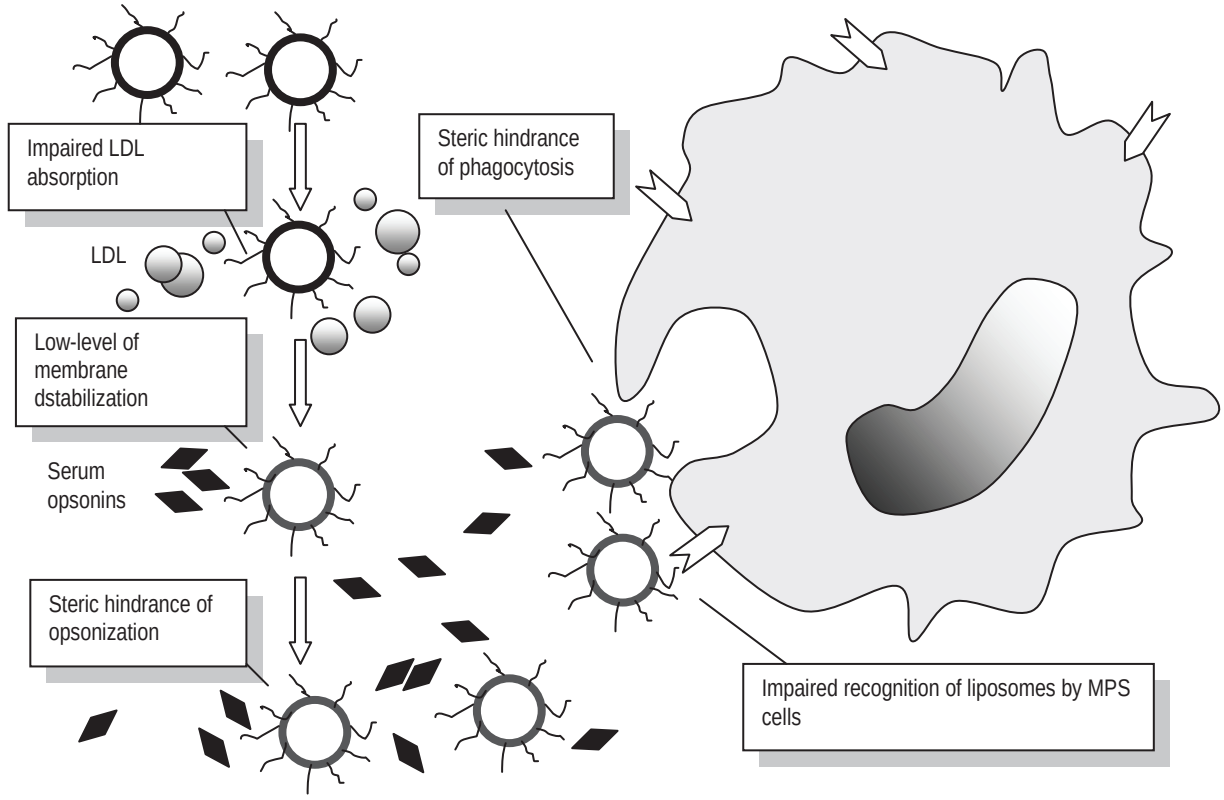

Fig.3 *Impact of steric stabilization against MPS recognition and sequestration of "stealth" liposomes. The densely packed and PEG-grafted bilayers resist LDL interactions with subsequent low level of opsonization and hampered recognition and phagocytosis by mononuclear phagocytes (See the text for details)*

Targeted Liposomes

The targeted liposome based drug delivery concept was fuelled by the established incapacity of non-decorated stealth liposomes to avoid exposure of non-malignant tissues and to eliminate the hallmark dose-limiting toxicities of cytoreductive chemotherapy [27]. These site-specific targeted liposomes are decorated with different types of homing moieties to increase the rate of liposomal drug accumulation in the ultimately targeted tissues/cells *via* interactions with cancer cell receptors/antigens (Fig. 4) [15, 85, 92].

Peptide, incl. peptide hormones and proteins have been also widely explored as targeting ligands for liposomal antineoplastic drugs. Among these special attention has been paid to transferrin-modified vesicles [61, 62, 143-148]. As with other targeting ligands, Tf could be conjugated or anchored directly to bilayer membranes of plain liposomes [16, 61, 62, 148, 149], or *via* the PEG-coating of stealth liposomes, a strategy which is has been increasingly employed in recent investigations as it allows to combine the plasma longevity of stealth liposomes with the enhanced cellular accumulation *via* Tf-receptor mediated endocytosis [44].

