EXPERT OPINION

Micro and nanosystems for delivering local anesthetics

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Introduction: One of the most common strategies for pain control during and after surgical procedures is the use of local anesthetics. Prolonged analgesia can be safely achieved with drug delivery systems suitably chosen for each local anesthetic agent.

Areas covered: This review considers drug delivery formulations of local anesthetics designed to prolong the anesthetic effect and decrease toxicity. The topics comprise the main drug delivery carrier systems (liposomes, biopolymers, and cyclodextrins) for infiltrative administration of local anesthetics. A chronological review of the literature is presented, including details of formulations as well as the advantages and pitfalls of each carrier system. The review also highlights pharmacokinetic data on such formulations, and gives an overview of the clinical studies published so far concerning pain control in medicine and dentistry.

Expert opinion: The design of novel drug delivery systems for local anesthetics must focus on how to achieve higher uploads of the anesthetic into the carrier, and how to sustain its release. This comprehensive review should be useful to provide the reader with the current state-of-art regarding drug delivery formulations for local anesthetics and their possible clinical applications.

Keywords: cyclodextrins, liposomes, local anesthetics, microparticles, nanocapsules, nanospheres, polymers

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1. Introduction

Local anesthetics (LA) have a wide variety of clinical applications, and are some of the most important agents employed to obtain analgesia during trans- and postoperative periods [1], as well as to control certain acute and chronic pain conditions [2,3]. In contact with the nerve fiber trunk, these agents promote a reversible interruption of the nerve impulses by binding to specific sites of sodium channels of the nerve membrane, resulting in decreased permeability to sodium ions [4].

Several reviews can be found in the literature that cover important aspects of LA pharmacology, their action on ion channels [5,6], and the variety of agents available commercially [1,7-10].

Figure 1 shows the chemical structures of clinically used LA. Some of these are the drugs of choice for ambulatory and surgical procedures (lidocaine and bupivacaine, respectively), in dentistry (lidocaine, articaine, prilocaine, and mepivacaine) or ophthalmology (proparacaine and oxybuprocaine), whereas others have been used in the past for short-term (procaine and chlorprocaine) and long-term (etidocaine and tetracaine) anesthesia, or are the active principles in topical (dibucaine, benzocaine, butamben, pramoxine, and oxethazaine) formulations. Due to their low molecular weight, LA molecules present fast systemic absorption. As a consequence, their

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Article highlights.

- Local anesthetics are very well studied drugs, used for pain control in medicine and dentistry procedures, but their effects last for no more than a few hours.
- Many types of LA-containing drug delivery systems have been proposed for infiltrative anesthesia, the most important being liposomes and polymer micro/nanoparticles. For these two carriers, studies in humans have confirmed the efficacy and safety of LA formulations, for infiltrative anesthesia.
- Difficulties in micro/nanoparticle production still restrict their use, while physical and chemical stability over time is the main technological challenge in liposome development.
- Cyclodextrins also have clear potential as LA carriers, due to their price, stability, and ease of scale-up. CDs are especially interesting for use with anesthetic agents whose solubility in water is limited.
- Drug delivery systems for local anesthetics have great clinical potential because they can provide prolonged sensory blockade, reduce local and systemic toxicity, and allow the loading of larger LA doses.

This box summarizes key points contained in the article.

anesthetic effect is of short duration [3,11], and the risk of systemic toxicity precludes the use of high bolus doses [12].

The use of both adjuvants and higher LA concentrations has been considered to prolong neural blockade; however, enhanced systemic (neurological and cardiac) toxicity has also been demonstrated [13,14]. Neural blockades prolonged for days are achieved only by using catheter techniques [15], with disposable pumps [16] or multiple LA injection.

Pharmaceutical formulations prepared with different carriers can prolong local anesthetic action, decrease plasma levels to safe ranges, or allow the achievement of analgesia equivalent to the common commercially available formulations with lower LA doses. Our group has been working on the development of novel LA drug delivery systems using different platforms (liposomes, polymers, and cyclodextrins, schematically represented in **Figure 2**), suited to the physicochemical properties of each local anesthetic agent.

Other drug delivery systems, such as nanostructured lipid carriers [17,18], third-generation liposomes (elastic or with lipid composition similar to the stratum corneum) [19,20], micellar carriers [20], adhesives [21], and hydrogels [22] have been tried for the improvement of LA activity. However, these systems are mainly used for delivery through the skin, and will be addressed in an upcoming review.

By choosing the proper carrier, improvements can be made in drug upload and bioavailability, as well as the control of drug release. For instance, because local anesthetics are amphipathic compounds, they are easily transported into the bilayers of liposomes, so that most of the commercially available LA should benefit from liposome-based formulations. Although the correlation between anesthetic hydrophobicity, lipid solubility, and narcosis has been known since the 19th century (Meyer-Overton rule), LA partitioning into liposomes is also determined by steric hindrances to its insertion between the lipids [23], uncharged/charged ratio at pH 7.4 [24], dipolar (ester vs. amide linkage) character, among other factors.

On the other hand, LA compounds with nonideal partitioning, which present partition coefficient (P) versus solubility (molar concentrations) values < 2 cannot be incorporated into membranes in sufficient amounts due to their limited water solubility [25]. At physiological pH, such LA (e.g., bupivacaine, tetracaine, etidocaine, and dibucaine) should benefit from complexation with cyclodextrins, or encapsulation into micro- or nanocarrier systems.

Here we present a review of the literature, covering the studies published so far concerning the three main drug delivery systems for the improvement of local anesthetic action (represented in **Figure 3**). Advantages and limitations are discussed, together with pharmacokinetic data that confirm the prolonged release provided by these formulations. The clinical section gives an updated review of the studies in humans, covering the published literature on pain control in medicine and dentistry, while the Expert Opinion section contains our overview of the field.

2. Polymeric micro- and nanoparticles

The number of reports of polymeric micro- and nanoparticles used as carrier systems for local anesthetics has increased in the literature [3,26]. Among the various LA tested so far, higher encapsulation efficiencies have been found for the more hydrophobic amide (bupivacaine, ropivacaine, and dibucaine) and ester (tetracaine and benzocaine) compounds, which show nonideal partitioning [25]. Accordingly, current formulations described in the literature preferentially employ the nonionized LA species, because many of the polymers used to prepare the carrier systems, such as poly(ε-caprolactone) (PCL), polylactide (PLA), and polylactide-co-glycolide (PLGA) are hydrophobic.

Polymeric particles can be described as spheres or capsules, where the spheres are structures with a polymer matrix, while the capsules consist of a core (usually an oily phase) covered by polymer (**Figure 2C and D**). The definition of micro- and nano- polymeric particles depends mainly on the size range of the material produced. Microparticles (which can be microspheres or microcapsules) are structures that vary from 1 to 200 µm, while nanoparticles (nanospheres or nanocapsules) have size ranges between 1 and 1000 nm [27]. The characteristics of such polymeric systems, and how they affect the loading and release of local anesthetics, will be described in this section.

The first published studies concerning polymeric systems and LA date from 1981 to 1982, when Wakiyama *et al.* prepared PLA microparticles for the encapsulation of butamben, tetracaine, and dibucaine. The authors found that the smaller the size of the microsphere, the faster the drug release *in vitro*.

