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REVIEW

Lipid-based carriers for the delivery of local anesthetics

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ABSTRACT

Introduction: There is a clinical need for pharmaceutical dosage forms devised to prolong the acting time of local anesthetic (LA) agents or to reduce their toxicity. Encapsulation of LA in drug delivery systems (DDSs) can provide long-term anesthesia for inpatients (e.g. in immediate postsurgical pain control, avoiding the side effects from systemic analgesia) and diminished systemic toxicity for outpatients (in ambulatory/dentistry procedures). The lipid-based formulations described here, such as liposomes, microemulsions, and lipid nanoparticles, have provided several nanotechnological advances and therapeutic alternatives despite some inherent limitations associated with the fabrication processes, costs, and preclinical evaluation models.

Areas covered: A description of the currently promising lipid-based carriers, including liposomes, microemulsions, and nanostructured lipid carriers, followed by a systematic review of the existing lipid-based formulations proposed for LA. Trends in the research of these LA-in-DDS are then exposed, from the point of view of administration route and alternatives for non-traditionally administered LA molecules.

Expert opinion: Considering the current state and potential future developments in the field, we discuss the reasons for why dozens of formulations published every year fail to reach clinical trials; only one lipid-based formulation for the delivery of local anesthetic (Exparel®) has been approved so far.

KEYWORDS

Local anesthetics; drug-delivery systems; liposomes; solid lipid nanoparticles; nanostructured lipid carriers; microemulsions; routes of administration

1. Introduction

Although the synthesis of new active molecules is an effective strategy for the treatment of many pathologies, limitations in physicochemical and pharmacological properties of drugs are driving technological innovations, such as the development of nanostructured carrier systems designed to overcome unfavorable properties of medicines, especially poor water solubility. A variety of nanocarriers have been designed, especially in the last three decades, during which research efforts have focused on the elucidation of their molecular and structural properties.

On the other hand, many concerns about the therapeutic efficacy of nanocarriers came to light after the expected performance of a variety of formulated DDS was challenged in preclinical and clinical trials. In general, the poor performance of nanocarriers *in vivo* can be attributed to a set of factors: i) the complexity of biological systems, including routes of administrations, DDS uptake capability by the bloodstream, and recognition by immunological system; ii) development of nanocarriers with a high degree of structural complexity and fabrication processes, which lacks in reproducibility, reducing their colloidal stability; iii) the use of some non-biocompatible components in nanocarrier formulations; and iv) the high financial costs related to the design of nanomedicines, including the scale-up steps [1-4]. All of these factors contribute to reducing nanocarriers' suitability to clinical applications.

Among the nanocarriers available, lipid-based DDSs offer a series of advantages over non-lipid carriers (such as biocompatibility, biodegradability, and non-immunogenicity, relatively low cost, and no requirement of organic solvents) in comparison to polymeric and inorganic carriers [5]. Lipid-based DDSs (liposomes, solid lipid nanoparticle, nanostructured lipid carriers, lipid-based micelles, nonionic surfactant vesicles, lipid-based polymeric systems) have attracted attention because they can encapsulate low aqueous solubility drugs, have colloidal stability, low toxicity, and are biodegradable [5,6]. Those encouraging results highlighted advantages regarding the pharmacokinetic profile of drugs, considering their enhanced bioavailability, protection against metabolization, as well as the possibility of systemic and local administration [7-9].

The last 30 years have witnessed tremendous growth in the development of nanostructured pharmaceutics, particularly lipid-based DDSs. According to Grewal and coworkers, nanomedicines are expected to account for 22% of the total pharmaceutical market in 2019, when the average compound annual growth rate for this class of products is estimated to be 14.5%, far above the expected (5.5%) for non-nanopharms [10]. Also, as stated by the Global Nanotechnology Drug Delivery Market, the nanomedicines business will have

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Article highlights

- Long-term/low toxic anesthesia is most likely to be achieved with local anesthetics in nanotechnological products
- The route of administration determines the clinical efficacy and relative systemic toxicity of LA-in-nanocarrier formulations
- Liposomes are the most traditional lipid-based DDS; Ionic-gradient liposomes improve the upload capacity of liposomes for the charged LA form
- Microemulsions are effective systems for skin delivery of local anesthetics
- Lipid Nanoparticles (SLN, NLC) have proven advantages for encapsulating high amounts of base LA forms
- Dozens of pharmaceutical dosage formulations are published every year yet do not reach clinical trials, nor hit the market due to the fabrication process, costs, and preclinical assessment limitations

This box summarizes the key points contained in the article.

grown from US$ 4.1 billion in 2014 to US$ 11.9 billion by 2023. Among the 78 nanostructured DDSs approved for medical application, 21 are lipid-based: 13 prepared with liposomes, seven nanoemulsions, and one surfactant-based [10]. Almost 35% of the nanostructured DDS global market is dedicated to chemotherapy agents. Accordingly, anti-cancer drugs are also the most frequent among the lipid-based DDSs, representing 89% of the liposomal formulations in clinical trials. Then, there are only three DDSs approved for pain treatment so far, carrying either morphine, propofol, or bupivacaine in liposomes [11].

These numbers let us question the reasons for the discrepancy between the wide variety of lipid-based nanocarriers and the non-proportional number of clinically available formulations. Additionally, connecting aspects related to biopharmaceutical properties, scale-up costs, biocompatibility, and pharmacological efficacy, what features are expected for an ideal lipid-based nanocarrier designed for pain treatment? How best to apply those concepts to the future clinical use of more effective and safer local anesthetics-DDSs? In other words, the development of lipid-based DDS for pain management continues to be a major challenge, with a growing need for renewal of knowledge along different research lines, considering important aspects such as the local anesthetic physicochemical properties, nanocarrier composition, routes of administration, long term stability, therapeutic efficacy, and systemic and/or local toxicity.

This review article is focused on lipid-based DDS, such as ionic gradient liposomes, nanostructured lipid carriers, and emulsions for different purposes in local anesthesia. Special attention is given to their application as nanotechnological strategies to overcome unfavorable local anesthetics properties, development processes, as well as their influence on pharmaceutical properties and clinical availability.