A recent contribution evaluated the cytotoxicity and cellular accumulation of Tf-modified cisplatin-loaded PEGylated liposomes in chemosensitive and cisplatin-resistant A2780 cells. Free cisplatin was 4 times less efficiently accumulated inside resistance cells, whereas the uptake of liposomal drug was comparable in both cell lines. Albeit the cytotoxicity of liposomal drug *vs*. free cisplatin was somewhat lower in the sensitive cell line, but was significantly higher in the resistant variant, in corroboration to the accumulation kinetics data [151].

Another paper reported cisplatin-loaded liposomes targeted at Tie2 - a receptor tyrosine kinase that plays important roles in vascular angiogenesis, and is highly expressed in vascular endothelial cells and a number of cancer cells. The delivery platform employed a novel peptide ligand PH1 peptide (TMG-FTAPRFPHY) selected by phage display library screening combined with surface plasmon resonance binding assays. The homing moiety was covalently conjugated to the distal end of DSPE-PEG(2000)-

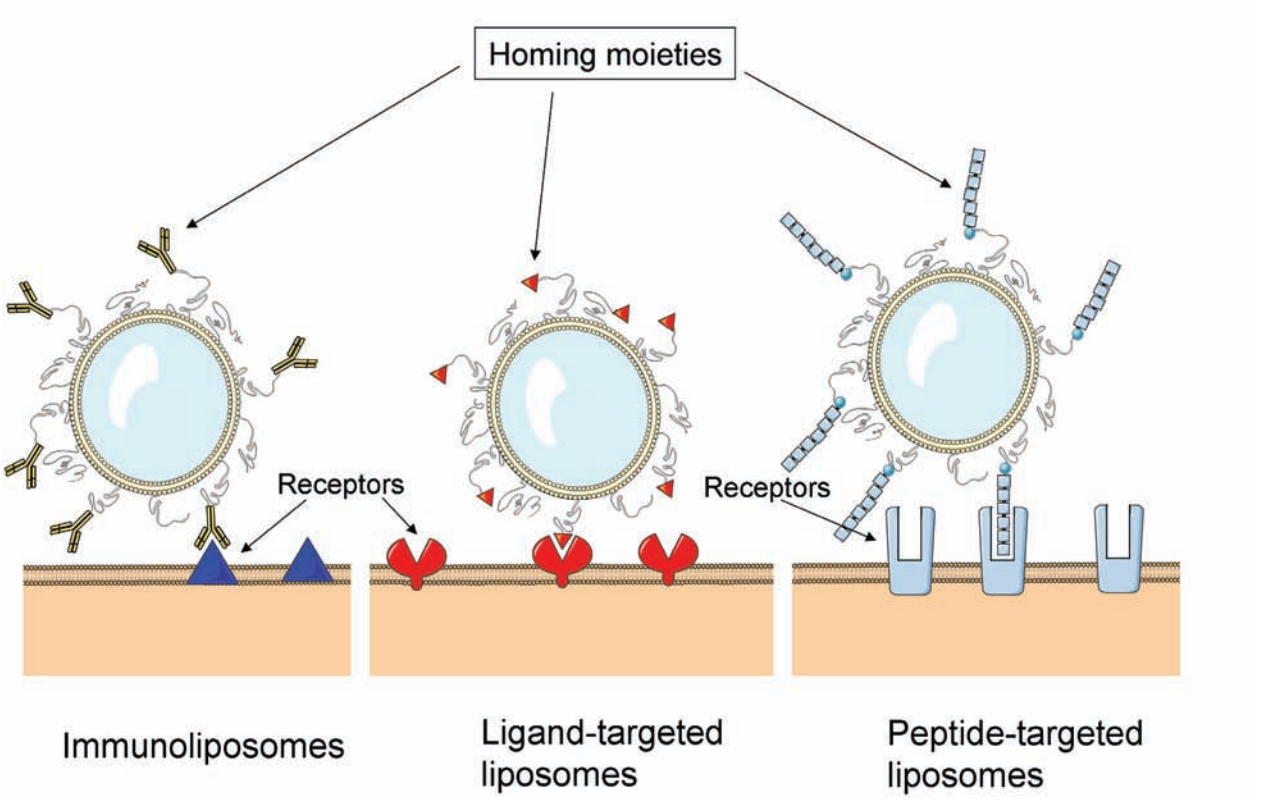

Fig.4 *Schematic representation of targeted stealth liposomes*

Maleimide lipid and grafted onto liposome membranes. These PH1-PEG-liposomes containing the anticancer drug cisplatin were showed to bind tightly to Tie2 positive cells, mediate active endocytosis of the drug containing liposomes, and result in much higher cell specific cytoxicities than mPEG coated liposomes [153].