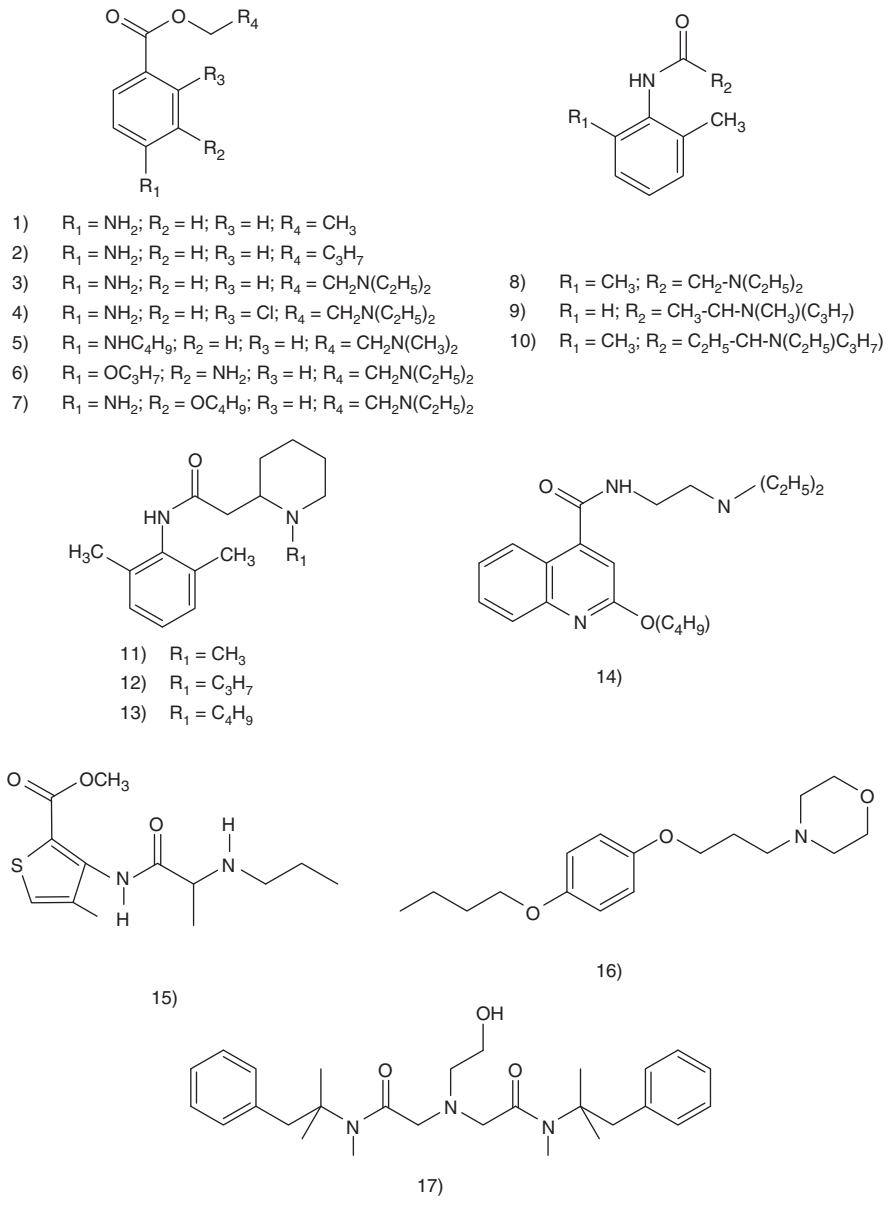

Figure 1. Chemical structures of clinically used local anesthetics: Aminobenzoic acid derivatives: i. Ester type: Benzocaine (1) Butamben (2); ii. Amino-ester type: Procaine (3), Chloroprocaine (4), Tetracaine (5), Proparacaine (6), Oxybuprocaine (7); Amino-amide type (with benzene ring): Lidocaine (8), Prilocaine (9), Etidocaine (10), Mepivacaine (11), Ropivacaine (12), Bupivacaine (13); Amino-amide with quinoline ring: Dibucaine (14); Amino-amide with thiophene ring: Articaine (15); Amino-ester type with morpholine group: pramoxine (16); Amino-amide (double group): Oxethazaine (17).

Their results were highly satisfactory, an example being the incorporation of dibucaine in microspheres, which resulted in analgesia in guinea pig skin that lasted for more than 300 h, compared to < 150 h in the case of free dibucaine [28,29].

In 1994 – 1995, the group of Le Verge and collaborators characterized PLA and PLGA microspheres containing the uncharged form of bupivacaine [30,31]. In the PLA microparticles, with blends of polymers of different molar masses, and size distributions between 1 and 5 µm, the drug content did not exceed 24%. However, in the PLGA microparticles (also containing different proportions of lactic and glycolic acids) of 1 – 10 µm size, drug contents reached 50%. The *in vitro* release profile of bupivacaine varied, with 50% of the drug being released after 54 h when the PLGA formulation was used. Drug release studies conducted *in vivo* showed that use of bupivacaine contained in PLA microspheres resulted in a reduced maximum drug plasma concentration, indicating a slower uptake of bupivacaine by the systemic circulation.

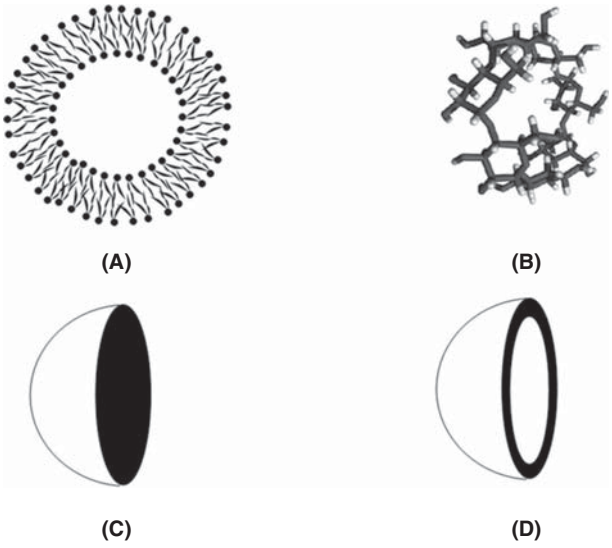

Figure 2. Schematic illustration of nanocarrier delivery systems for local anesthetics. (A) Liposome, **(B)** cyclodextrin, **(C)** polymeric nanospheres (cross section), and **(D)** polymeric nanocapsules (cross section). Representations, especially (C, D), are not on the same scale.

Curley *et al.* showed that microparticles prepared with PLGA (using different proportions of lactic and glycolic acids) could be loaded with up to 75% (w/w) of neutral bupivacaine. The average duration of sciatic nerve blockade in rats varied from 10 h to 5.5 days for these formulations, depending on the microparticle type, dosage, and the presence of dexamethasone as an additive. In addition, plasma drug levels were maintained four times lower than the threshold for CNS toxicity [32]. In a further study, these microparticles (25 – 125 μm size) were successfully tested in humans [33,34] (see Section 6).

The first papers describing the use of PLA and PLGA nanospheres for the delivery of LA were published in 1999 [35,36]. The size range of the particles produced was 250 – 820 nm, and polydispersity was low. Association rates for the larger particles were in the region of 30%, and the *in vitro* release of lidocaine was sustained for > 24 h. Modeling of the release profiles showed that the coefficients of diffusion of lidocaine from the nanospheres varied between 5×10^{-20} and 7×10^{-20} m^2/s, with the differences being related to the crystalline or dispersed forms of the incorporated lidocaine.

In the same year, Govender *et al.* investigated ways to increase the encapsulation efficiency of procaine hydrochloride (which is water soluble) in PLGA nanoparticles. Procaine hydrochloride was replaced by procaine dihydrate, and excipients such as the PLA oligomer poly(methyl methacrylate-co-methacrylic acid) (PMMA-MA) or fatty acids were used in the formulations. The use of procaine dihydrate or excipients (PMMA-MA, lauric acid, or caprylic acid) increased the encapsulation efficiency, without increasing either the size (~ 200 nm) of the nanoparticles or the pH of the aqueous phase (~ 9.3). Drug release from these formulations apparently took place in a rapid burst step followed by slower release [37].

The influence of microparticle size and porosity on the release profile of uncharged lidocaine from PLGA microparticles was investigated by Klose *et al.* [38]. The release of lidocaine diminished with increased particle size, while greater microparticle porosity altered the drug release mechanisms.

Moraes *et al.* developed PLGA nanosphere formulations containing ropivacaine hydrochloride. The particles showed good physicochemical stability and an average size distribution of 160 nm. The encapsulation efficiency was low (~ 4%), but sufficient to reduce the toxicity of ropivacaine against cultures of 3T3 cells [39].

In 2008, Holgado *et al.* compared PLGA microparticles containing uncharged lidocaine prepared by the solvent evaporation and flow focusing methods. The first methodology enabled the production of 3 – 8 μm microparticles of homogeneous size distribution, with greater encapsulation efficiency and slower drug release, opening up new avenues for the preparation of microparticles [40].