1.1 Local anesthetics (LA): from chemical structure to commercially available formulations for clinical use

After the discovery of cocaine and the synthesis of the first synthetic LA in 1905 (procaine), new agents have been developed throughout the twentieth century [12], as depicted in Figure 1. The commercially available LA agents, mainly from the ester/aminoester and aminoamide families [13,14], have different physicochemical and pharmacological properties and are used in a variety of doses (Table 1) and routes of administration. The effects of local anesthetics are relatively brief, lasting for hours after a single administration, which is insufficient for the treatment of prolonged, acute, or chronic pain (Table 1). Among them, bupivacaine (BVC) and lidocaine (LDC) are the most used agents worldwide, for infiltrative anesthesia in surgical and ambulatory procedures, respectively [15]. Benzocaine (BZC) prevails in topical formulations [16,17] for the skin, although the eutectic mixture of LDC and prilocaine

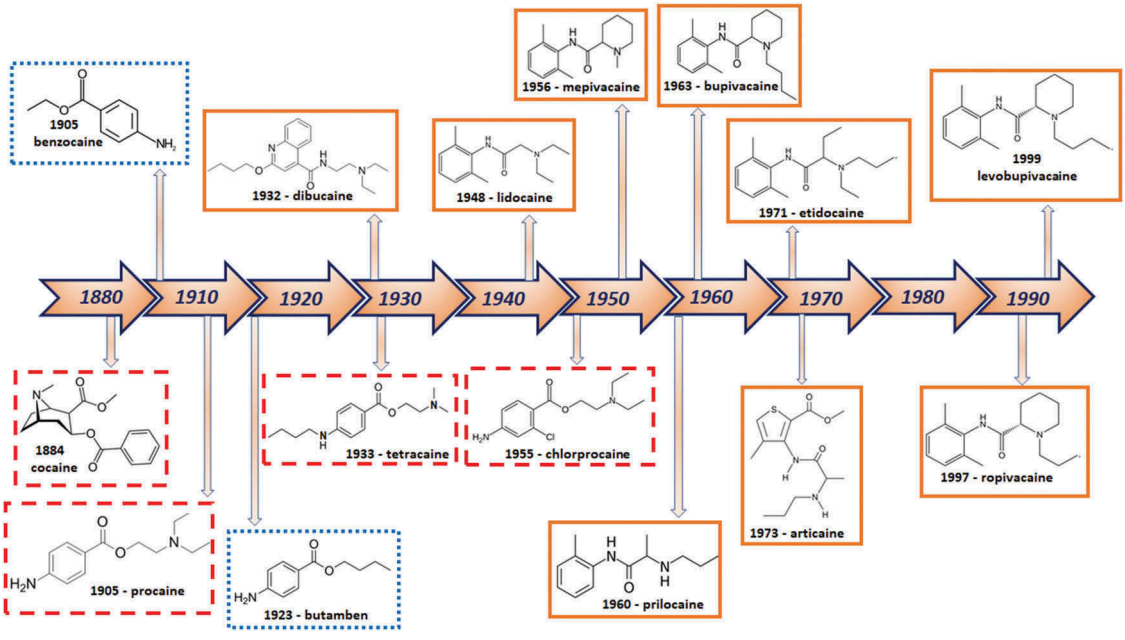

Figure 1. Chemical structure and chronology of local anesthetic agents. The colors identify ester (blue, dots), aminoester (red, dashes), and aminoamide (orange, straight line) families.

Table 1. Some physicochemical and pharmacological properties of local anesthetics from ester, aminoester, and aminoamide families.

Chemical classification	LA	pKa*	Sw (mM)	Partition coefficient	Potency	Duration of action (h)	Half-life (h)	Protein Binding (%)	Maximum dose (mg/kg)
Esters	***Benzocaine (BZC)***	–	4.4	253	–	0.5	0.3	–	3
	Butamben (BTB)	–	0.9	1136	–	-	1.5	–	–
	Procaine (PRC)	9.2	16.3	84	1	1	0.1	6	8
Aminoesters	***Chloroprocaine (CLP)***	9.2	2.0	250	4	0.75	0.1	83	12
	Tetracaine (TTC)	8.5	0.8	868	16	8	2.5	76	1.5
	Lidocaine (LDC)	7.8	13.1	144	4	1.5	1.5	64	5
	Prilocaine (PLC)	7.9	23.1	110	3	1.5	1.5	55	5
	Etidocaine (EDC)	7.7	0.2	1202	16	8	3.0	95	6
Aminoamides	***Mepivacaine (MVC)***	7.6	8.8	98	2	1.5	1.5	77	5
	Ropivacaine (RVC)	8.1	0.7[#]	592[#]	12	6	4.2	94	2.5
	Bupivacaine (BVC)	8.1	0.6	798	16	8	2.5	95	2
	Dibucaine (DBC)	8.3	0.03	2240	16	–	11.0	> 95	0.5
	Articaine (ATC)	7.8	62.1[#]	10	1.5	1.5	0.5	76	7

*pKa values at 25°C [13]; pKa of the *p*-aminobenzoate group of BZC, BTB, PRC, CLP, and TTC (~ 2.5) was not considered. [#] determined by us. Aqueous solubility (Sw) and Partition coefficients determined for the neutral (base) species, between egg phosphatidyl choline/buffer, at pH 10.5 [19,83,85] except for BZC, BTB (determined at pH 7.4) and ATC (pH 9.0). Potency, protein binding percentage, duration of action, half-lives, and maximum doses [20–29].

(PLC), known as EMLA®, has gained increasing importance for cutaneous anesthesia [18].

The fast metabolism of aminoesters by serum esterases can be blamed for the decline in chloroprocaine (CLP) and procaine (PRC) use over the years, although such rapid hydrolysis and minimal placental transfer justify CLP use in obstetric epidurals and its approval for intrathecal administration by the FDA [29]. From the aminoester family, only the most hydrophobic agent tetracaine (TTC) remained popular due to its potency, being the most used LA in ophthalmic procedures [30]. BZC and its butyl-derivative butamben (BTB) are also esters, but the lack of the amine group allows them to remain uncharged at physiological pH, explaining their low water solubility and restricted (topical) application [31].

Aminoamide agents are more stable than aminoesters because of their hepatic metabolism [32]. PLC, mepivacaine (MVC), and articaine (ATC) are short action agents with widespread use in dentistry [33], the latter having a particular structure – with an additional ester bond and thiophene instead of a benzene ring (Figure 1) – erroneously considered to increase its hydrophobicity [34]. The commercial appeal of dibucaine (DBC) is in the control of mucosal pain, mainly for the treatment of hemorrhoids [35,36]. Etidocaine (EDC) has been banned from the market because of its alleged systemic toxicity, although this is a matter of debate [37]. The youngest member of the aminoamide family is ropivacaine (RVC), synthesized in the less toxic levorotatory S(-) enantiomeric form, to overcome the systemic toxicity of racemic BVC in surgical anesthesia [38–40].

LAs have wide clinical application in regional blockades, postoperative analgesia, and treatment of acute/chronic pain because of their antiarrhythmic and other nonconventional effects, such as anti-inflammatory and anti-metastatic [14,41–43]. Therefore, several studies have reported differences in pharmacological effects, clinical efficacy, and systemic toxicity related to infiltrative, regional blockades, or topical administration (skin and mucosa application) [14,41].