pH-Responsive Liposomes

One of the hallmark issues associated with conventional liposomes is their propensity following cellular internalization to accumulate in certain subcellular compartments, mainly lysosomes, where the encapsulated drug is retained or even degraded, thus limiting its availability at the cytosolic target site (Fig. 5). This is of paramount importance in anticancer drug delivery as the majority of antineoplastic agents interact with pharmacological targets located inside cells or within the nucleus (e.g. genomic DNA) [16, 88]. An attractive and vastly explored approach to avoid lysosomal sequestration and degradation of entrapped materials is the use of pH-sensitive liposomes exhibiting considerable fusogenic activity at low pH and capacity to evade endosomal sequestration (Fig. 5) [17, 44, 80, 82, 154].

The typical pH-triggerable systems are composed from dioleoylphosphatidylethanolamine (DOPE) with cholesteryl hemisuccinate (CHEMs) or other acidic amphiphiles acting as bilayer stabilizers at neutral pH. DOPE comprises a compound with minimally hydrated and hence small headgroup, occupying relatively smaller volume *vs.* the bulky hydrocarbon chains [84]. This imparts a cone shape to the molecule, which is detrimental for bilayer assembly and supports the formation of inverted hexagonal phase micelles. Introduction of an acidic amphiphile which is negatively charged at physiological pH among DOPE molecules allows the formation of bilayer structures, and facilitates the construction of liposomes, stable at physiologically relevant pH and temperature [80, 84, 155]. In acidic pH the protonation of the carboxylic groups of the amphiphiles, reduces their stabilizing effect and this leads to liposomal membrane destabilization, since under these conditions PE molecules revert into their inverted hexagonal phase [80, 155]. More recently new classes of pH-sensitive systems based on fusogenic peptides [147], or surface modification of liposomes with pH-sensitive polymers [138, 154] have emerged, extensively discussed elsewhere [81, 155].

