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Review Article

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Publication date:
2018-07

Permanent link:
https://doi.org/10.3929/ethz-b-000268595

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Originally published in:
European Journal of Pharmaceutics and Biopharmaceutics 128, https://doi.org/10.1016/j.ejpb.2018.04.003

Funding acknowledgement:
147651 - Focused Ultrasound-Mediated Delivery of Encapsulated MGMT Antagonists for the Treatment of Temozolomide-Resistant Glioblastoma (SNF)
Pharmacokinetics of Lipid-Drug Conjugates Loaded into Liposomes

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Abstract
Drugs that are neither lipophilic nor suitable for encapsulation via remote loading procedures are generally characterized by low entrapment efficiencies and poor retention in liposomes. One approach to circumvent this problem consists in covalently linking a lipid to the drug molecule in order to permit its insertion into the vesicle membrane. The nature of the conjugated lipid and linker, as well as the composition of the liposomal bilayer were found to have a profound impact on the pharmacokinetic properties and biodistribution of the encapsulated drugs as well as on their biological activity. This contribution reviews the past and recent developments on liposomal lipid-drug conjugates, and discusses important issues related to their stability and in vivo performance. It also provides an overview of the data that were generated during the clinical assessment of these formulations. The marketing authorization of the immunomodulating compound mifamurtide in several countries as well as the promising results obtained with the lipid prodrug of mitomycin C suggest that carefully designed liposomal formulations of lipid-drug conjugates is a valid strategy to improve a drug’s pharmacokinetic profile and with that its therapeutic index and/or efficacy.

Keywords: lipid-drug conjugates, liposomes, cancer, pharmacokinetics, release kinetics
Introduction

A multitude of novel, potent, pharmacological agents are continuously identified by scientists working in early drug discovery. Nevertheless, the majority of these drug candidates fail in preclinical studies, and very few enter clinical trials [1, 2]. Poor biopharmaceutical/pharmacokinetic (PK) properties are one of the reasons for this unfortunate outcome [2, 3]. For example, rapid renal clearance or fast metabolism can prevent a drug candidate from exerting its therapeutic activity [4, 5]. Several formulation strategies have thus been implemented to overcome these issues and also improve the therapeutic indices of drugs. The development of nanoparticle-based drug delivery systems has especially allowed ameliorating the PK and biodistribution profiles of several compounds. A prominent example is Doxil®, the liposomal formulation of the anticancer but cardiotoxic drug doxorubicin, which was among the early FDA-approved nano-drugs [6]. To date, liposomes remain extensively used nano-carriers due to their ability to entrap and transport drug molecules with different physicochemical characteristics. However, despite the availability of several manufacturing procedures and encapsulation methods (e.g. reverse evaporation phase vesicles, pH gradient-driven loading, etc.), achieving satisfactory entrapment efficacy remains a challenge for certain categories of drugs [7-11]. Lipid-drug conjugation constitutes an efficient strategy to address this obstacle and enhance liposomal loading [12, 13]. In this approach, the drug’s solubility is modified by covalently linking the parent molecule to a lipophilic anchor via a cleavable or non-cleavable linker, thus strengthening its interaction with the lipid-bilayer of the liposome [14]. The resulting lipid-drug conjugates (LDCs) may be inactive prodrugs, entailing the release of the parent drug to display activity. Early liposomal-LDC (L-LDC) systems – prepared and tested more than 30 years ago – were often associated with a fast and rather uncontrolled drug release upon administration, limiting their further development [15]. Since then, many novel strategies to formulate and engineer L-LDCs have emerged, reinvigorating their translational potential [13, 14]. This review article aims to re-evaluate the benefit of the L-LDC approach in light of the PK and biodistribution data available to date, and discuss it in connection with therapeutic efficacy. The current state of L-LDC research is examined in terms of LDC design, possible L-LDC delivery routes and clinical success of L-LDCs. Orally administered L-LDCs will not be discussed here because of the high variability in PK compared to parenteral administration routes and the poor stability of the liposomes in the gastrointestinal tract [16].
2. Pre-considerations and fundamentals in L-LDC PKs

2.1 In vitro drug release of L-LDCs

The PK of a drug entrapped in liposomes is determined by an interplay of two individual processes: the PK of the carrier and the one of the released drug [17]. Additionally, in the case of prodrug-like LDCs, where the parent drug can be cleaved from its lipophilic anchor, the PK of the lipophilic LDC itself needs to be taken into consideration. As illustrated in Fig.1, two processes may be involved in drug release of L-LDCs before reaching the target tissue: (1) given their lipophilic nature, LDCs can be directly transferred from the liposomal bilayer to cellular membranes or to (lipo)proteins in the bloodstream; or alternatively, (2) LDCs might be converted to the parent drug and be thereafter released. As outlined below, liposomal drug release (immediate, sustained or delayed) can be tuned by modifying the LDCs’ anchor and linker designs.

