Expert Opinion

Review of prolonged local anesthetic action

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Importance of the field: Pain following surgery is often treated by local anesthetic agents. Duration of the analgesia can be extended safely following administration of encapsulated large doses of local anesthetic agents.

Areas covered in this review: This review considers formulations used for encapsulation of local anesthetic agents for prolonged anesthesia effect. All studies describing encapsulation of a commercial local anesthetic agent for providing prolonged analgesia were considered using the NCBI Medline site. of local anesthetic, prolonged anesthesia, polymers and liposomes were entered in order to retrieve appropriate articles and reviews from 1966 to 2010, with emphasis on the last 10 years. Reference pages were searched manually for other relevant articles. The topics covered include an overview of local anesthetic agents and a review of local anesthetic carrier agents, with emphasis on liposomes and polymer carriers. Articles were limited to the English language.

What the reader will gain: The current research areas for prolongation of local anesthetic effect are evaluated, along with their limitations. Each topic has been summarized, and the review has attempted to cover all current laboratory and clinical studies in a simple manner that should also be useful for readers without a pharmacology background. The direction of research is promising and exciting, and this review should be a useful up-to-date reference.

Take home message: Many formulations including polymer and liposome carriers have facilitated prolonged local anesthetic action for several days, although few clinical studies have been performed. This field promises a safe way to deliver local anesthetics for effect far beyond that of commercially available agents, with potential cost and health benefits for patients suffering chronic or postoperative pain

Keywords: liposomes, local anesthetics, polymers, prolonged action, sensory block

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1. Introduction

Local anesthetics are used clinically for analgesia either following surgery or for management of other acute and chronic pain. However, the duration of action of plain local anesthetic (LA) agents is limited. Alteration of physical or chemical properties of amide LA molecules has enabled decrease in toxicity or slightly prolonged action of LA agents; however, their length of action is still measured in hours [1]. Anesthesia prolonged for a period of days is at present best provided using catheter techniques [2] with disposable pumps [3] or multiple injections. Placement of these catheters requires skill, and there are extra requirements of monitoring, cost of equipment and the risk of infection from the indwelling catheter.

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Article highlights.

- Encapsulation of local anesthetic agents allows large doses to be released slowly and provide analgesia over a prolonged period
- Liposomes are versatile local anesthetic agent carriers, limited by lack of reliability and reproducibility during manufacture
- The most commonly used micro and nanoscale vehicles for drug encapsulation and delivery are micro and nanospheres. Microparticles are perfect for drug delivery as they remain at the depot site for long periods allowing slow prolonged release of the encapsulated drug.
- Limitations of encapsulated local anesthetic agents include neurotoxicity, myotoxicity, tachyphylaxis, motor block and viscosity
- Site directed prolonged local anesthetic action is possible. The few human studies have confirmed the potential safety and efficacy of prolonged LA formulations, demonstrating that this field has great clinical potential.

This box summarizes key points contained in the article.

A new agent, simple to apply by direct injection and without local or systemic side effects yet capable of providing local anesthesia for several days, would be invaluable. Pain following surgery is frequently treated by local anesthetic agents by direct infiltration, peripheral nerve blocks, or neuraxial techniques. Surgery, in particular day care surgery, requires longer term solutions than those now available. Use of regional analgesia has a major impact on improving post-surgical outcome both in terms of analgesia provided and in the reduction in postoperative complications. In addition, neuropathic pains and other chronic pain might be managed more effectively if a prolonged local anesthetic agent were available.

Most attempts to prolong LA action have so far only doubled or tripled plain drug effect time, using adjuncts to LA agents of readily available agents such as opioids and clonidine (which delays local anesthetic clearance from their site of action) [4]. A major leap in duration of action of LA agents occurs when LA agents are encapsulated, for example with liposomes or polymers. Massive doses of LA can thus be administered without toxicity owing to controlled release [5,6].

Encapsulation of LA agents delays their release, therefore prolonging the duration of action following injection beyond the period expected following injection of the plain solution. Following injection of a depot of the formulation, much of the LA agent is bound or carried inside another agent, therefore not immediately available. Several properties such as hydrophobicity and internal membrane pH affect encapsulated drug release rates [7-9]. The duration of the analgesia depends on the release rate of the LA agent from the carrier agent. Encapsulating agents include lipospheres [10,11], liposomes [12,13], cyclodextrins [14] and microparticles [15,16]. Some synergistic agents encapsulated with the main effective agent have been formulated, such as dexamethasone and clonidine, and these have increased the anesthesia time for several days [17]. This review considers the clinically available local anesthetic agents, and their integration into formulations for prolongation of their action following site-directed injection.