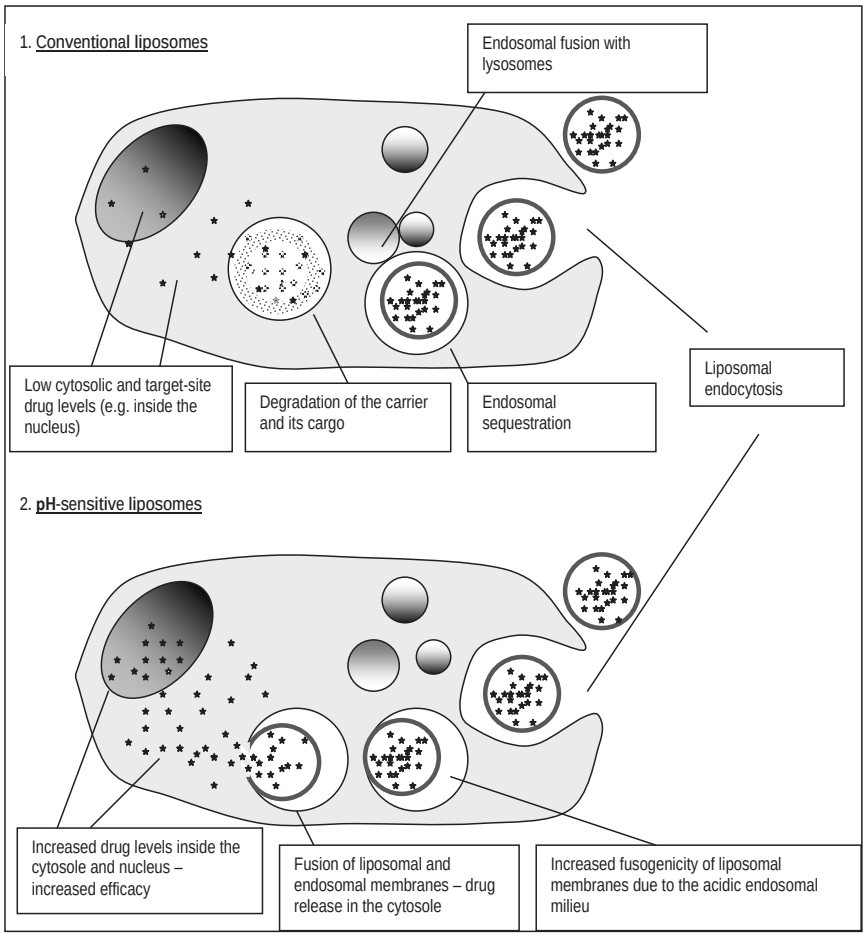

Fig.5 *Comparative schematic representation of the fate and trafficking of conventional liposomes, which are retained within the endosomal compartments (above) and pH-sensitive liposomes which become fusogenic inside endosomes and deliver their cargo in the cytosole (below)*

An exemplary novel DOPE:CHEMs system is a DSPE-PEG2000-modified formulation of cisplatin, developed as a sterically stabilized pH-responsive platform for intraperitoneal application [97] and optimized in terms of technological and formulation variables to obtain most favorable protocol for a reproducible and economically feasible large scale production [156]. These liposomes proved to be serum stable and to selectively accumulate and induce cytotoxicity in cisplatin-resistant small-cell lung carcinoma cell line (GLC4/CDDP), characterized with reduced drug uptake [97]. Specialized toxicological surveys of this formulation showed pronounced amelioration of the lethal toxicity (with significantly higher LD_{50} values compared to free drug), and elimination of cisplatin-related myelosuppression and hallmark nephrotoxicity following intraperitoneal [157] or venous application [158] in mice. The long circulating pH-sensitive liposomes of cisplatin proved to exhibit potent tumor-inhibiting properties following intraperitoneal application against Erlich ascites tumor with more marked increase in life span as compared to the free-cisplatin treated group. Moreover, in this therapeutic intervention the liposomal formulation demonstrated favorable safety characteristics vs. non-encapsulated cisplatin, in corroboration to the toxicological data [159].

Enzymatically Triggerable Liposomes

A sophisticated liposomal drug delivery system based on enzymatically assisted pro-drug activation and carrier destabilization is the LiPlasome® platform [85, 87, 164-167]. This elaborate strategy for triggered release of liposomal cisplatin or other antineoplastic agents is based upon the significant up-regulation of the secretory phospholipase A2 (sPLA2), peculiar for a variety of certain solid tumors [87, 164, 167-171]. To exploit this feature LiPlasome Pharma

A/S has developed liposomal drug delivery system (LiPlasomes®) composed of ether lipid prodrugs which are preferentially degraded within tumors, over-expressing sPLA2 [85, 87, 164-166, 168, 170]. The degradation of the liposomes is consistent with release of encapsulated drugs, and *de novo* formation of cytotoxic ether lipids and membrane disrupting fatty acid residues. The latter further destabilize the membranes of target cancer cells and facilitate the drug accumulation therein [30, 166-168]. The most advanced products of the company are PLA2-triggerable liposomal cisplatin (LiPlaCis®) which is subject to Phase I trials and oxaliplatin (LiPloxa®), currently under preclinical toxicological evaluation [172].

Sonosensitive Liposomes

Another feasible trigger for remote guided release of drugs from liposomes is ultrasound exposure. Ultrasonication has been documented to increase drug release from conventional and stealth liposomes with different phospholipid content [82, 175, 176]. Schroeder *et al*. have explored the possibilities for ultrasound assisted release of cisplatin from sterically stabilized liposomes. A pharmacokinetic study in Balb/C mice transplanted with C26 colon adenocarcinoma tumors in the footpad showed that the use of low-frequency ultrasound led to almost 70% of liposomal cisplatin release in contrast to 53% when there was no sonication [176]. These findings are especially intriguing having into consideration the fact that the unsatisfactory clinical performance of liposomal cisplatin (SPI-077) is at least partly due to the slow release of the drug from the sterically stabilized liposomes [8].