The relatively short duration of action of LA is due to their easy removal from the site of administration and systemic distribution. Despite the higher potency and membrane affinity of the LA base (neutral) form, most LA formulations are prepared with hydrochloride salts (i.e. with the charged or protonated LA form) due to their higher aqueous solubility compared to the neutral form. In association with the physicochemical properties of LA agents, the route of administration can also predict their pharmacological performance, with impacts on patient acceptance.

For example, regional anesthesia is one of the most important procedures since it is used to prevent persistent postoperative pain, avoiding the progression from acute to chronic pain after surgical procedures [14,44]. Even then, biological variables, such as gender, genotype, and phenotype, are related to the predisposition to postoperative pain, and regional anesthesia has proven long-term benefits to the patients when compared to pain management immediately after surgeries [44]. The main LA agents used for surgical procedures are BVC, RVC, and LDC (in short surgeries and dentistry). Additionally, postoperative analgesia is also achieved by peripheral nerve blockades with effective results compared, for instance, to intra-articular administration. Those differences in efficacy can also be attributed to the systemic distribution of the LA agent, acting on both the central and peripheral nervous system [45], but even that systemic distribution is currently associated with toxicity. Other studies reported that intravenous lidocaine infusions are effective for postoperative treatment, reducing the opioid consumption, and prolonging the duration of analgesia, especially in oncological patients [46–48]. All those routes of administration are considered for both hydrophobic (such as RVC and BVC) and hydrophilic (e.g. LDC and MVC) aminoamides LAs.

Among non-invasive methods of administration, in recent decades, topical application (skin and mucosa) of LA has also received considerable attention, especially during the early stages of the development of new formulations. In the skin, the main limitation is imposed by the stratum corneum and its complex lipid matrix, limiting the penetration of drugs. On the other hand, the main reason for suboptimal oral mucosa local anesthesia is the dilution on saliva, reducing the contact area of the formulation with the mucosa [17,49]. The most common topical LAs are BZC, PLC, and LDC as gels, ointments, creams

(benzocaine and the eutectic mixture of lidocaine and prilocaine), and patches (LDC), with the most diverse compositions. These formulations, intended for both adult and pediatric use, aim to promote drug permeation at the application site, evoking rapid onset of action and prolonging the anesthetic effect [18,50].

Studies have reported that BZC and LDC gels have been associated with insufficient analgesia [51,52] on oral mucosa and skin due to gel dilution by contact with saliva and the loss of adhesive properties during application to the gingival mucosa (in LDC-loaded patches, for example), which reduced the permeation surface and prolonged the time required for the onset of anesthesia [53]. Another important point is that some of these products, either for application to the skin or mucous membranes, have higher concentrations (20%) than most traditional topical gels (e.g. EMLA® with 2.5% lidocaine and 2.5% prilocaine), and therefore precautions are required regarding toxicity. To overcome those limitations, new formulations based on nanotechnological strategies have been developed. Some of these will be detailed in the next sections.

2. DDSs for local anesthetics

Since long-term anesthesia is a clinical need, efforts to obtain prolonged analgesia with local anesthetics agents instead of opioids in post-surgical pain management, or to decrease the systemic toxicity of the most potent LA agents during/after surgery or dental procedures, is of outermost importance. In this scenario, DDSs are very meaningful for clinical anesthesia, prolonging the time of action of LA from hours to days [36,54], and avoiding the side effects from systemic analgesia in post-surgical pain control [55] and dentistry procedures [56,57].

In a publication of 1997, Kuzma and coworkers reviewed the literature concerning long-acting DDSs for LA from 1970–1996 and complained that, although promising, none of them has shown "reliable and practical prolongation of anesthesia in humans" [58]. In fact, it was the breakthrough report of Grant and coworkers – of a 2-day anesthesia after subcutaneous injection with an ionic-gradient liposome formulation of bupivacaine in human volunteers [54] – that opened the way for the approval of the first LA-in-DDS (Exparel® Pacira Pharm. Inc.) in 2011 [10]. However, its clinical indication was to produce postsurgical local analgesia as well as postsurgical regional analgesia (in the case of interscalene brachial plexus nerve block), a clear and specific therapeutic use considering the wide variety of LA clinical applications.

The literature includes multiple good reviews that scrutinize patents [16] and publications [15,59–62], including all kinds of LA-in-DDSs, even those devoted to anesthetizing the skin [17] or the mouth [33,49]. It is worth noting that, due to its direct application at the desired area (regional nerve blockades, local infiltration, etc.), drug targeting is not a concern when preparing DDS for LA, as it is for other drugs. This is quite an advantage because preferential delivery to target cell/tissue is difficult to achieve [11,63], and it depends on the expression of specific molecules. Accordingly, and independently of the administration route, increased LA blood levels are only observed as side-effects due to inadvertent intravenous injections, and/or the clearance of LA agents from the administration site. Next, the most promising lipid-based formulations designed for local anesthetics are considered.

2.1 Liposomes

Liposomes consist of concentric, mono, or multi bilayers, formed from the organization of naturally occurring biocompatible lipids in water (Figure 2). The classification of liposomes depends on the number of lipid bilayers, size, surface charge, lipid composition, and methods of vesicle formation [63–66]. Phospholipids are the major liposomes components, and although cholesterol is not required for their lamellar ensemble, it has a prominent role in determining bilayer fluidity – a crucial factor for prolonged release of drugs, mainly in ionic-gradient liposomes [67].

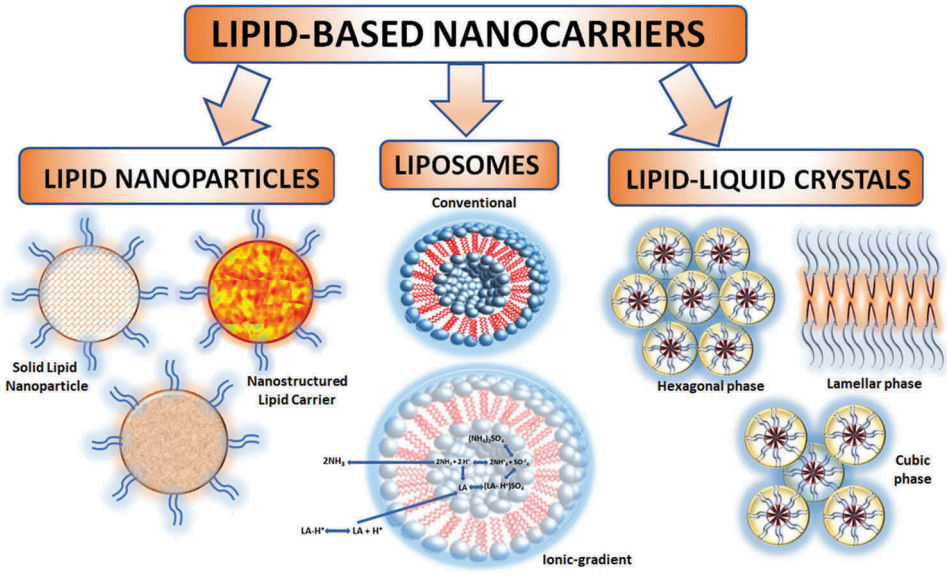

Figure 2. Representation of the lipid-based nanocarriers described as DDSs for local anesthetics.