Fig. 1. Schematic illustration of the release processes of an L-LDC.

Anchors such as fatty acids, steroids, glycerides, and phospholipids are commonly used to form LDCs [14]. The chemical structure of the LDC anchor plays a major role in the exchange rate of the liposome-associated drug towards plasma proteins or cell membranes, which can vary from a few minutes to several hours. Exchange rates are usually studied by incubating the L-LDCs with whole blood, plasma or serum [18], and, although these in vitro tests might not accurately reflect the in vivo performance of the L-LDCs, they provide valuable preliminary insights. In the case of alkylated LDCs, it was found that the exchange rate to biological membranes was inversely related to the alkyl chain length [19, 20]. Moreover, LDCs with only one alkyl anchor chain may not be stably anchored in the liposomal membrane, especially if the parent molecule is quite hydrophilic. For example, the lipophilic derivatives of cytarabine \( N^4 \)-hexadecyl-1-\( \beta \)-D-arabinofuranosylcytosine (NHAC) and \( N^4 \)-octadecyl-1-\( \beta \)-D-arabinofuranosylcytosine (NOAC) with a single alkyl anchor chain were substantially transferred to erythrocytes after 1 or 4 h incubation in human blood, respectively [5, 21]. The nature of the hydrophobic anchor plays a major role in the retention of LDCs in the bilayer. Tokunaga et al. [22] found that a cholesterol-anchored mitomycin C-based LDC exhibited higher liposomal retention than its stearoyl-anchored counterpart.
Already after 10 min incubation in rat serum, the stearoyl-anchored LDC was substantially transferred to plasma proteins, while the cholesterol-analogue remained completely associated to the liposomes. In addition, a more recent study reported that essentially no exchange was observed in vitro with the distearoylglyceride-anchored mitomycin C analogue 2,3-(distearoyloxy)propane-1-dithio-4’-benzyloxy carbonyl-mitomycin C (MLP) upon incubation in human plasma for 24 h [23]. Taken together, these studies suggest that LDCs with a cholesterol or a double fatty acid chain (di-acyl glycerol) anchor would be less prone to be exchanged compared to single alkyl chains.

The drug conversion rate of a LDC to its respective parent drug depends on the structure of the intermediate linker and on the environment that the LDC encounters. The most common linker in LDCs is by far the ester bond, which is easily cleaved by ubiquitous esterases in the body, followed by the amide linkage [14]. Other reported linkers include disulfide, thioether and carbamate bonds [14]. Not surprisingly, LDCs with thioethers are more stable in plasma than those with disulfide bonds [24], while carbamate bonds are cleaved faster than ester-bonds as reported by a study with mitomycin C cholesterol analogues [22]. In addition to the linker used, the drug conversion rate of an LDC is strongly dependent on the release medium, especially when the linker is cleaved by enzymes. For instance, Teshima et al. [25] showed that the LDC 21-palmitoyl-prednisolone was almost completely converted to prednisolone after 3 h of incubation in rat blood or rat liver homogenate, while the LDC was completely stable in an aqueous buffer at physiological pH. Parang and colleagues [26] observed different conversion rates for the LDC 5’-O-myristoyl-zidovudine in various tissues, and reported a 3-fold increase in its half-life in rat brain homogenate compared to rat plasma. Not surprisingly, the drug conversion rate for a given LDC may also differ between species [22, 27, 28]. A cholesterol-anchored mitomycin C-based LDC with an ester linker exhibited greater stability in mouse than in human serum [22]. It has also been reported that the liposomal incorporation of a LDC can reduce cleavage to a certain extent in comparison to the free form, which is most likely due to a shielding effect diminishing the interactions of the drug with the medium [22]. Moreover, it was shown that the liposomal composition can modulate the release rate [29]. When incubated with cysteine, the above-mentioned LDC MLP containing a disulfide-linker decomposed faster if incorporated in cholesterol-free liposomes based on hydrogenated soybean phosphatidylycholine (HSPC) than in cholesterol-containing ones [29]. This is probably the result of a superior membrane stability of the latter formulation.
It would be expected that the drug’s physicochemical properties such as hydrophobicity also influence its release profile. Two studies investigated the conversion rates of LDCs only differing by the drug’s structure, namely mitomycin C or cytarabine [22, 30]. It was found that mitomycin C was cleaved faster than cytarabine with a release of 78% after 22 h (calculated by degradation half-life of 10 h, pseudo-first-order kinetics) compared to 14% for cytarabine. However, the two studies differed in the assay medium (rat plasma and serum) which could also have impact on drug release as described in the paragraph above. With regard to exchange rates, we did not find any studies comparing drug release for systems differing only by the drug’s structure. Comparing results from studies varying profoundly in liposome formulation or release assay would, however, not allow precise statements on the effect of the drug on the LDC’s release profile.