Zhang *et al.* optimized the preparation of PLGA (50:50) microspheres containing uncharged bupivacaine, and modeled the drug release profile. They showed that particle size (~ 110 μm) could be controlled by adjusting the agitation speed and polymer concentration, and achieved 6 – 30% drug upload. The release profile was affected by the quantity of bupivacaine crystals loaded onto the surface of the microspheres, so that at low microsphere drug loadings the release mechanism followed the two-process Higuchi model, whereas at high loadings the release was in agreement with a first-order process [41].

Padera *et al.* found that association of the uncharged form of bupivacaine with PLGA microspheres increased the intrinsic myotoxicity of the anesthetic. Even though the carrier *per se* was non-myotoxic, the microspheres were thought to increase bupivacaine myotoxicity by two indirect mechanisms: rapid and sustained release of the LA was found *in situ* [41]. Nonetheless, even if myotoxicity occurs with increased duration of LA action, it appears to be reversible [3].

In 2010, Horie and coworkers prepared a release system for neutral lidocaine using PLGA microparticles, and tested the ability of the formulations to anesthetize the cochlea of guinea pigs. The microparticles (100 μm size) achieved 42% encapsulation efficiency and remained at high concentrations in the cochlea perilymph for more than 3 days after application, indicating their potential use for the sustained release of lidocaine in clinical applications [42].

Formulations of nanocapsules with oily nuclei (mixtures of caprylic and caproic oils), constructed using PLA [43], PLGA, or PCL [44-46] polymers, have recently been described for the encapsulation of different local anesthetics. In general, the formulations presented particle sizes in the range of 200 – 300 nm, with encapsulation efficiencies as high as 60% for benzocaine and 75% for uncharged bupivacaine.

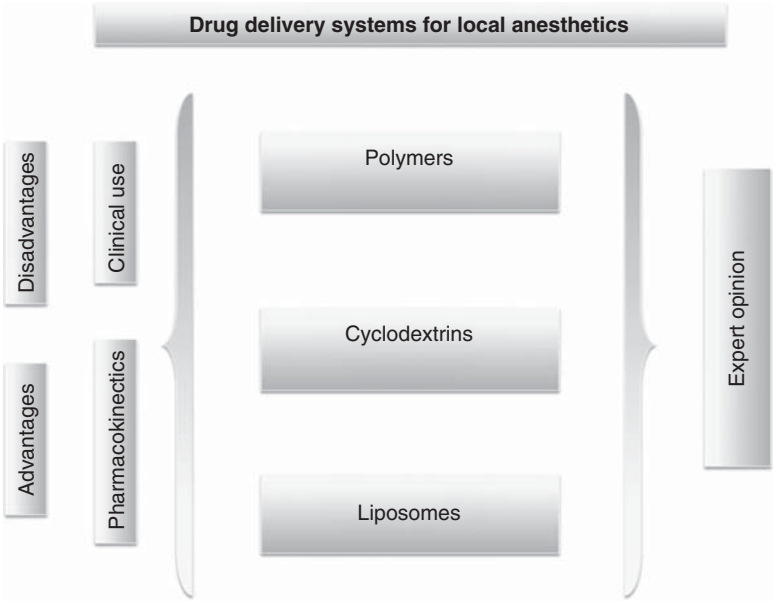

Figure 3. Topics covered in this review.

Association of these LA with nanocapsules prolonged the release profiles of the anesthetics over periods of 4 h or longer.

Alginate/chitosan and alginate/bis (2-ethylhexyl) sulfosuccinate (AOT) nanospheres were described for the encapsulation of bupivacaine hydrochloride (S75-R25), with encapsulation efficiencies of ~ 76 and ~ 88%, respectively. These formulations presented lower toxicity to 3T3 cells in cultures, and enabled longer motor/sensory blockade in sciatic nerves of mice, in comparison to plain bupivacaine [47].

Melo and coworkers showed that the anesthetic activity of benzocaine could be modulated by using PLA, PLGA, and PCL nanocapsules [48], with different oily nuclei (USP mineral oil, isopropyl myristate, and Cetiol) [49]. In tests using different polymers and the same oily nucleus, PLA nanocapsules provided the greatest increase (twofold) in the anesthetic effect of benzocaine, compared to plain benzocaine. The greatest intensity and duration of analgesia (up to 300 min analgesia in a sciatic nerve model) was provided by the PLGA-benzocaine formulation containing USP mineral oil in the nucleus.

2.1 Toxicity of polymeric systems

For the studies reported in this review the polymers used are, in most cases, biodegradable and biocompatible, in particular PLGA and PCL, which have already been approved by the Food and Drug Administration for use in humans. Rose *et al.* [2] reviewed different aspects of the toxicity of polymeric systems used for LA drug delivery. No hemodynamic alterations were observed following the administration of bupivacaine in microspheres, in either animal or human studies. Likewise, no axonal injury or demyelination was found in animal models, and no obvious human neurotoxicity was recorded up to 6 months after subcutaneous administration of bupivacaine in microspheres. Even with extremely large doses of bupivacaine, the safety profile for systemic LA toxicity was benign in animals and humans. However, more studies should be conducted to evaluate the toxicological aspects of these nanomaterials, because their small size, particle shape, and surface charge and/or reactivity could provoke toxicity. Induration and pruritus [33,34] reported after administration of bupivacaine in microcapsules could have been due to the microsphere matrix itself. In this context, further toxicity studies of these materials should be performed prior to commercial use of such formulations [2,50].

3. Local anesthetics in inclusion complexes with cyclodextrins

Cyclodextrins (CD) are cyclic oligosaccharides consisting of six or more α-1,4-linked D-glucopyranose units (**Figure 2B**) that are able to form inclusion complexes with various molecules, including local anesthetics [51]. The macrocyclic glycopyranose ring has a hydrophilic outer surface and a lipophilic central cavity, allowing CD to form reversible noncovalent inclusion complexes with many compounds [52,53], improving their solubility, stability, or bioavailability [54].

The three most abundant CDs, produced from degradation of starch by bacteria, are α-CD, β-CD, and γ-CD, which have six, seven, and eight α-1,4-linked α-D-glucopyranose units, respectively. β-CD is the most common natural CD, and has been extensively studied despite its inherent limited aqueous solubility [53,55,56]. The substitution of the hydroxyl groups located on the outer surface of the CD macrocyclic ring results in dramatic improvement in its solubility and

complexation abilities [51]. β-CD derivatives such as hydroxypropyl-β-cyclodextrin (HP-β-CD), maltosyl-β-cyclodextrin (G2-β-CD), methyl-β-cyclodextrin (M-β-CD), and sulfoalkyl ether-β-cyclodextrin (SAE-β-CD) have also attracted increasing interest due to their greater water solubility, improved complexation ability, and lower toxicity when compared to β-CD [52,54,57].

Most commercially available local anesthetics have a benzene ring that is easily accommodated inside β-CD or its derivatives. Even *o*-methyl-substituted amides, such as bupivacaine [58], ropivacaine [59], lidocaine [60], and prilocaine [61,] can easily fit inside the 7.8 Å cavity of the β-CD macrocyclic ring. The resulting association constants (K_a) are higher compared to the esters benzocaine [62], proparacaine [63], and tetracaine [64], which have non-ortho-substituted aromatic rings. As a result, most LA can form inclusion compounds with β-cyclodextrins, improving water solubility despite the noncovalent interaction and mild affinity (K_a < 1000) [58,59,61-64]. CD-based delivery systems have been proposed as potential new formulations for pain treatment, increasing LA bioavailability at the site of action, decreasing LA plasma concentration, and prolonging the duration of anesthesia.

In 1992, Meert and Melis showed that 80 μg bupivacaine plus 0.125 μg sufentanil in 10 or 20% HP-β-CD (for intrathecal or epidural administration, respectively) increased the duration of analgesia in rats [65].

Dollo and coworkers characterized inclusion complexes formed between LA (bupivacaine, etidocaine, mepivacaine, and prilocaine) and β-CD and its derivatives (HP-β-CD and M-β-CD) that led to increased solubility of the complexed anesthetics [55,66]. In 1998, the same authors completed these studies and demonstrated an improved bupivacaine therapeutic index in animals, after complexation with cyclodextrins [67]. In 2002, the formation of inclusion complexes between LA (lidocaine, prilocaine, proparacaine, and dibucaine) and methyl (M-β-CD) and hydroxypropyl (HP-β-CD) cyclodextrins was demonstrated using capillary electrophoresis and mass spectrometry. For both cyclodextrins, the association constants proved to be dependent on the steric and hydrophobic features of the anesthetic [68].