2. Conventional local anesthetic agents

Local anesthetic agents are applied directly, and their efficacy results from action on the nerve where the inward Na^+ current is blocked at the sodium ionophore during depolarization. For the duration of this blockage, analgesia ensues. Therefore, if the presence of the LA agent can be maintained for a prolonged period, the duration of LA action can be extended.

3. Chemistry of local anesthetic agents

Commercially available local anesthetic agents have a common chemical structure, consisting of a lipophilic aromatic ring, a link and a hydrophilic amine group, of which most are tertiary amines. They can be classified into two groups based on the nature of the link: amides [-NH-CO-] and esters [-O-CO-]. The amide group is the most commonly used clinically and includes lignocaine, prilocaine, levo-bupivacaine, bupivacaine, mepivacaine and ropivacaine. Speed of onset and duration of analgesia differ depending on location of injection of the LA agents (**Table 1**). Analgesia onset for neuraxial blockade is slower, and duration of action shorter. The ester group are weak bases, solubilized for injection as strong conjugate acidic hydrochloride salts (pH 3 – 6), and include cocaine, procaine, chloroprocaine and amethocaine [18].

The physicochemical properties of local anesthetics affect the potency, speed of onset, intensity and duration of local anesthetic action (**Table 2**). Chemical characteristics affect clinical characteristics directly. Drugs containing an ester group metabolize faster and are less toxic, for example, procaine and chloroprocaine [19]. The anesthetic potency of amide LA agents increases in the order of mepivacaine, ropivacaine and bupivacaine with lengthening the alkyl chains. Lignocaine and prilocaine are less potent than bupivacaine. It is generally considered that a high percentage of protein binding as well as high lipid solubility gives bupivacaine a longer effect in comparison with other aminoamide local anesthetics. Another view is that the only connection between protein binding and local anesthetics' duration of action lies in the fact that local anesthetics with greater lipid solubility are protein bound to a greater extent when they are rich the bloodstream, as no direct relationship exists between high drug binding to nonspecific proteins (glycoprotein and albumin) and local anesthetics binding to specific sites on sodium channels [20].

Absorption and distribution of amide-type local anesthetics depend on the degree of binding to plasma proteins, plasma pH and the physical properties of the anesthetic [21]. Relative potency is determined by lipophility, and pK_a values directly correlate with the onset of the local anesthetic effect.

Table 1. Major peripheral and neuraxial nerve block: onset time and duration of action for bupivacaine.

	Bupivacaine dose	Onset time (min)	Duration of action (min)
Sciatic nerve block [92]	30 ml, 0.5%	37 ± 27	880 ± 312
Femoral nerve block [93]	30 ml, 0.5%	22 ± 8	666 ± 210
Interscalene nerve block [94]	20 ml, 0.5%	28 ± 15	654 ± 240
Axillary brachial plexus block [95]	0.4 ml/kg, 0.25%	8 ± 8	896 ± 284
Epidural administration [96]	0.5% 15 – 30 cm^3	25	213

Table 2. Characteristics of commercially available amide local anesthetic agents [18,97,98].

Local anesthetic	Lignocaine	Mepivacaine	Bupivacaine	Ropivacaine	Levobupivacaine
Molecular mass	234	246	288	274	288
pK_a	7.8	7.7	8.1	8.1	8.1
Vdss (l)	91	84	72	59	54
Partition coefficient (octanol/buffer) pH = 7.4	43.0	21.0	346.0	115.0	346.0
Protein binding	64%	78%	95%	94%	95%
$t_{½}$ (min)	96	114	210	111	157
Clearance (l/min)	0.95	0.75	0.58	0.72	0.32
Commonly used application sites	Topical, infiltration, epidural, spinal, PNB	Infiltration, PNB, epidural	Infiltration, PNB, epidural, spinal	Infiltration, PNB, epidural	Infiltration, PNB, epidural, spinal, topical
Maximal doses (mg/kg)	3	6.6	2	3	2.5

PNB: Peripheral nerve block; Vdss: Volume of distribution at steady-state.

Both the size of the molecule and the value of the octanol/water partition coefficient affect the length of the effect (bupivacaine has the highest partition coefficient (~ 346) whereas the ropivacaine value is ~ 115.3) [22]. However, a better correlation to LA potency is found with the log P value of the uncharged LA species. Both partition coefficient and log P describe the ratio of concentrations of uncharged (neutral) compound between two solutions. log P is the logarithm of the ratio of the concentrations of the unionized solute in the solvents; however, the octanol/water partition coefficient may underestimate membrane partitioning for the charged, protonated species [23].