The distinctive ability of liposome to encapsulate drugs both in the inner aqueous compartment and lipid (bilayer) phases makes them attractive for the delivery of hydro-philic and hydrophobic drugs. Among the lipid-based DDSs, liposomes are by far the most common and investigated nanocarrier systems, with the largest number of products approved for human use [10]. In many of those formulations, liposomes have been PEGylated to form "stealth" vesicles that are able to escape from recognition and uptake by the reticuloendothelial system [68], or even be ligand-targeted (with aptamers such as proteins, vitamins, peptides, antibodies, and nucleic acids), which directs them to the desired tissues [63]. Drawbacks for the clinical effectiveness of liposomal preparations are mainly technological and related to instability of the colloidal systems, such as vesicles size control during preparation and drug leakage during storage, stability over time, and issues associated with scaling up production [69]. It is also important to mention the high price of synthetic lipids and the cost of preparation under sterile conditions [70,71], as well as concerns about the toxicity of long-term liposomal formulations of anti-cancer agents [72].

Beyond advantages such as biocompatibility, biodegradability, and nonimmunogenicity, liposomes can encapsulate amphiphilic drugs, such as local anesthetics [64,73]. Although the original description of liposomes (by Bangham) was made in 1965 [74], the first report of LA encapsulation into liposomes for drug delivery occurred in 1988, when the efficacy of liposomal tetracaine topically administered to humans was demonstrated [75]. The following reviews depict how, since then, liposomal LA formulations have been used to produce slow drug release, prolonged anesthesia, and reduced systemic and tissue toxicity following parenteral [15,59,60,76] or topical administration [17,33]]. The reader is encouraged to see Table 1 of the previously published revision [15] for a systematic description of pre-clinical studies conducted with LA into liposomes of different compositions, types (multi or unilamellar), and pH conditions. All of those studies report improved anesthetic effects, such as increased duration of anesthesia and sensory blockade.

Yet, a common feature of liposomal formulations is that LA encapsulation is not as high as those attained with other DDSs. Moreover, a fraction of the LA is intentionally left non-encapsulated, for the sake of fast onset of anesthesia. Other interesting points are that no targeting is necessary – since the drug is administered directly into the desired tissue – and no fine control of the particles size is required, also because liposomal LA formulations are locally applied. To exemplify that, an unusual ropivacaine formulation prepared as "pro-liposomes" and composed of hydrogenated phosphatidylcholine, castor oil, cysteine hydrochloride, and ethanol was described by Gynosar et al. [70]. According to the authors, the large multilamellar liposomes (in the micrometer range) that were formed after subcutaneous administration to human volunteers promoted 28 h of anesthesia, and the formulation was stable for 2 years at room temperature.

In 2011, the US FDA approved the use of Exparel®, a multivesicular bupivacaine liposomal formulation based on DepoFoam™ technology for regional anesthesia. These modified liposomes, composed of phospholipids (dierucoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine) and cholesterol, also contain tricaprylin, a triglyceride that seems to stabilize the bilayers, allowing the formation of giant multivesicular vesicles with an average diameter of 24–31 μm [11]. In DDSs, large particles are generally better for achieving high local drug level over prolonged periods – by creating a depot, as required for local anesthesia [77].

Exparel® is a long-duration formulation (> 72 h) used for wound infiltration and post-surgical pain management, after bunionectomy and hemorrhoidectomy, and recently approved by the FDA for interscalene brachial plexus nerve blockade [41]. Despite reports of a decrease in toxicity and improved anesthesia time [78,79], its effectiveness remains controversial. Different reports have described inconsistent results regarding its pharmacological effectivity, since the use of liposomal BVC did not induce clinically relevant analgesia compared to perioperative opiates [80]], reduce the postoperative opioid consumption [81]], nor decreased the number of days in hospital [76]. Additionally, meta-analysis reviews reported no prolonged duration of action over BVC in solution after knee arthroplasties [80]. However, this provided similar postoperative pain control to that obtained after femoral nerve block [82].

Changing liposomes composition is an important approach to adapt liposomal formulations for their route of administration (e.g. elastic liposomes for improved LA dermal delivery) [17,83] or to extend drug release (e.g. hydrogenated lipids plus cholesterol in ionic-gradient liposomes, IGL) [67].

2.1.1 Ionic-gradient liposomes for the delivery of LA

According to Gubernator et al., the active-loading of local anesthetics in ionic-gradient liposomes is the only way to achieve high drug:lipid ratios, thereby achieving an effective LA dose with liposomes [84]. From the physicochemical point-of-view, even that liposomes are versatile for the encapsulation of both hydrophobic (in the lipid "bilayer" phase) and hydrophilic (in their internal water core) drugs, they have a low drug upload capacity for hydrophobic and amphiphiles drugs, such as LA, because of the imbalanced proportion between lipid:water phases. In fact, the lipid:water volume ratio of a typical liposome formulation (5 mM or 3.9 g/L egg phosphatidylcholine vesicles) is 0.004 (4:1000 v:v, considering the lipid density equals to 1 g/mL) [85], which is insufficient to guarantee the loading of high amounts of hydrophobic compounds. This lipid:water misbalance of liposomes is also clearly expressed by the trapped aqueous volume: *ca*. 12 μL/μmol of lipids in 400 nm unilamellar vesicles [36]. In this scenario, IGL brings an elegant physicochemical setting to solve that problem, since the aqueous compartments of the vesicles serve as depots for the charged LA species, while the partition of the base form (neutral LA) into the membrane guarantees the permeation and sustained release of the anesthetic to the outer medium (Figure 2).

Ionic gradient liposomes for the encapsulation of bupivacaine were first described by Mowat et al. [86], who prepared liposomes with an acidic inner aqueous compartment (300 mM citrate, pH 4.0) and external pH of 7.4. The large unilamellar vesicles, composed of dioleoyl phosphatidylcholine:cholesterol

(55:45 mole%), were able to upload as much as 2% BVC and to promote sustained (14 h) release after intradermal administration in guinea pigs. Furthermore, BVC was incorporated in large multivesicular IGLs of hydrogenated soy phosphatidylcholine: cholesterol (2:1 mole%), in which the acidic pH in the aqueous inner compartments was provided by 250 mM $(NH_4)_2SO_4$ [54]. In both cases, the anesthetic was actively loaded into the liposomes from the outside medium (pH 7.4), due to the partitioning of the base LA form into the membrane, and its further redistribution and ionization in the inner aqueous compartment (Figure 2). The charged LA species – that predominates in the acidic IGL inner compartment is very water soluble, allowing entrapment of large amounts of the anesthetic [54]. This remarkable formulation developed by Barenholz' group was able to induce up to 48 h anesthesia after intradermal administration in six human volunteers. Further optimizations in the lipid composition, inner salt gradient (calcium acetate), average liposome size (diameters > 1 micron), and multilamellarity gave rise to injectable formulations known as Bupisome and Bupigel [87].