Bupivacaine has been the most extensively studied LA in relation to the formation of inclusion complexes with β-CD and its derivatives [60,65,67,69-72]. More recently, characterization and *in vivo* evaluation studies have shown promising results (increased anesthetic activity) for CD-based formulations prepared with levobupivacaine [71-73], ropivacaine [59], lidocaine [60,74], and tetracaine [64].

3.1 Toxicity of cyclodextrins

Although CD do not elicit immune responses and have low systemic toxicity [52], some studies in animals have suggested that nephrotoxicity could be an important toxic effect after parenteral administration [57,75]. In 1976, Frank and coworkers showed that β-CD induced renal damage, especially in the proximal tubule, which was characterized by vacuolation, cell disintegration, and mineralization. Histological studies have revealed the presence of β-CD microcrystals in the proximal tubules, indicating that nephrosis could be caused by intact β-CD tubular reabsorption and precipitation, due to its low aqueous solubility [76]. Frijlink and colleagues speculated that HP-β-CD, as well as β-CD, could form crystals due to CD-cholesterol inclusion complex formation and filtration through the glomerular basement membrane [77]. Nevertheless, electron micrographs detected only cholesterol and cholesterol-ester crystals after treatment with β-CD. Further studies revealed that HP-β-CD is well tolerated in humans [53,78,79], and that the use of other alkyl-derivatized cyclodextrins, such as sulfobutylalkylether (SBE-β-CD), is biologically safe [57,80].

In addition, HP-β-CD and SBE-β-CD are approved by the FDA for oral and infiltrative use [75]. In a recent study, Cereda and coworkers have shown that when bupivacaine and ropivacaine are complexed with HP-β-CD (1:1 mole%), the drugs present lower myotoxicity and similar cytotoxicity, compared to equivalent aqueous solutions of the anesthetics [81].

4. Liposomal drug delivery systems for local anesthetics

A significant advance in ultra-long-lasting action of local anesthetics was achieved after the introduction of drug delivery systems, especially liposomes [82-84]. The pharmaceutical application of liposomes has been considered because Bangham, in 1963, showed that vesicles were formed from phospholipids in an aqueous medium. Structurally, liposomes consist of microscopic spheres (**Figure 2A**) that can be produced using glycerophospholipids, with or without addition of cholesterol, nontoxic surfactants, sphingo- or glycolipids, long-chain fatty acids, or even membrane proteins. They possess one or more lipid bilayers, where the hydrophobic lipid tails are directed toward the core and the polar heads face the bilayer surfaces, in contact with the aqueous phase [85-88].

The composition of the lipids, their interactions, as well as the preparation methods employed, determine the pattern, size, and number of bilayers of the liposomes. The resulting structure guides the classification of liposomes in multivesicular systems (MVV), multilamellar vesicles (MLV), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUV), considering their size and number of bilayers. Other classifications consider the preparation method, composition, and application, including surface modification with polyethylene glycol (pegylated or *stealth* liposomes) to avoid recognition by the reticuloendothelial system (RES), to increase their blood circulation, or with antibodies for tissue targeting, as addressed in previous reviews [88-92]. These reviews reveal the versatility of liposomes as drug delivery systems for a wide range of drugs including antineoplastic, antifungal, antimicrobial, anti--inflammatory, and antinociceptive agents.

The structure, composition, and size of liposomes determine the pharmacokinetic, pharmacodynamic, and toxicity

profiles of the encapsulated drugs [84,86]. Vesicle size has an important effect on the destination and biodistribution of liposomes [88]. In the bloodstream, for instance, liposomes smaller than 500 nm can escape from the RES [86]. Following subcutaneous administration, vesicles smaller than 120 nm can pass into the capillaries, whereas larger liposomes (such as LUV) tend to remain at the site of application [89].

The degree of encapsulation of a drug into liposomes is determined by its hydrophilicity or lipophilicity, with polar compounds tending to remain in the aqueous phase (in the vesicle core and in bulk water), while hydrophobic drugs move into the lipid bilayer [93]. This is true for many commercially available LA, which partition into liposome bilayers according to their lipophilic character [24], but can also be trapped in the aqueous compartments of liposomes at relatively high concentrations (e.g., 2% bupivacaine, equivalent to a LA/lipid molar ratio of approximately 2:1), especially with the help of ionic gradients [12,94,95].

The encapsulation of LA into liposomes presents advantages such as slow release, prolonged duration of anesthesia, reduced plasma concentrations, and low toxicity to the central nervous and cardiovascular systems [1,3,94,96-99].

In laboratory animals, several studies have reported increased duration of anesthesia and sensory blockade after parenteral administration of bupivacaine encapsulated in MLV/MLVV prepared using different phosphatidylcholines (PC) and pH [98-108]; lidocaine in eggPC:Chol MLV at pH 6.0 [109]; or prilocaine [110], mepivacaine [111,112], lidocaine [112], and ropivacaine [113] in eggPC:Chol LUV at pH 7.4. Assays in humans have reported improved anesthesia after administration of bupivacaine encapsulated into PC:Chol multilamellar liposomes at pH 8.1 [114], 7.4 [115,116,] and 5.0 [95,102], or mepivacaine in eggPC:Chol LUV at pH 7.4 [117].

Table 1 summarizes the liposomal formulations reported for local anesthesia through infiltrative routes, and presents their main achievements.

4.1 Toxicity of liposome-based LA formulations

Liposomes do not present any risk of antigenicity, because their composition is similar to that of biological membranes [2,107], unless modifications are made in the chemical structure of the phospholipids, or additional compounds are included [118]. Although there are concerns about the potential for significant particulate embolization after massive intravenous bolus of liposomes, intravenous administration of plain liposomes has not been associated with any toxicity [119]. In general, liposomes are biocompatible, and do not cause (or cause very little) allergic [2,3] or toxic [92] reactions.

4.2 Liposome:cyclodextrin mixed systems

A novel strategy based on CD complexation and loading into liposomes has been evaluated for the development of new (double-loaded) LA delivery systems. We have studied ternary LA in β-CD in eggPC liposome systems for proparacaine [63] and prilocaine [120], using different NMR techniques, and have found higher association constants for both LA in the ternary than in the binary systems (LA in β-CD in liposomes > LA in β-CD > LA in liposomes). It was proposed that this new drug delivery strategy should increase anesthetic bioavailability, allowing the upload of higher effective doses (by combining increased water LA-CD complexation and membrane LA partitioning in liposomes).

Mura and coworkers tested the intensity and duration of the anesthetic effect in an animal model (dorsal muscle contraction) of a ternary drug delivery system (LA in HP-β-CD in large multilamellar vesicles composed of eggPC-Chol-stearylamine, 5.5:1.0:1.5 mole%) containing prilocaine (1 – 5%) for infiltrative use [121], as well as 1% benzocaine or 1% butamben in gels [122]. In all cases, the double-loaded formulation was the most effective, showing longer duration of anesthetic effect and shorter onset of action when compared to the single-loaded formulations. In addition, Vieira tested a ternary system (0.5% ropivacaine in HP-β-CD in eggPC unilamellar liposomes) in sensory blockade experiments in rats. A more prolonged (300 min) anesthetic effect of RVC was achieved using the ternary system, compared to either binary LA in liposomes (240 min), or free RVC (180 min) (Vieira, ALN *et al.* 2012, in preparation).

5. Pharmacokinetics of local anesthetics in drug delivery systems

In vitro release tests are used to assess the release profiles of drugs from pharmaceutical formulations, enabling comparison between the absence (free drug) and presence of a carrier. Despite the convenience of such tests, the results obtained may not correspond to the *in vivo* situation, because tests are typically performed under sink conditions [123] to remove the released drug. Differently, after *in vivo* administration of the drug delivery systems, free LA may be absorbed into adjacent tissues, decreasing the drug concentration.