Overall, the reviewed data indicate that drug release can vary strongly between different L-LDCs and depends on the anchor as well as the linker structure and - possibly - the drug itself. Although systematic in vitro studies might improve our understanding of underlying single processes, these cannot precisely inform about the in vivo release of the LDC from its liposomal carrier [19]. Animal studies are thus important to obtain a deeper understanding of the drugs’ de facto release behavior, as will be discussed below.

2.2 The AUC ratio - A means to assess an L-LDC

Liposomes alter drugs’ PK profiles and influence several of their PK parameters such as half-life, area under the plasma drug concentration-time curve (AUC), clearance, and volume of distribution [31]. The AUC represents the extent of exposure to a drug, and is often used to evaluate the biopharmaceutical benefit of a liposomal formulation over the free drug. The AUC ratio allows comparing different L-LDCs and is calculated as the AUC of the L-LDC group divided by an adequate control group. The nature of the control, however, influences the AUC ratio, making comparisons between different studies a difficult task. With regard to LDCs, the main controls used in literature are the free LDC or the free parent drug [4, 32]. Other controls, such as a liposomal formulation of the parent drug or LDC formulated with other types of carriers, have also been reported in comparative PK studies [33-35]. In addition, the free LDC is likely to require a suitable solubilization vehicle for its administration (e.g. dimethyl sulfoxide, poly(ethylene glycol) (PEG) 400, polysorbate 80) due to its lipophilic nature, which can also alter the PK [10, 25, 36-38]. However, systematic
studies on how such formulation vehicles affect the AUC of LDCs are missing. Finally, when comparing LDCs’ AUC ratios it is important to consider which entity has been tracked, namely the LDC itself or the released parent drug. Fig. 2 reports the chemical structures of the LDCs for which the AUC has been measured, and Table 1 provides their formulations’ properties, including the AUC ratios and the controls used in each case to calculate the ratios. While in the presented examples the liposomal formulation of the LDCs generally increased the circulation times, the ratios varied greatly ranging from up to 2-fold to several thousand-fold. This highlights the diversity of the observed L-LDC systems and illustrates their underlying complexity. Thus, the next section aims to identify factors determining the PK and the AUC of L-LDCs in vivo.

Fig. 2. Molecular structures of L-LDCs for which PK data are available. Compounds’ identification numbers are also listed in Table 1.

Table 1. AUC ratios of various L-LDCs.

3. PKs and biodistribution

3.1 Intravascular route of drug administration

The intravascular route of administration is the most frequently used route for liposomes in general and, with regard to clinical approval, the most successful one to date [53]. The applications of intravascularly injected L-LDCs are diverse, and include the treatment of cancer, human immunodeficiency virus infections and autoimmune diseases [23, 42, 54, 55]. Liposomes may simply serve as a solubilizer for the highly water-insoluble LDCs and/or as a carrier with controlled release properties. NHAC (Section 2.1) and elacytarabine, an elaidic acid ester of cytarabine [5, 21] constitute examples in which liposomes served primarily as solubilizers. These two cytarabine analogues were lipophilized to prevent cytarabine’s fast degradation to the inactive metabolite ara-U [56, 57]. With regard to the drug delivery function of liposomes, the L-LDC approach has been mainly applied to short-lived drugs with low therapeutic index such as cytostatic agents.
3.1.1 In vivo drug release of LDCs

Generally, in order to benefit from the favorable PK profile of its liposomal carrier, a LDC should remain associated to the liposome until it reaches the target site. However, upon i.v. administration, the drug is released over time to some extent, and the success or failure of the L-LDC will strongly depend on the release kinetics. The rational design of the LDC’s chemical structure, allows to modulate the release kinetics from the liposomal carrier and can significantly prolong the circulation time in vivo. Studying the in vivo release is thus key for a deeper understanding of any L-LDC system.

For LDCs conjugated to a single alkyl chain, in vivo liposomal release was shown to be fast. For instance, the cytarabine analogue NHAC with only one alkyl chain anchor rapidly leaved its liposomal carrier for plasma proteins and erythrocytes, and 5 min after injection into mice less than 10% remained liposome-bound [5]. As discussed in Section 2.1, cholesterol provides a stronger anchoring than single alkyl chains, which was also observed in in vivo studies [22]. Similarly, good retentions were observed for anchor structures that combined the structural characteristics of both, an alkyl chain with cholesterol, namely in a oleoyl-lithocholyl-anchored daunorubicin derivative (Fig. 2, compound 20) [52]. Gabizon et al. [4, 20518] provided further evidence using the mitomycin C prodrug MLP (Section 2.1) that having two alkyl chains interacting with the liposomal bilayer increased substantially the retention. This drug had indeed the highest calculated AUC ratio versus free mitomycin C (more than 3000, Table 1, compound 14), which could be attributed to a suitable prodrug design [4]. Besides an anchor with two alkyl chains that stabilized the drug in the liposomal bilayer, the drug included a disulfide linker attached to an aromatic ring (Fig. 2, compound 14) that was highly stable in normal blood (Fig. 3B) but cleavable upon exposure to reducing environments, such as those encountered in tumors [58].