These transmembrane-gradient liposomes (pH 7.4 outside/acidic pH inside the vesicles) have been also described for the encapsulation of 2% RVC [88,89], 0.012% DBC [36]], and 1.5% EDC [37]. In the case of RVC, the gradient liposomes provided higher encapsulation efficiency than conventional liposomes, and significantly prolonged the duration of anesthesia (*ca*. 10 h) after subcutaneous administration of 0.5% ropivacaine in mice. As for dibucaine, the formulation allowed its parenteral administration, surpassing the extremely low water solubility of DBC, to induce ultra-long anesthesia (27 h) after infiltrative administration of 0.012% DBC in mice. Additionally, both the *in vitro* (cell culture tests) and *in vivo* (local and systemic, in zebrafish) toxicity of the anesthetic were decreased after encapsulation into the IGL [36]. Another interesting IGL application was reported for etidocaine, an LA agent that had been discontinued in 2008 because of a supposed lack of efficacy and security (US FDA 2008) [37], which remains a matter of controversy. Encapsulation into IGL promoted prolonged *in vitro* release (24 h) of etidocaine, reducing its intrinsic cytotoxic effect over human fibroblasts in culture [37] and promoted safe, prolonged anesthesia after administration of as much as 1.5% EDC in the sciatic nerve of rats (unpublished results).

Liposomes are the most well-known DDSs for local anesthetics because of their capacity to encapsulate LA both in the bilayer and aqueous phases, being relatively easy to prepare, showing biocompatibility, biodegradability, and minimal loss of encapsulated drug volume [62]. Nevertheless, issues related to cost, shelf stability, and low drug-to-lipid uptake remain challenging for liposomal formulations [87]. Modified liposomes, such as IGL, have allowed increased LA upload, by using large, multivesicular vesicles and by entrapping high amounts of the charged LA species in their acidic water compartments.

Another strategy to favor the encapsulation of large amounts of LA is the use of carriers with more balanced lipid: water ratios than liposomes, such as microemulsions and nanostructured lipid carriers, as discussed in the following sections.

2.2 Microemulsions for local anesthesia: from dermal to injectable administration routes

Microemulsions are one of the most well-described delivery systems for LA. Traditionally used for transdermal delivery (in skin biopsies, collagen injections, laser surgery, venous cannulation, and punctures), their use as injectable systems has also been considered due to the pharmaceutical stability and high upload capacity of both hydrophobic and hydrophilic drugs [90,91].

Emulsions have been used as DDSs since the beginning of the 20th century, but it was only after the advent of biophysical techniques (such as dynamic light scattering, static light scattering, transmission electron microscopy) that determination of their physicochemical features and colloidal properties (such as size distribution and morphology) allowed their differentiation as microemulsions and nanoemulsions. In this section, we will present some systems described in the literature as microemulsions.

Microemulsions are composed of aqueous (water or buffer solution) and oily phases stabilized by an interfacial layer of surfactants; they can form thermodynamically stable suspensions under well-defined components concentration and environmental temperature. Then, in this easy-to-prepare system, the oily and aqueous phases coexist in equilibrium [91,92], identifying them as good candidates for drug-delivery.

Among the LA agents used as microemulsions preparations, BZC, TTC, LDC, PLC, DBC, and – more recently – BVC and RVC have been described for topical and injectable use.

Comparisons in the literature reveal that enhanced anesthetic effect is associated with variations on microemulsion compositions. The presence of isopropyl palmitate in combination with Tween 20, Tween 80, and Chremophor EL favored the formation of a stable formulation for 2% BZC, even in a wide range for droplet sizes (from 60 to 140 nm) [93]. In another report, co-delivery of benzocaine and indomethacin evoked a synergistic analgesic effect when topically administered in eucalyptus oil, Tween 80, ethanol and water microemulsion formulation [94].

In an attempt to obtain more effective and non-cytotoxic LDC base formulations, Yuan et al. [95–97] developed systems based on the interactions between hydrophilic (sodium caprylate) and hydrophobic (caprylic acid) linkers, stabilized by egg phosphatidylcholine. Those three reports presented systems with best permeation performance across reconstructed human skin models, considering relationships between the highest surfactant concentration and the best drug transdermal diffusion profile.

On the other hand, the skin permeation profiles were also evaluated for the LA hydrochloride form, as observed by Dogrul et al. [98]. Results indicated that the water/oil type (Miglyol®/lecithin/ethanol/water) microemulsion and the presence of long and medium-chain length triglycerides (such as olive oil and caprylic/capric triglycerides) were able to enhance the skin permeation of lidocaine, with faster and more efficient local anesthetic effects. More recently, remarkable anesthetic/analgesic effects were also obtained for LA not traditionally used for topical administration, such as ropivacaine hydrochloride [99].

In fact, the influence of the base LA form on skin permeation and its relationships with pharmacological effects have been reported in the literature. Differences in LA forms and physicochemical features were associated with the modulation of skin flux values, as described for LDC, TTC, and DBC. Those results showed that the partition coefficient of the LA agent could modulate its transdermal flux when administered at the same formulation composition, such as microemulsions containing isopropyl palmitate, dioctyl sodium sulfosuccinate, or Brij 97 as tensoactives, together with 1-butanol [91].

Both microemulsions composition and LA physicochemical properties are considered as essential factors for obtaining topical long-duration anesthesia. Then, new formulations have also been developed for providing alternatives to well-known commercially available compositions, such as EMLA®.

In this sense, some efforts have been devoted to the eutectic mixture of lidocaine and prilocaine (both as base forms), such as its encapsulation in a mixture of hydrogenated soy phosphatidylcholine stabilized by propylene glycol monolaurate and Tween 80 [92]. However, comparisons regarding formulation aspects must be considered since small droplet sizes (~ 20 nm) were obtained with other surfactant mixture compositions (Tween 80 and poloxamer 331) [100], with impacts on microemulsion stability and morphological aspects.

Studies on microemulsion innovations and their structural characterization have also aimed at developing safer formulations. In fact, enhanced LA skin permeation flux is achieved by using, sometimes, high concentrations and/or non-biocompatible components (oily phases or even surfactants mixtures), which limits the systems administration by other routes.

In this context, different works have proposed *in situ* depot microemulsions, such as lipid-liquid crystalline phases in biological medium (Figure 2) for injectable use by the subcutaneous route, especially with bupivacaine [101,102]. Those new systems are based on the tendency of biologically relevant lipids to self-assemble into well-defined phases, such as cubic, inverted hexagonal or lamellar, upon temperature treatment and hydration [103]. Their main advantage is because there is no need for additional chemical modifications for depot formation. Besides, the *in situ* depot formation was not determined by the incorporation of temperature-sensitive gellators, highlighting the essential contribution of structural characterization and physicochemical aspects on future pharmaceutical formulation performance.