Several studies have assessed the distribution of drug delivery systems for local anesthetics in animals and volunteers. Typically, drug delivery systems provide more constant and lower plasma concentrations when compared to the free anesthetic, suggesting that carriers delay LA transfer to the bloodstream. This delayed redistribution to plasma serves as a good indication of the depot-related slow-release profile of LA delivered locally through different carriers. Although various different types of carriers have been used in the reported studies, most were shown to be able to alter the pharmacokinetic behavior of local anesthetics, as described below.

5.1 Pharmacokinetics of local anesthetics in liposomes

Mashimo *et al.* studied the pharmacokinetics of free and liposome-encapsulated 2% lidocaine in dogs after epidural administration. There were no significant differences in plasma lidocaine concentrations between liposomal (multilamellar,

Table 1. Liposomal local anesthetic formulations described in the literature for infiltrative use.

Anesthetic agent	Liposomes	pH	Administration route	Subjects	Antinociceptive test	Results	Ref.
Lidocaine (2%)	MLV (EggPC + Chol)	6.0	Epidural	Dogs	Somatosensory evoked potential test	Longer anesthesia duration, reduced release rate, and higher local availability	[109]
Bupivacaine (0.25%)	MLV (EggPC + Chol)	8.1	Endovenous	Rabbits	Electrocardiographic test	Decreased toxicity in comparison to 0.5% bupivacaine with or without vasoconstrictor	[99]
Bupivacaine (0.25%)	MLV (EggPC + Chol)	8.1	Brachial plexus blockade	Rabbits	Serum levels	Slower release rate in comparison to 0.5% bupivacaine with or without vasoconstrictor	[98]
Bupivacaine (0.5%)	MLV (EggPC + Chol)	8.1	Epidural	Humans	Pain scale and motor blockade evaluation	Increased duration of analgesia without side effects in comparison to 0.5% bupivacaine with vasoconstrictor	[114]
Bupivacaine (0.25%)	MLV (EggPC + Chol)	7.4	Brachial plexus blockade	Humans	Pinprick	Pain relief for 40 h	[115]
Bupivacaine (0.5%)	MLV (DMPC + Chol)	Not given	Injections at the root of the tail	Mice	Tail-flick	Prolonged analgesia in comparison to 0.5% bupivacaine	[100]
Bupivacaine (0.25%)	MLV (EggPC + Chol)	8.1	Extradural	Rabbits	Radioactive images	Slower systemic distribution than bupivacaine solution	[103]
Bupivacaine (0.75 and 2%)	LUV (DOPC + Chol)	7.4	Intradermal	Guinea pig	Allergic cutaneous reaction	Increased drug upload and neural blockade duration	[94]
Bupivacaine (0.25%)	MLV (EggPC + Chol)	7.4	Epidural	Humans with lung neoplasia	Pinprick	Analgesia improved (threefold) in comparison to bupivacaine with vasoconstrictor	[116]
Bupivacaine (2%)	MLV (DSPC + Chol)	5.3	Infiltration in wounds	Rats	Von Frey filament test	Prolonged anesthetic effect	[101]
Bupivacaine (0.5%)	MLV (EggPC + Chol + α-tocopherol)	8.1	Intracisternal	Rabbits	Motor blockade and morphological evaluation	Longer motor blockade without neurotoxicity	[105]
Bupivacaine (0.5%)	MLV (EggPC + Chol + phosphatidic acid)	6.4	Injections at the root of the tail	Rats	Tail-flick	Prolonged anesthetic effect	[108]

Table 1. Liposomal local anesthetic formulations described in the literature for infiltrative use (continued).

Anesthetic agent	Liposomes	pH	Administration route	Subjects	Antinociceptive test	Results	Ref.
Bupivacaine (0.75, 0.375, 0.25, 0.125, and 0.065%)	MLV (EggPC + Chol + α-tocopherol)	8.1	Epidural	Rabbits	Motor blockade and blood pressure	Increased motor blockade with hemodynamic changes depending on the LA concentration	[106]
Bupivacaine (0.5, 1, and 2%)	MLV (Hydrogenated soyPC + Chol)	Not given	Intradermal	Humans (forearm)	Pinprick	Improved anesthesia	[15]
Bupivacaine (0.5%)	Dehydration, rehydration MLV (DMPC and DSPC)	4 - 5.5	Subcutaneous injection	Mice	Cutaneous electrical stimulation and vocalization threshold	Increased sensorial blockade in comparison to bupivacaine in solution	[89]
Bupivacaine (0.5%)	MLV (EggPC + Chol + phosphatidic acid)	6.35	Injections at the root of the tail	Rats	Tail-flick	Improved anesthesia with identical serum levels	[104]
Bupivacaine (0.5, 1, and 2%)	LMVV (Hydrogenated soyPC + Chol)	4 - 5.5	Infiltration (back)	Humans	Pinprick	Dose-dependent prolongation of anesthesia	[95]
Mepivacaine (2%) and bupivacaine (0.5%)	Unilamellar (EggPC + Chol + α-tocopherol)	7.4	Sciatic nerve blockade	Mice	Paw-pressure test	Liposomal mepivacaine but not liposomal bupivacaine improved anesthesia in comparison to plain solutions	[111]
Prilocaine (3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Infraorbital nerve blockade	Rats	Infraorbital nerve blockade test	Improved sensorial blockade in comparison to prilocaine in solution, and equivalent anesthesia to vasoconstrictor containing prilocaine	[110]
Prilocaine, lidocaine, mepivacaine (2%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Infraorbital nerve blockade	Rats	Infraorbital nerve blockade test	Improved anesthetic effect, more evident for mepivacaine	[112]
Ropivacaine (0.5%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Sciatic and infraorbital nerve blockade	Mice and rats	Paw-pressure plus infraorbital nerve blockade test	Longer and increased anesthesia in comparison to ropivacaine in solution	[113]
Prilocaine (3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraplantar and intraoral injections	Rats	Paw edema test and oral mucosa histological analysis	No inflammatory effects on the paw and less inflammatory reaction in oral mucosa than prilocaine with vasoconstrictor	[141]

Table 1. Liposomal local anesthetic formulations described in the literature for infiltrative use (continued).

Anesthetic agent	Liposomes	pH	Administration route	Subjects	Antinociceptive test	Results	Ref.
Mepivacaine (2 and 3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraoral injection	Rats	Pharmacokinetic analysis (liquid chromatography - tandem mass spectrometry) and oral mucosa histological analysis	Reduced C_{max}, prolonged AUC and t½ in comparison to mepivacaine with vasoconstrictor. Protective effect in tissue against local inflammation evoked by mepivacaine or vasoconstrictor	[117]
Ropivacaine (0.5%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraoral injection	Humans	Pharmacokinetic analysis (HPLC)	Similar pharmacokinetic profile in comparison to ropivacaine with vasoconstrictor	[124]
Mepivacaine (2 and 3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraoral injection	Humans	Electrical pulp tester and visual analog scale (VAS)	Increased anesthesia duration and reduced injection discomfort in comparison with mepivacaine with vasoconstrictor	[139]
Prilocaine (3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraoral injection	Humans	Electrical pulp tester	Similar anesthetic efficacy in comparison to prilocaine without vasoconstrictor. Lower anesthetic efficacy in comparison to prilocaine with vasoconstrictor-associated prilocaine formulation	[136]
Mepivacaine (3%)	LUV (EggPC + Chol + α-tocopherol)	7.4	Intraoral injection	Humans	Pharmacokinetic analysis (liquid chromatography - tandem mass spectrometry)	Similar systemic absorption in comparison to mepivacaine with vasoconstrictor	[125]
Bupivacaine (0.5 – 2%)	LMVV HSPC100 + C16-SoyPM + Chol	5.5	Abdomen injection	Mice	Vocal response to electrical stimulation	Prolonged analgesia in comparison to bupivacaine without vasoconstrictor	[142]

PC:Chol, 1:1 mole%) and non-liposomal formulations, with the latter showing a tendency toward higher lidocaine plasma levels. The area under the curve (AUC) and the time to reach maximum concentration (T_{max}) were greater in animals given LA in liposomes, suggesting that encapsulation did not reduce drug absorption, but reduced the rate of release and increased lidocaine availability in the epidural space. Although differences were not statistically significant, the plasma half-life (t½) and mean residence time (MRT) tended to be longer in the liposomal group when compared to lidocaine in solution [109].