Not only anchor structures bear the potential to control the release (see Section 2.1), LDC transfer can also be modulated by using different linker structures. In a study involving zidovudine-based LDCs, it was shown that 30 min after i.v. injection the LDC with an amine linker was less prone to distribute to erythrocytes than its amide-linked counterpart (Fig. 2, compounds 7 and 24) [42]. In addition, besides controlling exchange rates, linker structures also determine the cleavage of LDCs and by adequately engineering them, it is possible to avoid premature cleavage. This is well illustrated by the already mentioned study comparing the two zidovudine-based LDCs, which indeed showed fast cleavage for the amide-linked
derivative. Thirty minutes after systemic injection, 40% of the amide-linked zidovudine-based LDC had already been metabolized, while only 10% of the amine-linked analogue was converted to the parent drug [42]. This could account for the observed differences in AUC ratio between the two L-LDCs (4.9 to 7.0 of the amide-linked to the amine-linked conjugate, respectively, Table 1, compounds 7 and 24). Another example of immediate cleavage was provided in a study with a methotrexate-based LDC conjugated to a dioleoylglyceride-anchor with a linker structure containing two ester and an amide bond (Fig. 2, compound 13) [46]. Upon systemic injection into healthy mice, the L-LDC rapidly released the parent drug indicated by a higher AUC of the parent drug compared to the one of the LDC (AUC ratios were 2.1 versus 0.9, respectively, Table 1, compound 13) [46]. This was not in line with an earlier in vitro study where the conjugate was said to be stable in human plasma for more than 24 h when entrapped in liposomes, and was explained by differences in interspecies enzyme activity [59]. Anyhow, the L-LDC revealed an increased therapeutic index compared to free methotrexate despite the rapid conversion to the parent drug possibly due to a sustained-release effect.

Fig. 3. Illustration of the different administration routes described for L-LDCs (A). Examples of PK profiles of L-LDC and their respective controls at the various administration sites (B-E). C and D represent the total drug amount remaining in the injected thigh muscle and knee joint, respectively. Full name of LDC in panel B is specified in Fig. 2. B, C, D, and E were adapted with permission from [4], [11], [60], and [35], respectively.

3.1.2 Impact of the liposomal carrier on L-LDC PKs

Aside from the nature of the lipophilic moiety and the linker of a LDC, the composition and physicochemical properties of the liposomes also have a major impact on the PK and biodistribution. While long-circulating PEGylated liposomes are generally preferred, there are therapeutic applications for which the extensive uptake of non-PEGylated vesicles by the mononuclear phagocyte system (MPS) may be beneficial [61]. This is the case of 1,2-dipalmitoyl-glycero-3-muramyl tripeptide-phosphatidylethanolamine (MTP-PE) or mifamurtide (compound 15, Fig. 2), a lipophilic derivative of a component found in bacterial cell walls called muramyl dipeptide. Both parent drug and LDC exhibit immunomodulating properties by inducing macrophages’ tumoricidal activity, but the L-LDC has proved to be superior over the free parent drug due to a reduced renal clearance [62]. The mifamurtide
liposomal formulation is special in that in addition to its relatively large size (2.0 to 3.5 μm), it holds a negatively charged phosphatidylserine-based lipid that increases MPS uptake [63-25565]. Thus, this L-LDC is one of the few examples in Table 1 (compound 15) with an AUC ratio of < 1, which can be explained by its fast capture by the MPS (mainly in liver, lung, and spleen) [47, 66].

Prolonged systemic circulation of liposomal carriers is especially important for targeting disease sites that profit from the enhanced permeation and retention effect, which is predominantly found in cancer or inflamed tissues [67]. A docetaxel-based LDC formulated in PEGylated liposomes achieved higher levels in the tumor and reduced deposition in non-MPS organs such as the heart and lungs compared to the control docetaxel solution [68]. Teshima et al. [25, 40] systematically investigated in rats the impact of PEG coating on the PK of a prednisolone-based L-LDC with a palmitoyl-anchor (Fig. 2, compound 4), and found that compared to the non-PEGylated counterparts, the PEGylated egg phosphatidylcholine (EPC)-based and distearoylphosphatidylcholine (DSPC)-based L-LDCs exhibited significantly increased AUC (2.5- and 2.2-fold, respectively, Table 1, compound 4). However, circulation time is not the only parameter determining efficacy as shown by a study investigating the tumor efficacy of the cisplatin-based L-LDC cis-bis-neodecanoato-1,2-diaminocyclohexane-platinum(II) (NDDP) (Fig. 2, compound 19) [51, 69]. By comparing two long-circulating liposomal formulations, one with PEG and one with monosialoganglioside, the authors found that the latter formulation had reduced clearance compared to the PEGylated-counterpart. In spite of this, the highest anti-tumor efficacy was achieved with the PEGylated liposomal NDDP, as already indicated by preliminary in vitro cytotoxicity tests, which could be explained by an enhanced release of active parent drug [69, 70].