The crystalline phases formed by associating glyceryl monooleate and medium chain triglycerides have modulated the release rate of BVC hydrochloride [104]. In particular, different phase organizations are associated to low viscosity and adequate syringeability, such as liquid lamellar phases, while hexagonal and cubic structures show high viscosity to form *in situ* gel systems, modulating the release of BVC hydrochloride [102]. On the other hand, LA skin permeation seems to be a process explained not only by shifts on phase organization, but especially by the hydrophobicity of the anesthetic agent, as observed for LDC, PLC, and their eutectic mixture after incorporation into glyceryl monooleate-water or sphingomyelin-water, that formed cubic and lamellar phases respectively [105].

The literature includes another interesting use of lipid microemulsions, beyond DDSs: for the treatment of systemic toxicity induced by local anesthetics. In this specific case, the treatment is based on the LA uptake from the blood circulation after emulsions administration by intravenous route. Originally developed in the 1960s for parenteral nutrition, this combination of free fatty acids was effective for reversion of unexpected ventricular arrhythmia and cardiac arrest evoked by hydrophobic local anesthetics, such as BVC, levobupivacaine, and RVC [106]. Commercially available as various preparations (e.g. Intralipid®, Lipofundin®, Lipovenoes®, Medialipid®, and ClinOleic®), the main components of the lipid emulsions includes long-chains fatty acids, such as linoleic acid (~ 55%), oleic acid (~ 25%), palmitic acid (~ 15%), alpha-linolenic acid (~ 5%), and stearic acid (~ 5%). Other compositions have medium and long-chain triglycerides from coconut oil (~ 50%) and soybean, olive, or fish oils (~ 50%) [106–108]. Despite several studies, the molecular mechanisms of these reverse LA transporters remain unclear, since an association of features is considered as essential for the uptake of local anesthetics, e.g. i) the drug partition coefficient, with more pronounced binding percentages for hydrophobic molecules (BVC ~ levobupivacaine > RVC) [109]; ii) the differential cellular mechanisms for preparations containing linolenic acid and stearic acid to attenuate the sodium current across cardiac channels [110]; and iii) reduction of vasodilatation evoked by the ATP-sensitive potassium channels and inhibition of nitric oxide release [111,112]. All of those properties contribute to the reversion of heart damage and cardiac function restoration. The clinical use of this special kind of lipid-based nanocarrier (designed for the uptake of local anesthetics) has clinical relevance and has generated opportunities for the development of similar new carriers with possible LA dose control.

2.3 Lipid nanoparticles

Lipid nanoparticles are innovative nanocarriers. Their first and second generations, the so-called solid lipid nanoparticles (SLN, launched in 1990) and nanostructured lipid carriers (NLC, 2000) (Figure 2), contributed to the upload of hydrophobic molecules and long-term stability of lipid-based formulations [113]. Generally, these nanocarriers are prepared by hot emulsification-ultrasonication or high-pressure homogenization methods, with suitable scalability [114]. Such easy-handling formulations are based on a lipid core (major component) stabilized by a surfactant, that confers them superior drug upload capacity for hydrophobic molecules than that of liposomes. Generally, there is no use of organic solvents in the preparation method, and the used excipients are approved by regulatory agencies such as the FDA [115]. The main difference between SLN and NLC resides is in the lipid core composition: solid lipids in SLN, while NLC is formed by a blend of solid and liquid lipids at room temperature. The blended-type internal matrix of NLC prevents drug expulsion over time [116].

In fact, some efforts are still necessary to determine how lipids, surfactant, and drugs are structurally arranged into these lipid nanoparticles. In this sense, Ribeiro and coworkers estimated the ratio of drug and excipient molecules per particle, from which a schematic representation of individual

nanoparticle was proposed [117]. Based on this, NLCs are covered by relatively few surfactant molecules that stabilize a large lipid core that contains at least two orders of magnitude more lipid molecules than surfactant [118]. Radaic et al. highlighted the importance of the lipid nanoparticle structural organization in the design of specialized SLN/NLC formulations, emphasizing the incorporation of ligands, functional groups, and/or coatings in the surface of such systems. Cationic nanoparticles, for instance, are useful for the sustained release of genes, and also favor electrostatic interactions with mucous tissues, while PEGylation decreases the potential plasma protein interaction of SLN/NLC [119]. Also, Barbosa et al. [120] employed a set of methods, such as infrared spectroscopy, electron paramagnetic resonance, and small-angle X-ray scattering, for the interpretation of the structural organization of lipid nanoparticles. Their results confirmed that the lipid matrices composed of cetyl palmitate, myristyl myristate – and a blend of caprylic/capric triglycerides in the case of NLC – could adopt a lamellar arrangement, in which DBC (base) was successfully entrapped, increasing the lipid packing [120]. This was also corroborated by the determined coefficient partition levels at pH 8.2 (> 10^4), which was at least one order of magnitude higher than traditional egg phosphatidylcholine-based liposome for DBC delivery. The encapsulation efficiency of such lipid nanoparticles is directly related to partition coefficient of the drug, so that all the formulations in Table 2 employed the base LA form [120].

In general, due to their hydrophobic character, the base LA forms have a high affinity for being loaded into nanocarriers. The LA-based SLN/NLC formulations exhibited excellent physicochemical properties, biocompatibility, prolonged drug-release, and higher efficacy [123–126]. The most relevant findings in the last years are discussed below and depicted in Table 2. Encapsulation of lidocaine (LDC) or the eutectic mixture of lidocaine and prilocaine (LDC-PLC) has been extensively studied as topical anesthetics for the oral mucosa and skin administration. Ribeiro *et al*. reported the development of NLC for the sustained release of 2% LDC [127] and the eutectic mixture of LDC-PLC (5%) [117] to be applied as topical anesthetics. In both cases, SLN/NLC formulations composed of synthetic or natural lipids plus poloxamer displayed long-term stability, with excellent structural properties. In the natural lipids-based NLC paper, the authors demonstrated the safety and efficacy improvement in comparison to free LDC, as observed in the cell viability and the tail flick test in mice, respectively [117,127]. Indeed, the optimized NLC loaded with LDC-PLC formulation [117] was employed as the lipid component of lipid-biopolymer hydrogels [128] and films [129], in order to facilitate the transbuccal application of the anesthetics. Such hybrid forms induced four times higher topical anesthesia (7–8 h, as evaluated through the tail flick test in mice) than the commercially available forms used as controls [128,129]. Formulations of SLN/NLC co-loading LDC-PLC were compared in biological assays, and the authors concluded that both systems were promising when applied in topical skin anesthesia. SLN formulations exhibited better skin permeation capacity than NLC, and both formulations were more effective than free

Table 2. The state-of-the-art of lipid nanoparticle (SLN, NLC) DDSs for the sustained release of local anesthetics.