Polymer-based drug delivery systems

Polymeric micelles and macromolecular pro-drugs

Polymer-drug conjugates are water-soluble tailor-made structures designed to modulate the pharmacokinetic properties of the therapeutic agent [19, 32, 37, 177]. On the one hand, particulates with a larger size than glomerular excretion threshold value (42–50 kDa for water-soluble polymers) may provide a prolonged blood circulation of the conjugated drug. On the other hand, polymeric carriers having a size smaller than 200 nm and a hydrophilic and biocompatible surface may avoid the recognition by the reticuloendothelial system [27, 28, 32, 35, 37, 38, 178]. Besides optimization of the rate and duration of drug delivery the conjugation strategy can provide drug targeting to specific cells or tissues and control of the release of highly toxic drugs as an effective way to minimize the adverse side effects. In addition, the hydrophilic polymer carrier can impart favorable physicochemical properties, e.g. increasing the solubility of lipophilic drugs or the stability of labile agents from chemical or proteolytic degradation [27, 28].

It is well-appreciated that cisplatin and its analogues react with a variety of nitrogen- and sulphur-containing biomolecules by ligand exchange reactions [3, 179]. In blood a high fraction of cisplatin is bound to plasma proteins, including albumin, transferrin and g-globulins that reduce its therapeutic concentration. The ligand exchange kinetics of platinum compounds is largely determined by the nature of the leaving groups. Carboxylate groups possess low nucleophilicity and therefore they are able to undergo the reverse exchange reaction with chloride ions to regenerate cisplatin at physiological salt concentrations [4, 5].

The property of carboxylate ligand as a good leaving group has been exploited to design cisplatin delivery systems based on carboxylate-containing polymers. Polymer–drug complex micelles were spontaneously formed on mixing of cisplatin with PEO-poly(aspartic acid) or PEO-poly(glutamic acid) block copolymers in an aqueous solution [180-184]. The cisplatin-incorporated micelles were extremely stable in distilled water whereas in physiological saline the micelles showed dissociation into unimers, accompanied with sustained platinum (II) complexes release. The micelles formed from PEG-b-poly(aspartic acid) underwent fast structural decay (~30 h) that caused liver and spleen accumulation and comparable antitumor activity to free cisplatin despite restrained nephrotoxicity. The time scale of decaying of the micelles was prolonged to 50 h when PEO-b-poly(glutamic acid) copolymers were used for cisplatin conjugation which improved the selectivity and efficiency in tumor targeting [184].

The metal ligand coordination was also utilized to incorporate the drug into the cross-linked micelles with ionic poly(methacrylic acid) cores and a hydrophilic shell of PEO chains [185]. The size of the loaded micelles was about 150 nm and the drug content was determined to be 22%(w/w). Cisplatin was encapsulated in nanoparticles formed by hydrophobically modified chitosan [186] or thermosensitive polymer carriers [187, 188].

In contrast to the particulate carriers such as micelles, water-soluble polymers allow drug molecules to interact with a single macromolecule rather than a large particle. The polymer carriers take advantage

of EPR effect without accumulating into the liver and spleen. However, linear polymers have limited drug payload capacity. For instance, binding of cisplatin to homopolymers and alternating copolymers bearing carboxylate moieties often results in the formation of poorly soluble cisplatin-polymer conjugates when the molar ratio of cisplatin to carboxylate residues in the polymer exceeded 0.2 [189].

Dendrimers

Dendrimers comprise a class of globular, highly branched, synthetic macromolecules with tunable size and architecture [52, 190, 191]. They encompass multiple layers with large number of chemically active surface groups, also known as generations, which emanate out of an initiator core, denoted as generation zero (G_0). Typically the size of dendrimer particles ranges 1–15 nm and these are characterized by significant homogeneity in terms of size distribution and morphology [191]. Dendrimers have many attractive properties which make them advantageous drug carriers as compared to both linear and hyperbranched polymer-based systems [191-193]. The unique highly regular branching architecture and the multiple arms of dendrimers provide a large multivalent backbone whereby anticancer drugs, targeting moieties or solubilizing groups can be feasibly attached through covalent conjugation or electrostatic adsorption. Moreover, drugs could be also loaded within the cavities of the core regions, either covalently or *via* hydrophobic, hydrogen, or van der waals bonding. In addition, the low level of polydispersity of these macromolecules is a prerequisite for reproducible pharmacokinetic and biodistribution behavior which is of paramount importance for their actual applicability as drug delivery systems [191, 194].

The research on dendrimer-drug deliver systems is focused on biodegradable backbones, e.g. the polyaryl ether dendrimers, polyester dendrimers based on 2,2-bis(hydroxymethyl)propionic acid, glyceryl-succinate polyester dendrimers, and especially polyamidoamine (PAMAM) dendrimers [27, 52, 190, 191]. The latter have been exceptionally widely studied as drug delivery platforms in line with their excellent biocompatibility, water solubility, and abundance of large number of active functions suitable for coupling of chemotherapeutic agents and targeting ligands [52, 190, 191].