Active targeting via liposome-decorating targeting moieties has been shown to influence L-LDCs’ PK [52, 71-73]. Mori et al. [71] reported a first example of an actively targeted L-LDC system in mice using liposomes decorated with the monoclonal antibody 34A, which targets capillary endothelial cells of the lung. The liposomes were loaded with a LDC of floxuridine and showed an up to 50-fold higher drug accumulation in the lungs over time compared to the control group treated with non-targeted liposomes. A survival study with mice bearing lung metastasis supported the biodistribution data, revealing a significant increase in survival time for the group treated with the active targeted L-LDC [71]. In a follow-up publication, the authors evaluated the PK of PEGylated liposomes loaded with the
same LDC in healthy and tumor-bearing mice [72]. In this case, the liposomes were coupled to a monoclonal antibody targeting colon adenocarcinoma tumor cells. In the presence of the antibody, clearance was reduced in healthy mice while in tumor-bearing mice it was enhanced, likely due to an increase in tumor uptake. Another study evaluated a L-LDC system of apolipoprotein E-associated liposomes loaded with a daunorubicin-based LDC (Fig. 2, compound 20) [52]. The liver uptake of the daunorubicin conjugate was shown to be increased by 5-fold in rats with up-regulated LDL-receptors in the liver compared to the non-targeted L-LDC control.

The nature of the phospholipids in the bilayer also influences the fate of the L-LDCs. The AUC is generally higher for liposomes prepared with high phase transition lipids such as D二级 PC (T$_m$ = 55.1 °C [74]). Floxuridine-dipalmitate loaded into liquid crystalline EPC liposomes was cleared faster than when incorporated into more rigid DSPC vesicles [75]. Interestingly, Teshima et al. [25, 40] reported less predictable results with the 21-palmitoyl-prednisolone LDC (Fig. 2, compound 4). For non-PEGylated as well as for PEGylated liposomes with low PEG levels, no significant differences in AUC were found between EPC- and DSPC-based liposomes (Table 1, compound 4). However, at high PEG concentrations (10 mol%), the AUC of DSPC-based liposomes was 1.4-fold lower than that of EPC-based liposomes, which could be explained by the formation of unstable micelle-based structures [76]. The use of sphingomyelin over phosphatidylcholine (PC) is another approach to further increase the LDC half-life, as it was demonstrated in a study with a 6-mercaptopurine-analogue conjugated to a stearoyl glycerol-anchor (Fig. 2, compound 12) [45, 77], where the AUC ratio increased by 1.5-fold for the L-LDC containing sphingomyelin (Table 1, compound 12). The addition of cholesterol in the membrane is also a well-established approach to reduce clearance [78]. Its insertion in the mitomycin C-based LDC MLP formulation led not only to an increase in half-life and AUC of liposomal MLP but also revealed superior anticancer efficacy in a pancreatic carcinoma mouse model compared to a formulation without cholesterol [18].

Finally, the anchoring of LDC in the liposomal membrane can have an impact on the in vivo fate of liposomes by altering their surface and membrane structure, and increase their clearance by the immune system. This is in fact a major drawback of the L-LDC approach, since it might impede the use of liposomes with high payloads. For instance, the incorporation of a phospholipid-anchored LDC of 2’-C-cyano-2’-deoxy-1-β-D-arabinofuranosyl-cytosine-3’-phosphate (compound 3) into liposomes would further increase their AUC.
pentofuranosylcytosine was reported to reduce the blood circulation time of liposomes compared to unloaded vesicles [33]. Similarly, conventional liposomes loaded with 10 mol% floxuridine-dipalmitate were cleared faster than those containing 2 mol% of the drug [75].

3.2 Extravascular route of drug administration

The extravascular administration of L-LDCs is performed for localized or systemic actions. L-LDCs can be used for the local treatment of diseases or pathologies such as osteoarthritis or lung inflammation to avoid drug exposure of healthy tissues and reduce systemic toxicity [54, 32555]. A prerequisite for this is the stability and retention of the liposomes at the injection site, combined with a slow drug release profile. Additionally, given that after extravascular injection small liposomes are slowly absorbed into the blood circulation, L-LDCs have also been employed to achieve sustained systemic exposure and prevent high peak blood concentrations [49].