Boogaerts *et al.* compared plasma concentrations after administration of 0.25% bupivacaine, either free or liposome-encapsulated (multilamellar, eggPC:Chol, 4:3 mole%), during axillary block in rabbits. The highest average concentration of bupivacaine was the same in both groups, but the plasma concentration profiles were different: animals given liposomal bupivacaine had lower plasma levels during the first 10 min and then higher levels after 24 h. The authors reported that only the free fraction of bupivacaine was able to diffuse into the blood and that liposomes provided a local depot for bupivacaine, slowly releasing their contents [98].

Yu *et al.* studied the pharmacokinetics of free and multilamellar liposomal (PC:Chol:phosphatidic acid, 1:0.4:0.1 mole %) bupivacaine formulations, injected into rat tails. C_{max} was five times smaller using liposomes than for the nonencapsulated anesthetic, although both formulations presented the same T_{max} (30 min). The AUC value for the liposomal formulation was less than half that for the bupivacaine solution. The t½ of liposomal bupivacaine was longer when compared to the non-liposomal bupivacaine. The absorption of free bupivacaine was almost complete (> 93%) after 45 min, whereas for liposomal bupivacaine there was less than 54% absorption after 480 min. The pharmacokinetics of bupivacaine was greatly altered by entrapping this LA into liposomes, with the liposomal bupivacaine producing a small peak followed by steady plasma levels over several hours [104].

Tofoli *et al.* determined the pharmacokinetic parameters of liposomes (unilamellar, eggPC:Chol:α-tocopherol, 4:3:0.07 mole%) loaded with 2% mepivacaine ($MVC_{2\%LUV}$) after intraoral anesthesia in rats, and compared the results with those obtained using commercial mepivacaine formulations (2% mepivacaine with 1:100,000 epinephrine – $MVC_{2\%EPI}$ and 3% mepivacaine – $MVC_{3\%}$). As expected, $MVC_{3\%}$ induced higher plasma concentrations than $MVC_{2\%LUV}$ after intraoral injection, for up to 240 min following administration ($p < 0.05$). No statistical differences between $MVC_{2\%EPI}$ and $MVC_{2\%LUV}$ were observed for plasma concentrations, C_{max}, and AUC_{0-420} values. These results indicated that encapsulation of mepivacaine in liposomes altered the absorption of LA in a similar way as that caused by addition of epinephrine. Interestingly, $MVC_{2\%LUV}$ induced longer t½ values than $MVC_{3\%}$ and $MVC_{2\%EPI}$, which means that the liposomal LA presented a delayed elimination [117].

Franz-Montan *et al.* determined the pharmacokinetic parameters of liposomal 0.5% ropivacaine after dental anesthesia in 14 healthy volunteers. In a randomized, double-blind and crossover study, the volunteers received maxillary infiltration of liposomal (eggPC:Chol:α-tocopherol, 4:3:0.07 mole%) ropivacaine, and ropivacaine with 1:200,000 epinephrine, in two different sessions. No differences between the formulations were observed with respect to C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, t½, and plasma ropivacaine concentrations, indicating that liposome-encapsulated ropivacaine had a similar pharmacokinetic profile to that shown by ropivacaine associated with epinephrine [124].

The pharmacokinetics of bupivacaine loaded into large multivesicular PC:Chol liposomes (BUPISOME™), prepared using a pH gradient to achieve a high drug/phospholipid mole ratio (1.8), was evaluated by Davidson *et al.* [12]. Eight volunteers received subcutaneous injections of 2% liposomal bupivacaine or plain 0.5% bupivacaine. Similar C_{max} values were measured for both formulations, despite the fourfold increase in total bupivacaine dose of the liposomal preparation. Higher T_{max} and t½ values were registered for the liposomal bupivacaine preparation, as expected for a slow release formulation. The authors concluded that the liposomal formulation allowed the administration of higher bupivacaine doses without increasing either peak plasma concentrations or the risk of systemic toxicity.

Tofoli *et al.* assessed the pharmacokinetic profile of a liposomal (unilamellar EPC:Chol:α-tocopherol, 4:3:0.07 mole%) mepivacaine formulation in relation to commercial preparations of the same anesthetic salt. In a randomized crossover study, 15 volunteers received commercial anesthetic solutions (2% mepivacaine with 1:100,000 epinephrine – $MVC_{2\%EPI}$, and plain 3% mepivacaine – $MVC_{3\%}$) and 2 and 3% liposomal mepivacaine formulations ($MVC_{2\%LUV}$ and $MVC_{3\%LUV}$). No differences were observed between the drug plasma levels for $MVC_{2\%LUV}$ and $MVC_{2\%EPI}$ at any time, except at 120 min. $MVC_{3\%}$ and $MVC_{3\%LUV}$ induced higher drug plasma concentrations, C_{max}, and AUC than the 2% formulations, at all times. No advantages were found for the 3% formulations; however, $MVC_{2\%LUV}$ exhibited the properties of a slow-release formulation, reducing the plasma concentrations of mepivacaine in a similar way to the addition of a vasoconstrictor [125].

5.2 Pharmacokinetics of cyclodextrins and polymer–LA drug delivery systems

Cyclodextrins are also able to alter the pharmacokinetics of local anesthetics. Estebe *et al.* studied the pharmacokinetics and pharmacodynamics of bupivacaine:SBE7-β–CD (1:1 mole%) complex in five nonpregnant Lacaune sheep, following randomized crossover epidural administration (20 mg) with a 4-day wash out. The pharmacodynamic effects were evaluated from the motor activity of the animals, with the injection of BVC-SBE7-β–CD producing a significant increase in the intensity of motor block as a function of time. Pharmacokinetic analysis showed no significant difference in C_{max} between the two formulations. However,

T_{max} was significantly extended after injection of the BVC-SBE7-β–CD complex, suggesting a decrease in the absorption rate of the anesthetic into the systemic circulation, explaining the prolonged antinociception effect observed after epidural administration [70].

The use of biopolymer carriers in drug delivery systems also alters the release of LA and their pharmacokinetics. Kranz *et al.* investigated the *in vivo* release of bupivacaine hydrochloride (5 mg) from an injectable PLGA system that formed microparticles *in situ*. After injection of this emulsion system, bupivacaine was released from the internal polymer phase in a controlled fashion. Sprague–Dawley rats received intramuscular injection of such a system (0.25:1, polymer: oil phase) or a polymer solution (40% PLGA in 2-pyrrolidone) into the right *musculus rectus*. Lower C_{max} and higher T_{max} were observed with the emulsion system, a sign of reduced initial drug release and systemic uptake of the LA, indicating a sustained release of the drug [126].

Ratajczak-Enselme and coworkers studied the pharmacokinetics of ropivacaine-loaded PLGA microspheres (PLGA-RVC) and compared this system with epidural administration (in bolus or infusion) of ropivacaine solution. Twelve nonpregnant Lacaune ewes were divided into three different groups. Six received ropivacaine-loaded microspheres (500 mg/15 mL), three received a bolus of ropivacaine (30 mg/15 mL) followed by a 6-h infusion of ropivacaine (2 mg/mL, 10 mL/h), and the last three animals received three successive boluses of ropivacaine (50 mg/15 mL) separated by 2-h intervals. After epidural administration of ropivacaine-loaded PLGA microspheres, C_{max} in the plasma was *ca.* 100 ng/mL, whereas epidural and intrathecal C_{max} were close to 600 and 150 µg/mL, respectively. The authors concluded that the epidural administration of appropriate PLGA microsphere formulations of ropivacaine led to reduced systemic absorption, allowing higher drug uptake through meninges [127].

Table 2 summarizes the most important pharmacokinetic parameters reported for local anesthetic formulations described in the literature.

6. Clinical studies with local anesthetics in drug delivery systems

Many additives, such as α_1- and α_2 adrenergic agents, dextrans, opioids, hyaluronidase, bicarbonate, and vasopressin derivatives, have been associated with local anesthetics to increase anesthesia duration [128]. Drug delivery systems for local anesthesia are only commercially available for topical application on the skin, while a few studies have addressed infiltration/block anesthesia in humans.