LA	Carriers	Lipid Composition	Size (nm)	%EE	Stability (months)	Pharmacological Tests	Route Year	References
Lidocaine (2%)	SLN/NLC/hydrogels	Compritol®, Precirol®	78	~ 97%	6	Pin prick in pigs	Skin 2009	[123]
Lidocaine (1%)	SLN	Monostearin, glyceryl palmitostearate, stearic acid	265	-	-	Epidural block in rats	Parenteral 2012	[134]
Ropivacaine (1.5%)	Nanocapsule	Lipoid®, Labrafac®	63	~ 93%	-	Permeation in mice skin	Skin 2014	[143]
Lidocaine-prilocaine (0.04/0.05%)	NLC	Soy lecithin, stearic acid	265	~ 60%	2	-	Buccal/skin 2015	[125]
Benzocaine (0.2%)	SLN/hydrogel	Cetyl palmitate	300	~ 20%	-	Tail flick in rats	Skin 2015	[121]
Ropivacaine (0.2%)	NLC	Soy lecithin, stearic acid	205	~ 81%	-	Writhing test in mice	Skin 2015	[132]
Lidocaine-prilocaine (2.5/2.5%)	SLN/NLC	Cetyl palmitate, myristyl myristate, Miglyo®l	290	~ 50%	14	-	Oral mucosa 2016	[117]
Bupivacaine (1%)	NLC	Cetyl palmitate, Capryol®	166	~ 55%	12	Paw pressure test in rats	Parenteral/skin 2017	[40]
Dibucaine (0.5%)	NLC	Myristyl myristate, coconut oil	218	~ 81%	12		Skin 2019	In preparation
Lidocaine (2%)	NLC	Beeswax, copaiba oil, or Shea butter, almond oil, or Cocoa butter, sesame oil	214 231 209	~ 79% ~ 86% ~ 84%	12	Tail flick in mice	Skin/parenteral 2017	[127]
Lidocaine-prilocaine (2.5/2.5%)	NLC/film	Cetyl palmitate, myristyl myristate, Miglyol®	-	-	-	Tail flick in mice	Oral mucosa 2017	[129]
Lidocaine-prilocaine (2.5/2.5%)	SLN/NLC	Compritol®, Precirol®	150	~ 90%	4	Tail flick in rats	Skin 2017	[130]
Dibucaine (0.1%)	SLN/NLC	Myristyl myristate, cetyl palmitate, Miglyol®	210	~ 80%	8	Tail flick in mice	Skin 2018	[136]
Lidocaine-prilocaine (2.5/2.5%)	NLC/hydrogel	Cetyl palmitate, myristyl myristate, Miglyol®	-	-	6	Tail flick in mice	Oral mucosa 2018	[128]
Bupivacaine (0.2%)	NLC	Linoleic acid, hyaluronic acid	150	~ 90%	3	Tail flick in rats	Skin 2018	[131]
Lidocaine (1%)	NLC	Stearic acid, glycerin monostearate, castor oil, vitamin E derivative	168	~ 86%	1	Tail flick in rats	Skin 2018	[126]
Articaine (2%)	SLN/hydrogel	Glyceryl tripalmitate	250	~ 65%	4	-	Skin 2018	[122]

drugs. NLC showed higher *in vivo* analgesic action than SLN in tail flick tests in rats [130]. In general, the encapsulation of lidocaine by SLN/NLC was slightly higher than that of prilocaine - probably due to the higher hydrophobicity of LDC over PLC – resulting in a more sustained release profile and long-lasting anesthesia.

Other LA, such as benzocaine, procaine, and bupivacaine, have been successfully encapsulated in SLN/NLC formulations intended for use in transdermal anesthesia [124,125,131]. Ropivacaine (RVC) was efficiently encapsulated into optimized NLC formulations prepared with a less usual technique (emulsion evaporation-solidification at low temperature) that provided homogeneously dispersed nanoparticles of less than 200 nm. The authors correlated RVC permeation levels with histopathological analysis of miceskin tissues and suggested that NLC was able to penetrate the corneocyte layers of the skin, facilitating the transdermal penetration of RVC, which improved the antinociceptive effect in more than 80% in comparison to the free drug [132].

An interesting strategy to improve LA penetration into the *stratum corneum* barrier, and, consequently, its antinociceptive effect, was recently adopted. First, the authors synthesized a novel liquid lipid, from the conjugation of propylene glycol with hyaluronic and linoleic acids. Then, modified NLC formulations were formed with Precirol® and Compritol® (as solid lipids), the propylene glycol conjugated lipids and polysorbate 90, to encapsulate BVC for transdermal delivery. The modified-NLC formulations presented desirable physicochemical properties and stability, prolonging the BVC release for up to 72 h. The *in vitro* percutaneous penetration of BVC from such modified-NLC was higher than those measured with non-modified NLC or free BVC trough rat skin. In accordance, this system prolonged anesthesia since at the end of the experiment (75 min), the maximum possible effect was around 60%, as assessed by tail flick test in mice [131]. Another modified cationic NLC – composed of tocopheryl polyethylene glycol 1000 succinate (a vitamin E derivative), stearic acid, castor oil, glycerin monostearate, and dimetyldioctadecylammonium bromide – has been devised to improve LDC permeation and antinociceptive effect. The electrostatic interaction between the positively charged nanoparticles and the negative, superficial charges of the *stratum corneum* was planned. This approach contributed to the excellent *in vitro* permeation and *in vivo* efficacy performance by the cationic NLC in comparison to free LDC, with no cytotoxicity [126]. These results highlight the relevance of a deep understanding of the physicochemical features of the drug, nanocarrier, and target for improving the design and function of DDSs [133].

Beyond topical administration, LA-based lipid nanoparticles have been designed for other goals. Leng *et al*. [134] proposed LDC-loaded SLN formulations to prolong the epidural anesthesia time. The authors tested different solid lipids, and the most appropriate SLN composition – showing the most prolonged LDC release profile (~ 48 h) – was composed of glyceryl palmitostearate and poloxamer 188. The formulation provided a 12-h epidural block in rats, against the 2 h elicited by LDC in solution [134]. In another study, bupivacaine in enantiomeric excess (S75:R25, Novabupi®) was successfully encapsulated in NLCs designed for injectable administration. The miscible lipid internal matrix, elucidated by Raman mapping, allowed high encapsulation efficiency (55%) and long-term stability (1 year at 25°C). The blockage duration of the sciatic nerve of rats with the NLC formulation was twice as high (> 8 h) than that induced by free bupivacaine, after injection in the popliteal region. Authors have claimed that this formulation minimizes the toxicity of bupivacaine by decreasing the required dose for anesthesia in clinical practice [40].