Pharmacological effects related to structure of the agents are accounted for both by selective affinity of drugs to membrane receptors and enzymes and also hydrophobic interaction of drugs with membrane lipids.

The most widely used available amide LA agent is bupivacaine, a racemic mixture of two stereoisomers. However, the risk of cardiovascular and central nervous system toxicity can be reduced by the use of ropivacaine, a pure (-)-(*S*)-enantiomer homologue of bupivacaine and levobupivacaine, the pure (-)-(*S*)-enantiomer of bupivacaine [24]. The potency of bupivacaine is proportional to the toxic effects on the cardiovascular system and central nervous system in that the (-)-(*S*)-bupivacaine is less toxic than racemic bupivacaine, which in turn is less toxic than (+)-(*R*)-bupivacaine.

Cardiovascular system toxicity is related to direct action of the LA agents on the heart and peripheral blood vessels as well as indirect blockade of sympathetic or parasympathetic activity. Both the action potential duration and the effective refractory period are decreased by local anesthetics [25]. Local anesthetics have a dose-dependent negative inotropic effect on cardiac muscle [26] and they may depress myocardial contractility by affecting calcium influx and triggered release [27]. Depression of conduction and contractility appeared at lower doses and plasma concentration with bupivacaine than ropivacaine [28] or levobupivacaine [29].

3.1 Mechanism of action

The mechanism of action may be more complicated than blockage of the inward Na^+ current, as calcium, potassium and G-protein-regulated channels may also be blocked. Lignocaine binds and dissociates rapidly from the channel, whereas bupivacaine binds rapidly but dissociates more slowly. This has little effect on neuronal block, but assumes greater importance when referring to effects on cardiac toxicity [18]. (*R*)- and (*S*)-enantiomers of local anesthetics have been demonstrated to have a different affinity for the different ion channels of sodium [30], potassium and calcium, which accounts for the significant reduction of central nervous system and cardiac toxicity of the (*S*)-enantiomer as compared with the (*R*)-enantiomer [31].

The speed of onset of block is related to the concentration of molecules of local anesthetic in the free base or non-ionized state. This depends on the initial dose and the dissociation constant (pK_a) of the local anesthetic, and the pH of the tissues [18]. In humans the duration of anesthesia is influenced markedly by the peripheral vascular effect of the local anesthetic drug. Many local anesthetics have a biphasic effect on vascular smooth muscle: at low concentration, these agents tend to cause vasoconstriction, whereas at clinically used concentrations, they cause vasodilatation. However, differences exist in the degree of vasodilator activity produced by various drugs. In spinal cord, the pial vessels are dilated by bupivacaine, but they are constricted by ropivacaine, a finding suggesting a stereoselective effect on vascular tone independent of nerve block *per se* [32]. Onset of action of LA agents differs, for example ropivacaine was faster than levobupivacaine for sciatic nerve surgical block [33].

4. Encapsulating carrier agents for prolongation of LA effect

Encapsulation of local anesthetic agents allows large doses to be released slowly and provide analgesia over a prolonged period. Without encapsulation, delivery of such large doses of local anesthetic agents would be lethal. Following injection, a drug delivery system should have minimal tissue reaction, a reliable drug release profile, and well-defined degradation rate for biodegradable carrier until all non-toxic products are excreted. The local anesthetic is either encapsulated into a carrier envelope or matrix serving where LA drug is evenly dispersed in the matrix.

Biodegradable implants are advantageous because they do not require surgical removal following the termination of therapy, however the dose cannot be adjusted following delivery. Some biodegradable polymers degrade over a period of weeks and others may remain for years. Release of the LA agent from the polymer matrix occurs in steps. First the drug releases from the matrix, then the drug diffuses into the aqueous environment just outside the matrix. Drug release can be modified by remodeling the polymer system, and studying the drug fate through release. Altering the polymer-to-polyester ratio resulted in longer degradation periods compared with poly (ester-anhydride). This prolonged the duration of release of the incorporated drug [34]. Modification of drug release was also achieved with PLGA microspheres. Burst effect was modified by drug/PLGA ratio and particle size was controlled by the stirring rate and polymer concentration [35].