The first use in humans of a local anesthetic associated with a drug delivery system, namely a multilamellar liposome suspension of bupivacaine, was described by Boogaerts *et al.* Epidural injection of the liposomal formulation almost doubled postsurgical anesthesia in patients submitted to major surgery, compared to plain bupivacaine [114]. However, the liposomal formulation was not suitable for anesthesia during surgery, but was used to control the pain after surgical procedures, as described in subsequent published studies and case reports. After this study, two case reports of multilamellar liposomal bupivacaine use in humans were published; one showed that pain relief in an algodystrophic-arm patient lasted 40 h, compared to 12 h for a plain bupivacaine solution [115]. In another case, relief of the pain of chronic cancer lasted for 11 h after injection of a liposomal formulation, compared to 4 h for plain bupivacaine [116].

Increased duration of anesthesia in volunteers was also observed after subcutaneous injection of multivesicular bupivacaine (BUPISOME™) at 0.5, 1, and 2% concentration levels. These formulations produced anesthesia in the lower back for 19, 39, and 48 h, respectively, whereas plain 0.5% bupivacaine provided only 1 h of anesthesia [95].

More recently, it was shown in a study of 184 patients submitted to hemorrhoidectomy that an injection of a single dose of 300 mg of encapsulated bupivacaine in an extended-release injectable suspension (EXPAREL™, approved by the FDA), administered intraoperatively through wound infiltration, significantly reduced pain over 72 h, compared to a placebo [83].

Another release system, PLGA microcapsules loaded with bupivacaine and dexamethasone (see Section 2), has been shown to extend bupivacaine anesthesia for up to 96 h after subcutaneous injection. Side effects such as mild pruritus and local induration have been reported, possibly due to the quantity of polysaccharide microcapsules injected, which therefore demands further investigation [33,34].

In addition to their use in postsurgical analgesia, drug release systems have also been investigated for dental anesthesia.

6.1 Drug delivery systems and local anesthetics in dentistry

In contrast to the majority of the other human organs, the nerve fibers responsible for human teeth sensibility lie inside osseous cavities of the face, which increases the difficulty of access of local anesthetic solutions. These solutions must reach the nerve fibers through cortical bone, which is usually not particularly dense in the maxilla, but very dense in the mandible. Faster penetration of larger amounts of local anesthetic solution into the cortical bone cavities could enhance the duration and decrease the onset time of local anesthesia.

Few studies regarding the local anesthetic effect of drug delivery systems in dentistry are found in the literature. Most of the reported studies concern topical anesthesia, which is out of the scope of this review. In any case, procedures for noninvasive buccal mucosa anesthesia prior to LA infiltration aim to provide comfort to the patient. Among the topical formulations available, EMLA™, an eutectic mixture of 2.5% lidocaine and 2.5% prilocaine [129,130], deserves mention for being the most effective topical anesthetic in dentistry, despite the fact that among dentists benzocaine is the most used and popular topical anesthetic agent [131].

Table 2. Pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, t½) measured for the local anesthetic in solution (F) and in liposomes (L), cyclodextrins (CD), or polymers (P) drug delivery formulations.

	Pharmacokinetics Parameters (in plasma)					
Formulation(s)	**AUC_{0-t}**	**$AUC_{0-\infty}$**	**Cmax**	**Tmax**	**t ½**	**Ref./Units**
Free (F) and liposomal (L) 2% lidocaine MLV(PC:Chol)	119.5 ± 77.5 (F); 214.4 ± 76.1 (L)*	197.0 ± 146.8 (F); 330.3 ± 104.4 (L)*	1.64 ± 0.64 (F); 1.93 ± 0.63 (L)	11.4 ± 3.8 (F); 17 ± 4.8 (L)*	99.8 ± 43.0 (F); 157.6 ± 100.4 (L)*	[109]‡,¶
Free (F) and liposomal (L) 0.5 % bupivacaine MLV (PC: Chol: phosphatidic acid)	96.5 ± 2.9 (F); 42.0 ± 2.2 (L)*	109.5 ± 7.6 (F); 120.6 ± 9.9 (L)	0.65 ± 0.04 (F); 0.12 ± 0.02 (L)*	30.0 ± 0.0 (F); 30.0 ± 0.0 (L)	176.6 ± 42.8 (F); 745.5 ± 149.1 (L)*	[104]‡,¶
2% mepivacaine with 1:100,000 epinephrine (F1); 3% mepivacaine (F2) and liposomal (L) 2% mepivacaine LUV (eggPC:Chol:α-tocopherol)	66.7 (61.2 – 115.9) (F1); 241.4 (221.0 – 248.9) (F2); 92.7 (72.3 – 111.9) (L) *F1 vs. F2; F2 vs. L	60.1 (46.5 – 65.2) (F1); 349.3 (257.0 – 454.7) (F2) ; 776.1 (424.1 – 1221.5) (L) *F1, F2 vs. L	0.40 (0.33 – 0.48) (F1); 1.9 (1.24 – 2.42) (F2); 0.27 (0.21 – 0.33) (L) *F2 vs. L	90 (60 – 195) (F1); 120 (120 – 120) (F2); 150 (120 – 195) (L)	94.9 (89.7 – 226.5) (F1); 145.2 (49.0 – 315.9) (F2); 1465.2 (911.6 – 2822.4) (L) *F1, F2 vs. L	[117]§,¶
0.5% ropivacaine with 1:200,000 epinephrine (F) and liposomal (L) 0.5% ropivacaine LUV (eggPC:Chol:α-tocopherol)	32.4 (20.1 – 44.0) (F); 40.4 (26.3 – 55.2) (L)	78.5 (4.9 – 102.6) (F); 71.9 (28.1 – 138.6) (L)	93.4 (63.2 – 114.7) (F); 92.9 (82.7 – 97.7) (L)	37.5 (30.0 – 45.0) (F); 30.0 (15.0 – 56.3) (L)	868.0 (142.0 – 1498.0) (F); 869.0 (349.0 – 1512.0) (L)	[124]§,#
0.5% bupivacaine (F) and liposomal (L) 2% bupivacaine MLVV(PC:Chol)	Not applicable	150 ± 74.1 (F); 1410.0 ± 759.0 (L)	0.87 ± 0.45 (F); 0.83 ± 0.34 (L)	37.5 ± 16.0 (F); 262.0 ± 149.0 (L)*	131.0 ± 58.0 (F); 1294.0 ± 860.0 (L)*	[12]‡,¶
2% mepivacaine with 1:100,000 epinephrine (F1); 3% mepivacaine (F2); liposomal 2 % (L1) and liposomal 3% (L2) mepivacaine LUV (eggPC:Chol:α-tocopherol)	32.30 ± 9.04 (F1); 50.01 ± 16.47 (F2); 26.60 ± 13.77 (L1); 47.65 ± 14.11 (L2) *L1 vs. F2; F1 vs. L2; L1 vs. L2	41.38 ± 13.77 (F1); 63.75 ± 25.19 (F2); 34.25 ± 21.74 (L1); 58.55 ± 22.87 (L2) *L1 vs. F2; F1 vs. L2; L1 vs. L2	620.34 ± 126.23 (F1); 1073.28 ± 225.51 (F2); 606.92 ± 289.16 (L1); 1037.93 ± 262.76 (L2) *L1 vs. F2; F1 vs. L2; L1 vs. L2	41.00 ± 42.22 (F1); 26.00 ± 16.49 (F2); 32.00 ± 41.61 (L1); 37.00 ± 41.61 (L2)	149.32 ± 37.15 (F1); 143.43 ± 36.92 (F2); 129.79 ± 57.75 (L1); 128.72 ± 46.54 (L2)	[125]‡,#

*Significant differences ($p < 0.05$).

‡Data expressed as mean ± SD.

§Data expressed as median (lower and upper quartiles).

¶Units: AUC_{0-t} and $AUC_{0-\infty}$ $\mu g.min^{-1}.mL^{-1}$; C_{max} - $\mu g.mL^{-1}$; T_{max} and t ½ - min.

#Units: AUC_{0-t} and $AUC_{0-\infty}$ $ng.min^{-1}.mL^{-1}$; C_{max} - $ng.mL^{-1}$; T_{max} and t ½ - min.