Intramuscular (i.m.) injections of L-LDCs have been used to deliver anticancer drugs to surrounding lymphatic tissues and treat lymph node tumoral metastasis [79]. Sasaki et al. [11] showed that the liposomal formulation of a 5-fluorouracil-based LDC with an octadecyl anchor and an amide linker was extensively retained in the thigh muscle of rats after i.m. injection (Fig. 3C). The L-LDC enabled high drug concentrations in a regional lymph node for a prolonged period. It also minimized systemic exposure when compared to the liposomal formulation of the parent drug 5-fluorouracil, which was readily absorbed in the blood circulation. Similar findings were obtained in an earlier study by the same group with a mitomycin C-based LDC conjugated to a nonyl anchor via an ester bond [37].

L-LDCs have also been administered via the intra-articular (i.a.) route for the treatment of joint diseases. Lopez-Garcia et al. [60] injected i.a. 21-palmitoyl-triamcinolone acetonide entrapped in conventional multilamellar liposomes to rabbits and monitored the drug concentrations in the knee joint. While free triamcinolone acetonide was cleared immediately, the L-LDC could be detected for more than 40 h after its administration (Fig. 3D). Overall, the prolonged local PK profile led to a considerable decrease in knee joint inflammation. In another study investigating drug retention in the joint, liposomal size proved to be a key parameter. Bonanomi et al. [80] compared liposomes of 160 and 750 nm loaded with the LDC palmitoyl-dexamethasone and found that efficacy was enhanced with the larger ones.
This could be explained by the 2.6-fold higher drug concentration that was detected in the joint 48 h after i.a. injection most likely due to a decreased liposomal drainage. Similar results were obtained with a methotrexate-based phospholipid analogue entrapped in 100-nm small unilamellar vesicles and 1.2-μm multilamellar vesicles (MLVs) [54]. Local L-LDC-based therapy for joint diseases seems promising with regard to the above-illustrated therapeutic outcomes. However, according to Burt et al. [81] the long-term release of drugs applied via i.a. injection should last from weeks up to months to minimize the frequency of injections, which can be associated with adverse side effects. Achieving such long releasing kinetics at the target site is, however, a challenging task with L-LDCs.

Intraperitoneal (i.p.) administration has been investigated in multiple cases for the delivery of L-LDCs given its applicability for both local and systemic treatments [82, 83]. This administration route was recently employed to improve the metabolism of overdosed ethanol following the administration of enzymatic L-LDC in the peritoneal cavity [84]. Depending on their lipid composition and size, liposomes can leave the peritoneal cavity relatively quickly and reach the blood compartment, achieving systemic exposure [85-87]. Vesicles with sizes greater than 1000 nm largely remain in the peritoneal cavity and it has been shown that PEGylation as well as positive charges favor liposomes’ prolonged retention in the peritoneal fluid [85, 86]. Vadiei et al. [51] directly compared the i.p. and i.v. routes of administration in rats using the liposomal cisplatin-based LDC NDDP, and found that the C\textsubscript{max} was 2.2-fold lower for the i.p. injected liposomal NDDP group. Due to the large size of the liposomes in the range of 1 to 5 μm, the group postulated that liposomal migration towards the blood circulation was low and that the NDDP fraction reaching the blood compartment after the i.p. injection was probably released from the liposomal carrier and associated to plasma proteins.

Other less common routes of administration that have been tested with L-LDCs include the intrapulmonary and intravitreal routes. For instance, Wijagkanalan et al. [55] succeeded to decrease systemic glucocorticoid exposure by delivering dexamethasone palmitate entrapped in liposomes directly into the lungs via microspraying. Intravitreal injection of the LDC O-palmitoyl-tilisolol loaded in liposomes was investigated to increase the retention in the vitreous body, showing significantly higher concentration of the L-LDC compared to tilisolol-bearing liposomes (Fig. 3E) [35].
4. From preclinical studies to clinical trials

Despite the promising results obtained in preclinical studies, only five L-LDCs have entered clinical trials to date. The very first clinical studies were performed in the mid-1980s with liposomal mifamurtide (L-MTP-PE, described in Section 3.1.2, structure shown in Fig. 2, compound 15), later known under the trade name Mepact®. L-MTP-PE has been tested in humans for several indications including melanoma, soft tissue sarcoma, and osteosarcoma. Combined with chemotherapy, L-MTP-PE showed promising benefits particularly in osteosarcoma patients, and received the orphan drug designation in 2001 by the FDA and in 2004 by the EMA. Five years later, the EMA granted full market approval to MTP-PE as Mepact® for the treatment of high-grade resectable non-metastatic osteosarcoma. MTP-PE has, however, not been approved in the U.S. as the antitumor effect of L-MTP-PE in the treatment of osteosarcoma remains under debate [88]. PK investigations in healthy humans following the infusion of 4 mg revealed a biphasic clearance pattern with a fast decline in drug serum concentration in the first hour, indicating a fast uptake by the MPS [89]. Generally, PK of L-MTP-PE was not affected in patients with renal or hepatic impairment [90, 91]. L-MTP-PE therapy is well tolerated with minor side-effects (headache, chills, tachycardia, nausea, and pyrexia) [89], and in 2016 the EMA granted it orphan designation for two additional indications, namely the treatment of hepatocellular carcinoma and echinococcosis. However, Mepact® has not received market approval for the latter indications, yet.