Considering the ubiquitous abundance of biotin as micronutrient especially in rapidly proliferating cells such as cancer cells Yellepedi *et al.* designed biotinylated PAMAM dendrimers as a targeted carrier for antineoplastic agents, including platinum drugs. The effect of generation and the mechanism of cellular uptake of biotin-PAMAM-G_4 in ovarian cancer (OVCAR-3) and human embryonic kidney (HEK 293T) cells was determined by fluorescent microscopy and flow cytometry. The cellular uptake of biotin-PAMAM was significantly higher in the cancer cell line, as compared to the non-malignant HEK293T cells. Mechanistic studies demonstrated that the cellular uptake of biotinylated-PAMAM was mediated by biotin receptor-mediated endocytosis and charge-mediated adsorptive endocytosis. The cytotoxicity of biotinylated-PAMAM-G_4 in the HEK 293T cells was comparable to that of the parent PAMAM dendrimers [54]. To further address the applicability of the show potential as nanocarriers in targeted drug delivery the same group developed cisplatin-loaded biotinylated PAMAM dendrimers. The systems were investigated for encapsulation efficiency, *in vitro* cytotoxic activity and cellular accumulation of cisplatin in a panel of chemosensitive (OVCAR-3, SKOV-3, A2780) and one cisplatin-resistant (A2780/CP70) ovarian cancer cell lines. The PAMAM dendrimers displayed relatively low encapsulation efficiencies of cisplatin ranging *ca.* 5- 21%. The dendrimer loading however significantly augmented the cytotoxic effects of the drug as evidenced by the significantly lower values of the IC_{50} values thereof *vs.* those of the free drug. The cytotoxicity data were corroborated by an *in vitro* accumulation assay which showed that the PAMAM-G_4 NH_2 dendrimer complexes of cisplatin display far more efficient, approximately ten fold higher uptake in both A2780 and A2780/CP70 cells as compared to the free drug. These finding point out for the feasibility of biotinylated PAMAM dendrimers as potential targeted nanoplatforms of cisplatin in ovarian cancer [55].

Multi-arm Star-Like Polymers

In spite of the undisputable advantageous characteristics of dendrimers, e.g. their well-defined and homogenous size distribution patterns controlled branching architecture and drug loading feasibility their synthesis is time consuming and tedious stepwise procedure. Thus an emerging alternative are the core–shell type star polymers bearing hyperbranched cores and multi-arm shell of linear polymers bearing active end functionalities [195, 196]. These new macromolecules based upon various branched core architectures exhibit “unimolecular micelle” behavior in water solution, whereby the covalently linked interior and shell domains remain stable independently

of concentration, abundance of interactive solutes and temperature [197-202]. The structural stability and multifunctionality of the stars conditions the significant scope for their elaboration as drug delivery systems, since they provide opportunities for either chemical/ electrostatic immobilization, or physical encapsulation of anticancer drugs [197, 202].

Another recent report has presented the formulation and evaluation of a core–shell type star polymer with a branched hydrophobic polystyrene interior and covalently attached poly(acrylic acid) arms, as a drug delivery system for cisplatin. This architecture proved to afford several advantages as cisplatin carrier such as high density of carboxylate functions that are able to reversibly immobilize the drug, exceptionally high drug payload, stability in aqueous milieu upon storage and sustained release of the agent under physiological conditions.

The system displayed prominent capability for intracellular uptake and exhibited concentration and time- dependent cytotoxicity in a panel of human tumor cell lines [203]. This cisplatin -formulation has been further developed to a reversibly PEGylated nanocarrier. The design strategy was based on functionalization of the polyacrylate arms *via* a PEGylated cisplatin analog, allowing for detachment of the coating following hydrolysis in biological milieu. The formation of PEG shell resulted in higher drug payload and improved drug release profile of the nano-conjugates. The *in vitro* bioassay confirmed that the PEGylated conjugates exhibited higher cytotoxicity compared to the non-PEGylated cisplatin -loaded stars [204].