Also, in an attempt to reduce its intrinsic toxicity, DBC was encapsulated into SLN and NLC formulations. The safety of these formulations was confirmed by cytotoxicity tests against 3T3 and HaCat cells lines [135,136]. The *in vivo* efficacy test in mice was performed for DBC-loaded SLN, which was able to double the recovery time (> 130 min) in comparison to the control (0.05% free DBC). In our group, the encapsulation of 4% TTC into NLC formulations optimized by Experimental Design has also given interesting results, extending the *in vitro* release of the anesthetic to more than 48 h (*in preparation*).

Another kind of lipid nanoparticle, known as lipid nanocapsules (LNC), was first described in 2000 [137]. They are prepared without organic solvents by the phase-inversion method. In comparison to SLN and NLC, their lipid core is shielded by greater amounts of surfactant, thereby achieving very small particles (< 100 nm) in which drugs can be encapsulated in the core or adsorbed in the shell/water interface [138,139]. Phospholipids can be used as excipients for LNC, playing a special role for transdermal drug delivery. LNC has been employed for the sustained release of different classes of drugs [140–142]. This system establishes specific interaction with lipid bilayers, overtaking the gaps of the *stratum corneum*, due to their small particle sizes [142,143], which makes LNC especially interesting for skin administration.

Despite such remarkable properties, there is only one published report so far on the encapsulation of local anesthetics in LNC. Optimized RVC-loaded LNC (63 nm) was obtained by response surface design, with high RVC encapsulation (93%); the system exhibited a biphasic release profile, with an initial RVC burst release (~35%) followed by sustained release rate, over 25 h. Applied to the excised skin of mice the RVC-loaded nanocapsules showed enhanced cumulative retention (2×), as well as *in vivo* permeation and higher plasma levels, in comparison to ropivacaine in propylene glycol [143].

Overall, considering the discussed results, lipid nanoparticles have been shown to be promising nanocarriers to encapsulate different LA agents for many purposes, being more effective than the existing commercially available forms. However, SLN/NLC formulations have not reached the market yet. Therefore, clinical trials are the next step for these innovative systems to move beyond academia and to be commercialized as efficient nanostructured LA-based formulations.

3. Conclusion

This review has covered different kinds of formulations for LA: liposomes, microemulsions, and nanostructured lipid carriers. Current trends in lipid-based LA-in-DDSs were revealed, considering the different administration routes and purposes.

Liposomes (1965) are the oldest and the most popular lipid-based carrier, for various drugs, including LA. Despite their versatility to carry hydrophilic/hydrophobic drugs, liposomes are limited by lack of reliability and reproducibility during manufacture [59], and because of their low lipid:water balance, which limits the upload of high amounts of LA. Ionic-gradient liposomes circumvent that limitation by entrapping large amounts of the charged (more soluble) LA form in their inner aqueous compartments.

On the other hand, microemulsions are presented as systems able to entrap large amounts of hydrophobic LA agents. However, their main limitation is the use of non-biocompatible or high concentrations of some excipients. Preferably used for local skin anesthesia, microemulsions have been identified as an alternative for improving the therapeutic performance of LA as hydrochloride forms, especially by injectable routes.

Other lipid-based carriers, such as SLN and NLC (the 1990s), are younger than liposomes, so it is reasonable to expect that they will become more known and spread in the coming years. These alternative lipid-based carriers also have advantages, such as relatively low cost, easy preparation, superior LA upload capacity (than liposomes), and colloidal stability. From the lipid-nanoparticles, NLC is a remarkable carrier for LA in the base form.

Nevertheless, just one ultra-long-acting local anesthetic (ULALA) lipid-based formulation has been approved for clinical use. Many reasons account for why the dozens of formulations published every year fail to reach clinical trials, such as non-ideal fitting among LA agents, drug carriers, and administration routes, but mainly because of pharmaceutical technology issues related to the colloidal stability of the formulations during scaling up and storage.

4. Expert opinion

Recent years have witnessed the recognition of lipid-based nanocarriers as potential delivery systems. Due to their versatility of chemical composition and physicochemical features, lipid-based nanocarriers can be applied on the skin, mucosa, or parenteral injection. Biocompatibility associated with the ability to improve the solubility of some LA agents are the main advantages of those systems. On the other hand, what explains the discrepancies among the variety of new lipid-based systems and the discrete therapeutic efficacy reported in preclinical and clinical studies? To answer this question, many issues must be considered, including the physicochemical properties of LA agents to formulation processes, costs for scale-up, nanocarriers physicochemical stability, and non-adequate preclinical models for different routes of administration. So, the pathway from the bench to the bedside seems long for LA lipid-based delivery-systems.

The literature has addressed the pharmacological performance of several lipid-based DDSs for LA, a wider group of formulations developed for skin application than for parenteral injection. A variety of available components for skin delivery systems and the differences in the costs for parenteral formulation design have contributed to this scenario. Additionally, the development of formulations for ophthalmic, oral mucosa application have gained attention due to other limitations. For example, the existing preclinical models for the pharmacodynamic evaluation are not representative of practical conditions of use, since the dilution by saliva and lacrimal fluid provide different environments for DDS application, where the formulation viscosity is rapidly changed by washout effect. In this sense, such LA-based DDSs have been evaluated using non-specific topical models, such as tail flick and paw pressure tests (Table 2). Their performance in these tests can be misapprehended due to the distinct histological organization of skin and mucosa (oral, nasal, and eye tissues are less resistant to drug permeation).

Other important points to be considered are related to the DDS and their production processes. What features are expected for the best clinical performance of LA-in-DDS formulations? i) the lipid components must be biocompatible, even if not entirely nontoxic; ii) the formulation effectivity on inflamed tissues, surgical wound repair, injured mucosa or skin, associated to the ability of the formulation to remain at the application site, has to be appropriately evaluated, considering real conditions of applications (e.g. dilution or removal by saliva); iii) properties related to the colloidal stability are essential to maintain the nanoparticles' structure (which should be achieved without enhancing the surfactant or co-solvent concentration, in order not to affect the formulation toxicity, nor the fabrication costs).

Based on the information available on the NIH clinical trials website [144], there are 25 records of studies involving local anesthetics and liposomes, but none with microemulsions or nanostructured lipid carriers. All but one of those involve aminoamide local anesthetics: 19 for BVC (seven comparing Exparel® with other LA and opioid agents), three for RVC, and one each for LDC, PLC, and MVC.

These data confirm that researchers are currently betting on ionic-gradient liposomes for the parental delivery of the long-acting agent bupivacaine, but – considering the advantages and limitations of the existing lipid-based LA-in-DDS (here exposed) and our experience in the field – we predict that nanostructured lipid carriers will soon reach clinical trials and produce effective new DDSs for local anesthetics.

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