Table 2. Pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, t½) measured for the local anesthetic in solution (F) and in liposomes (L), cyclodextrins (CD), or polymers (P) drug delivery formulations (continued).

	Pharmacokinetics Parameters (in plasma)					
Formulation(s)	**AUC_{0-t}**	**$AUC_{0-\infty}$**	**Cmax**	**Tmax**	**t ½**	**Ref./Units**
Free (F) and SBE7-β-CD complexed (CD) 20 mg bupivacaine	Not available	Not available	Not available	11.2 ± 3.6 (F); 46.0 ± 13.1 (CD)	Not available	[70]‡,¶
PLGA:peanut oil injectable *in situ* forming microparticle system (ISM) and *in situ* implant of PLGA microparticles (P), both containing 5 mg bupivacaine	Not available	Not available	5.57 µg/mL (P); 1.06 µg/mL (ISM)	60 min (P); 90 min (ISM)	Not available	[126]
Free ropivacaine (50 mg/15 mL) given in three successive boluses separated by 2-h intervals (B1, B2, B3); and ropivacaine-loaded PLGA microspheres (500 mg/15 mL) (P)	Not available	Not available	≈100 (P); 85 ± 26 (B1); 97 ± 57 (B2); 102 ± 64 (B3).	60 (P); 24 ± 19 (B1); 19 ± 23 (B2); 12 ± 3 (B3)	148 ± 185 (B1); 265 ± 159 (B2); 446 ± 187 (B3) P not available	[127]‡,#

*Significant differences ($p < 0.05$).

‡Data expressed as mean ± SD.

§Data expressed as median (lower and upper quartiles).

¶Units: AUC_{0-t} and $AUC_{0-\infty}$ $\mu g.min^{-1}.mL^{-1}$; C_{max} - $\mu g.mL^{-1}$; T_{max} and t ½ - min.

#Units: AUC_{0-t} and $AUC_{0-\infty}$ $ng.min^{-1}.mL^{-1}$; C_{max} - $ng.mL^{-1}$; T_{max} and t ½ - min.

Considering infiltrative anesthesia, only liposomal formulations have so far been reported. The characteristics of liposome-encapsulated local anesthetics, such as prolonged anesthesia, reduced release, and lower toxicity in both cardiovascular and central nervous systems, are well known [99,132-134]. For dentistry purposes, it is interesting that some liposomal formulations have shown the same effectiveness in soft tissue as vasoconstrictor-associated LA formulations [115].

At the usual concentrations employed, all local anesthetics used in dentistry are vasodilators, and the duration of anesthesia in dental procedures is very short in the absence of an associated vasoconstrictor [135]. Vasoconstrictors are utilized to extend the time of contact between the local anesthetic and the periosteum. Thus, greater numbers of molecules are available to cross the cortical bone and reach the nerve fibers [136]. Despite their safety and effectiveness, some patients cannot tolerate vasoconstrictors [137,138].

Liposomal anesthetic formulations have been proposed to replace those associated with vasoconstrictors. Tofoli *et al.* observed the effects of a buccal infiltration of mepivacaine (intraoral injection of 1.8 mL) into the upper right canine region, using formulations with or without epinephrine or liposome encapsulation. They concluded that liposome-encapsulated 3% mepivacaine resulted in longer pulpal anesthesia, compared to a plain 3% solution, and that even in a lower concentration (2%) the liposome-encapsulated formulation resulted in pulpal anesthesia that was similar to that of the plain 3% solution. However, a solution of 2% mepivacaine with 1:100,000 epinephrine showed longer pulpal anesthesia than all the other formulations [139].

Wiziack-Zago *et al.* compared the anesthetic efficacy of liposome-encapsulated prilocaine with both plain and vasoconstrictor-associated formulations (1.8 mL). The formulations were infiltrated into the buccal sulcus of the maxillary right canine of volunteers. It was observed that the liposomal formulation presented similar anesthetic efficacy as the plain formulation, but showed lower efficacy compared to the vasoconstrictor-associated prilocaine formulation [136].

As discussed in Section 4, liposomal formulations of lidocaine, prilocaine, and mepivacaine were able to increase the duration of anesthesia, compared to their corresponding plain solutions, following infraorbital nerve block in rats [110-112]. The same efficacy is not always observed during dental anesthesia in humans. Soft tissue anesthesia is usually easy to achieve with the majority of local anesthetic solutions, because the formulations are injected very close to the gingival nerve endings. However, to achieve pulpal anesthesia, the formulation needs to cross the dense cortical bone to reach the terminal nerve endings of the tooth apex [136].

Liposomes have no intrinsic vasoactive properties, and the vasodilatation induced by the local anesthetic molecules could be the cause of the similar behavior of the vasoconstrictor-free and liposomal anesthetic solutions. Under these conditions, the number of molecules released from the liposomes may be not enough to induce a significant duration of dental anesthesia. The factors influencing this phenomenon are the liposome encapsulation efficiency and the degree of vasodilatation induced by a specific local anesthetic [136].

A randomized double-blind and crossover study in volunteers showed that encapsulation in liposomes did not improve the anesthetic efficacy of ropivacaine injected into oral mucosa. The combination of epinephrine and ropivacaine or lidocaine provided longer duration of pulpal anesthesia than the liposomal formulation [140].

7. Conclusions

Several different drug delivery systems have been reported that provide a safe way to deliver local anesthetics. Advantages over the commercially available LA agents include prolonged anesthetic activity, sustained release, and reduced toxicity.

Technological restrictions still limit high LA uploads and the development of an ideal carrier system. Nevertheless, the existing drug delivery systems for LA have considerable clinical potential, which is supported by a small number of studies that have confirmed their safety and efficacy in humans.

8. Expert opinion

Despite the unavailability of an ideal formulation for the sustained release of LA, new strategies have been developed aiming to achieve a fast onset of anesthesia, prolonged pharmacological efficacy, and decreased toxicity.

It is important to observe that for regional anesthesia purposes there is no need to consider the targeting of the drug delivery system, because the administration of local anesthetics is directly onto the nerve trunk. In addition, a prolonged lifetime in the circulation is not desirable, because the anesthetic is expected to stay at the site of injection/application, and absorption into the bloodstream is part of the clearance mechanism. Research must therefore focus on improving drug upload to overcome the properties that limit LA potency (lack of chemical stability, and water or lipid solubility), and the combining of each LA agent with a carrier compatible with its physicochemical features (lipid solubility, molecular shape, polarity, charge, etc.).

Considering the polymers, PLGA and PCL are approved by the FDA for applications in drug delivery systems. Nevertheless, only two studies using polymers and LA in humans have so far been described in the literature [33,34]. We wonder if drawbacks such as the high cost of polymers (especially when compared to the low cost of the LA agents), or technological limitations in the scale-up process, make polymer formulations economically uncompetitive. Despite these disadvantages, some progress has been made, especially in relation to the release profiles of local anesthetics and increased anesthesia times. In the near future, formulations for local anesthesia utilizing carrier systems consisting of micro- and nanoparticles will probably become available, offering safer and more efficient alternatives to the existing local anesthetic formulations.

Cyclodextrins are able to improve the solubility of most LA by forming *host–guest* inclusion complexes. Improved drug stability and residence time at the site of injection are also important characteristics of LA-CD drug delivery systems. CD derivatives are safe for human use, and offer additional advantages such as relatively low cost, simple sterilization, and ease of production scale-up [26,52].

Regarding liposomes, a reasonable number of pharmaceutical studies have been performed, although clinical studies remain scarce. Novel liposomal formulations, designed to improve drug loading by entrapment of the charged LA species using an ion gradient inside impermeable liposomes, or with blends of donor/receptor vesicles (see [26] for a review), have been proven to successfully upload almost two bupivacaine molecules per lipid molecule. However, physical (size increase by vesicle fusion) and chemical (lipid peroxidation) instability during storage remains a drawback in liposome technology, affecting sterilization procedures and process scale-up in the case of these drug delivery formulations.

Drug delivery systems provide a safe way of administering local anesthetics, with pharmaceutical effects that far exceed those obtained with the current commercially available agents. These systems offer potential health benefits for patients suffering from chronic or postoperative pain.

Declaration of interest

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