Molecular hosts as drug delivery systems for platinum metallodrugs

The supramolecular interactions of macrocyclic hosts with different types of small guest-molecules leading to formation of stoichiometric inclusion complexes have been subject to intense research during the last several decades [25, 205-208]. Due to their exceptional generic properties, macrocycles can be considered as an important class of drug delivery vehicles, able to accommodate drugs within their structure thus affording a steric barrier to drug degradation and/or deactivation. Moreover, the size of a macrocycle can be tailored to control the rate of drug release and binding strength of the host-guest complex [207, 209-212]. A number of important types of macrocyclic molecular hosts have been developed and characterized, including crown ethers [213], cyclodextrins [207], calix[n]arenes [208] and cucurbit[n]urils [25]. These macrocyclic compounds offer an interior concave surface available to accommodate guest molecules such as drugs and diagnostic agents. Moreover, these macrocyclic molecular hosts can be chemically modified to allow tailoring of their physicochemical and complexation properties according to desired application [205, 212].

Cyclodextrins

Cyclodextrins (CDs) comprise a class of crystalline, nonhygroscopic, cyclic oligosaccharides derived from starch, containing at least six D-(+)-glucopyranose units attached by (α1,4) glucoside bonds [207, 214]. Among the most commonly utilized members of this class are the naturally occurring a-, b-, and g-cyclodextrin, which comprise respectively 6, 7, and 8 glucose units [215]. Cyclodextrins are 'bucketlike' or 'conelike' toroid molecules, with a rigid structure and a central cavity, the size of which varies according to the CD type. Based on the molecular architecture and the specific arrangement of polar hydroxyl functions the internal surface of the CD's cavity is hydrophobic, whereas the outside of the torus is hydrophilic [207]. These structural peculiarities allow the CD to accommodate a guest molecule within the cavity, forming an inclusion complex. CD interactions with drug molecules result primarily in enhancement to dissolution characteristics and bioavailability owing to enhanced solubility and improved chemical and physical stability [215]. Although it is the least soluble analog b-cyclodextrin is the most widely used agent from this class of excipients, because it is the least expensive, is commercially available from a variety of sources, and is capable to accomodate a number of guest-molecules of pharmaceutical interest.

In a new report a water-soluble trans-platinum complex was synthesized by inclusion complexation with beta-cyclodextrin. The complexation was confirmed by ^{1}H NMR, FT-IR, TGA, and XRD as well as by SEM and EDX. It was shown that the encapsulation with cyclodextrin allowed to solubilize the otherwise porly soluble metal compound to a solubility value of 1.6 mg/mL. Moreover, the cytotoxicity in vitro of the novel inclusion complex indicated a much higher activity after encapsulation [216].

Cucurbit[n]urils

Cucurbit[n]urils (CB[n]), comprise a relatively new family of macrocyclic molecular hosts that has exhibited promising results in improving anticancer drug delivery [24-26, 217, 218]. Partial or complete encapsulation of drugs within the homologues CB[6],

CB[7] or CB[8] can hinder untoward interactions with small molecule solutes or proteins and impart enhanced chemical and biopharmaceutical stability, with concomitant improve drug solubility and control drug release [24, 25, 217, 219]. These favorable features are concomitant with low intrinsic toxicity potential of the CB[n] family of nano-containers [24, 26]. Moreover, by means of modification with homing moieties CB[n] could be transformed from simple molecular hosts to targeted delivery systems, as recently documented for CB[6]-hyaluronate conjugates and lectin-seeking sugar-decorated CB[6] derivatives [220, 221].

CB[7] has been shown to effectively accommodate oxaliplatin with consequent enhancement of the stability of the drug. Most notably the encapsulated oxaliplatin is far less reactive to L-methionine [222] and thus the inclusion of platinum drugs in cucurbit[7]uril could be considered a vital strategy towards hindering untoward reactions with nucleophiles, as eventually justified with other platinum metallodrugs [217].

cisplatin has been also s effectively loaded in CB[7] occupying the central cavity of the molecular host whereby platinum atom and both Cl-ligands are located inside the macrocycle [5, 223]. *In vitro* studies have shown that CB[7] has no effect on the in vitro cytotoxicity of cisplatin in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant sub-lines A2780/cp70 and MCP1. Nevertheless the CB[7] inclusion greatly modulates the *in vivo* potency of the drug against human tumor xenografts. Thus while cisplatin-CB[7] is just as effective on the chemosensitive A2780 xenografts compared with free cisplatin, whilst in the cisplatin -resistant A2780/cp70 model the inclusion complex markedly inhibited tumor growth. Delianation of the mechanistic aspects of this ability of CB to overcome resistance *in vivo* pointed out that this is due to a pharmacokinetic effect. Whilst the peak plasma level and tissue distribution are the same for cisplatin -CB[7] and free cisplatin, the total concentration of circulating cisplatin-CB[7] over a period of 24 hours is significantly higher than for free platinum drug when administered at the equivalent dose, suggesting for intensified exposure of malignant lesions [223]. CB[n] proved to effectively encapsulate novel preclinical multinuclear platinum complexes without greatly compromising their cytotoxicity [218, 219, 224, 225].