In the late 1980s, L-NDDP a liposomal LDC of cisplatin (Section 3.1.2, structure shown in Fig. 2, compound 19) was tested for the first time in humans. PK profiles after i.v. infusion revealed biphasic patterns at concentrations of 312.5 mg/m² or below, as seen before in rats and rabbits [92]. At doses of 390 mg/m², the clearance followed a monoexponential curve indicating MPS saturation and a transition from linear to non-linear PK (Fig. 4A) [92, 93]. Administering L-NDDP via the i.v. route, however, only achieved moderate responses in patients with refractory colorectal cancer [94]. Other clinical studies with L-NDDP have focused on its local administration such as intrapleurally and i.p. [95, 96]. These administration routes led to a significant decrease in systemic NDDP exposure and allowed higher maximum tolerated doses (MTDs) compared to i.v. administration. L-NDDP (Aroplatin™) was designated as an orphan drug by the FDA in 1999 for the treatment of malignant mesothelioma but did not reach the market. Clinical trials evaluating its efficacy in malignant pleural mesothelioma indicated a response rate of 42% but suggested that lesions
spreading into the surrounding tissue might not be affected by the local therapy [97]. In 2005, a clinical phase 1 trial was initiated in patients with advanced solid malignancies and B-cell lymphoma with a new, undisclosed formulation of Aroplatin™. However, this product was later discontinued and never reached market approval. Multiple reasons might have accounted for the lack of success of L-NDDP [98]. Firstly, NDDP was not a single compound but an isomeric mixture with lipophilic anchors of different chain lengths (Fig. 2, compound 19) [98]. Secondly, upon preparation of the infusion solution by rehydrating the lyophilized powder with isotonic saline solution, NDDP rapidly converted into its parent drug and after 1 h more than 50% was released from the liposomes [98, 99]. Thirdly, the liposomes used in the L-NDDP formulation were conventional and not PEGylated liposomes, which could have been beneficial in the systemic therapy of solid tumors [69, 100].

Three L-LDCs of cytarabine analogues have progressed into clinical trials. A N^4-acyl derivative of cytarabine with an ester linker, N^4-oleyl-ara-C, appeared to be rapidly degraded to the inactive metabolite ara-U with a half-life of merely 25 min as shown in a pilot phase I/II study [101]. These results prompted the development of the more stable N^4-alkyl derivatives with amide linkers, such as NOAC (Section 2.1). NOAC was less prone to deamination as determined in a phase I study [102]. Doses of 600 mg/m^2 in patients were well tolerated without unexpected side effects and led to long plasma half-lives of up to 16 h (versus a few minutes for free cytarabine) [102, 103]. However, to the best of our knowledge, this formulation did not enter clinical phase II. Various clinical studies have been performed with the LDC of elacytarabine (Section 3.1) [104, 105]. Its L-LDC formulation, a combination of liposomes and micelles with size ranges of 5-45 nm, gained orphan drug designation in the EU and the USA in 2007 and 2008, respectively [57]. Its elimination was found to be linear at doses up to 1500 mg/m^2 [104]. Encouraging pilot efficacy results led to an international randomized phase III trial for patients with relapsed or refractory acute myeloid leukemia which, however, concluded that liposomal elacytarabine did not exert any benefit on the overall survival compared to the standard therapy [105].

More recently, the new promising L-LDC PL-MLP was evaluated in a phase I study under the trade name Promitil® (Sections 2 and 3, Fig. 2, compound 14). The study confirmed its prolonged circulation time and good tolerability (3-fold increase in MTD) compared to the parent drug [106]. Linear PK were observed in humans with doses from 0.5 to 2.5 mg (Fig. 4B). PL-MLP is indeed the first long-circulating or PEGylated L-LDC that has progressed
into clinical trials. An expanded phase Ib/IIa study is currently ongoing in patients with advanced colorectal cancer to investigate its safety and efficacy [107].

**Fig. 4.** NDDP (A) and MLP (B) dose *versus* AUC. Green area indicates the linear PK range and red area indicates the non-linear. Data are adapted from [92] and [106], respectively.

### 4505. Conclusion

Since the first formulation attempts in the 1970s, L-LDCs have undergone important development in the past forty years. However, the combination of a lipid-based drug derivative associated to a liposomal carrier might not be an obvious first-choice approach to modulate the PK or biodistribution of challenging drugs. The laborious and costly development implied, together with the extra testing procedures required for the new lipophilic drug candidate are possibly the main reasons why L-LDC formulations have been mainly investigated for drugs with poor encapsulation yields by conventional active or passive loading methods. Notwithstanding, lipophilic drug derivatization has one great advantage over all other loading methods. As long as conjugation *via* a linker is possible, the physicochemical properties of the drug are not as crucial to ensure a good entrapment efficiency. As illustrated by various examples in the present review, the L-LDC approach is not only effective to disperse lipophilic drugs, but can also substantially alter a LDC’s PK. In this regard, the structure of the conjugated lipid and linkage- whether cleavable or not- as well as the liposomal formulation have an important impact on the *in vivo* L-LDCs’ performance.

The new generation of L-LDC with long-circulating properties and suitable designs in terms of anchor and linker seem promising drug delivery systems. In several studies, L-LDCs were shown to be active while exhibiting a better safety profile than the free drug. However, with respect to their application in humans, clinical trials have so far been quite disappointing with only one product achieving market approval in selected countries. The initial lack of success of early L-LDC formulations may be attributed to insufficient characterization and the use of sub-optimal liposome formulations. The efficacy results expected in the near future for the first PEGylated mitomycin-based L-LDC tested in humans might revive the interest of the pharmaceutical industry for the L-LDC approach.
Acknowledgements

Financial support from the Swiss National Science Foundation (Sinergia program, CR15I3_147651) and Lipoid GmbH (endowment to the University of Jena for P. L.) is acknowledged. The authors thank Dr. Jong Ah Kim for her critical reading and editing of the manuscript.

References


diaminocyclohexane platinum(II) and cisplatin given i.v. and i.p. in the rat, Cancer Chemother. Pharmacol., 30 (1992) 365-369.


Figure 1
1 Linoleoyl-SN38  
« LA-SN38 »

2 Cis-diamminedioleato-platinum(II)/cis-diammine-1-chloro-2-oleato-platinum(II)  
« OA-CDDP »

3 5'-O-stearoyl-8-chloroadenosine  
« 8CAS »

4 21-Palmitoyl-prednisolone  
« Pal-PLS »

5 N^4-stearoyl-gemcitabine

6 5'-O-myristoyl-zidovudine  
« AZT-M »

7 N^4-palmitoyl-zalcitabine-(3'→5')-zidovudine  
« N^4-pamdC-AZT »

8 N^4-[N-(cholesteryloxycarbonyl)glycyl]-cytarabine  
« COCG-Ara-C »

Figure 2.1
9. \( N^\prime \)-cholesteryloxy carbonyl-gemcitabine  
   « Gem-Chol »

10. \( N \)-(cholesteryloxy carbonyl)glycyl-mitomycin C

11. Cholesteryl oxy acetyl-mitomycin C

12. 1-Stearoyl-glycero-3-succinyl-6-mercaptopurine  
   « 6-MP »

13. 1,2-Dioleoyl-glycero-3-\( \beta \)-alanyl-\( N \)-carbonylmethyl- 
    methotrexate  
   « MTX-DG »

14. 2,3-(Distearoxyloxy)propane-1-dithio-4' -benzyl oxy carbonyl- 
    mitomycin C  
   « MLP »

15. 1,2-Dipalmitoyl-glycero-3-muramyl tripeptide-phosphatidylethanolamine  
    « MTP-PE, mifamurtide »

16. 1-Palmitoyl-2-(myristylaminecarbonyloxy-oxaliplatin)succinyl- 
    glycero-3-phosphatidylcholine  
    « Oxalilipid »

Figure 2.2
17
dichloro-(n-lauryl)-propanediamine-platinum(II)
« DDPP »

18
cis-bis-N-decyliminodiacetato-1,2-diaminocyclohexane-platinum(II)
« N-decyl-IDP »

19
cis-bis-neodecanoato-1,2-diaminocyclohexane-platinum(II)
« NDDP »

R₁, R₂, R₃ are aliphatic groups of 2 to 4 carbons

20
N-[3-O-(oleoyl)-lithocholyl]-alanyl-leucyl-alanyl-leucyl-daunorubicin
« LAD »

21
α-tocopherylthiodiglycolyl-docetaxel
« DTX-s-VE »

22
α-tocopherylthiodiglycolyl-docetaxel
« DTX-ss-VE »

23
Nonyloxycarbonyl-mitomycin C

24
N⁴-palmityl-zalcitabine-(3'→5')-zidovudine
« N⁴-hxddC-AZT »

« » Trivial name or widely used abbreviation.
## Table 1.1

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Drug, measured entity in the L-LDC group.
Control, measured entity in the control group.
LDC, lipid-drug conjugate.
Parent, parent drug.
DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine.
SPC, soy phosphatidylcholine.
POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine.

---

**Table 1.3**
Figure 3
Figure 4

A.

Dose (mg/m²)

AUC (µg Pt/mL/min)

B.

Dose (mg/kg)

AUC (mg/L/h)