Calix[n]arenes

Among the macrocyclic hosts calixarenes have received special appraisal because of their unique three dimensional structure, facile large scale synthesis and the exceptional possibilities to undergo further synthetic elaboration and functionalization [22, 208, 211, 212]. Calix[n]arenes are macrocyclic compounds composed of phenolic units linked by methylene or sulfur groups at the 2,6-positions. They encompass a “cup”-like rigid conformation, with defined lower and upper rims and a central annulus, with a central cavity large enough to accommodate small molecules and ions [208, 212]. Moreover, their chemical transformability and the possibilities for upper and lower rim modification can allow supramolecular interactions and complex-formation with larger molecules, including proteins and nucleic acids, further broadening the biomedical applicability of calixarenes [209, 210, 226]. As far as drug delivery is concerned however, despite of the numerous favorable characteristics of calix[n]arenes these share the distinction of being water insoluble, which greatly limits their practical utility. On these grounds considerable efforts have been focused on approaches to increasing the water solubility of these compounds *via* introduction of polar functional groups or moieties such as sulfonates [210, 227], phosphonates [228], amines and amino acids [22, 226, 229], guanidinium [230], peptides [211], saccharides [231, 232], or polyethylene oxide (PEO) [233-235], either directly or *via* linkers to the upper or lower rims of calix[n]arenes [212].

The encapsulation of three platinum(II)-based anticancer complexes with DNA-intercalating phenanthroline motifs in p-sulfonatocalix[4]arene (s-CX[4]) has been examined. All three metal complexes formed 2:2 inclusion complexes with s-CX[4] where the two metal complexes stacked in a head-to-tail configuration and were capped by the s-CX[4] molecules. Encapsulation of the metal complexes in either CX[4] significantly decreased the metal complexes’ rate of diffusion and protected the guest molecule from degradation by reduced L-glutathione. *In vitro* growth inhibition assays using the LoVo human colorectal cancer cell line showed no significant change in the cytotoxicity of one of the encapsulated complexes when encapsulated by CX[4] host and hence it could be regarded as a suitable drug delivery systems for platinum coordination compounds [236].

Moreover, substituted calixarenes have been recently described as feasible drug delivery platforms for efficient accommodation of a series of cytotoxic dinuclear platinum complexes [237].

Conclusions and future prospectus

The landmark progress in our understanding of human genomics, cellular and molecular biology and their relevance to neoplastic disease during the last decades have led to unprecedented delineation of the signal-transduction pathways and their precise role in malignant transformation and tumor biology with concomitant identification of new therapeutic targets. These advances have fuelled much research efforts upon the rational design of targeted, patient-friendly anticancer drugs. While the undisputable advantages these innovative strategies pose, relative to the classical chemotherapeutic armamentarium, they are by no means devoid of limitations, concerning their limited capacity to eradicate malignant populations, the emergence of resistance with monotherapy and their potential for pharmacokinetic drug-drug interactions.

Thus to this end the conventional "heavy-duty" anticancer drugs appear to be an inevitable component of cancer management. On these grounds the opportunities offered by state-of-the-art delivery technologies in terms of profound improvement of the biodistribution and toxicity profiles of existing drugs condition the exceptional and long-standing research efforts towards formulation design for targeted delivery of chemotherapeutics, including cisplatin and its congeners.

In line with the unique characteristics of the nanosized drug delivery systems significant development has been reported on development of nanopharmaceutical platforms for cisplatin and other platinum complexes. Using surface modification with polymers and/or homing fragments or chemical functionalization it is feasible to tailor the generic properties of nano-scale objects such as micelles, liposomes, macromolecular pro-drugs, supramolecular complexes etc. for optimal targeting and remote drug release. Nevertheless, the elaboration of these sophisticated delivery strategies is associated with different problems such as hampered drug release, and unfortunate cost-effectiveness, which have hampered the progress of the field beyond the experimental stages, and recall for further more detailed characterization of nanocarriers as possible delivery vehicles for platinum drugs.

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