5. Microparticles and nanoparticles

5.1 Liposomes

Lipid vesicles are sealed sacs containing a lipid bilayer, usually phospholipids. There are three types of liposome: multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). Lipid-soluble drugs can be carried in the bilayer itself, and liposomes may contain one or more bilayer. Alternatively, aqueous drugs can be carried inside the aqueous compartment contained inside the bilayer [36].

5.1.1 *Liposome characteristics*

The pharmacokinetics of liposomes can be manipulated by the administration route combined with lipid size. Liposomes vary in size and lipid composition. A smaller vesicle can be produced by fractionation and this has improved the pharmaceutical techniques. Drug load efficiency is important because the loaded drug must be incorporated in high does and it must be possible to retrieve the loaded drug easily.

5.1.2 *Duration of effect*

Many routes of drug administration have been described, including central neuraxial administration [37] and peripheral nerve administration [10]. Release of bupivacaine from the liposome is prolonged (**Figure 1**), resulting in prolonged release. Injection of liposomal bupivacaine compared with plain 0.25% bupivacaine allowed injection of a 100% higher dose of bupivacaine in rabbits without toxicity [38]. Liposomes have also been injected epidurally in rabbits, with increasing dose of liposomal bupivacaine causing faster onset motor blockade [39]. Interestingly, decrease of the internal liposomal pH prolonged the duration of sensory analgesia by slowing the bupivacaine release [8,38].

5.1.3 *Limitations of liposomes*

Liposomes suffer lack of reliability and reproducibility during manufacture owing to oxidation and hydrolysis, which results in leaking of the encapsulated drug [40]. In addition the low weight–volume ratio decreases the payload that can be attached.

Liposome metabolite compounds have been found to be neurotoxic [41]. Also, uncontrolled leakage of drug may occur following breakdown of the liposomes. The mechanism for this may be lethicin fatty acid oxidation. Malinovsky *et al.* did not demonstrate such toxicity, possibly owing to added stability of the liposome from the addition of cholesterol and α-tocopherol in the phospholipid bilayers [42]. Another limitation is that at present the cost of liposome raw materials is very high for liposome production [1].

5.2 Lipospheres

The lipospheres are stable structures consisting of a solid hydrophobic fat core such as triglycerides or fatty acid derivatives, stabilized by a monolayer of phospholipids, which are easy to prepare. The size of the early reported lipospheres limited these formulations to implantation (**Table 3**), therefore limiting clinical application. However, release of bupivacaine was sustained and clinical effect very prolonged [10]. More recently, a nanoliposphere has been developed that does not gellify and is suitable for injection [43]. There are not yet any reports of *in vivo* use of this injectable liposphere bupivacaine formulation.

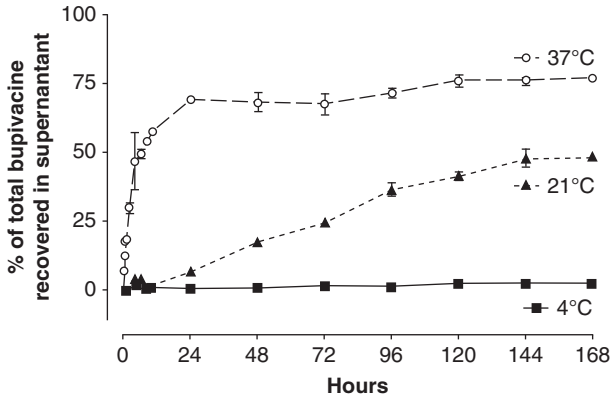

Figure 1. Release of bupivacaine from liposomes *in vitro* at different temperatures expressed as per cent of total encapsulated bupivacaine versus time.
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5.3 Lipid-protein-sugar particles

Lipid-protein-sugar particles (LPSPs) have also been used to encapsulate local anesthetics. The natural occurrence of LPSPs at the injection target site is potentially advantageous over the polymers [44], which may take a long time to degrade because the carrier is removed more speedily. Their action, like that of microspheres, was prolonged by the addition of a glucocortocoid-dexamethasone [45]. A prolonged sensory blockade was also achieved using 3 or 60% dipalmitoylphosphatidylcholine (DPPC) spray-dried LPSPs containing 10% bupivacaine, plus 0.05 or 0.1% dexamethasone (**Figure 2**). Their optimum form gave sensory block for rat sciatic nerve for a maximum of 610 min [46]. Thus, the addition of dexamethasone doubled the duration of the sensory and the motor block. No residue of particulate formulation was seen after 2 weeks. However, the overall duration of block, even following addition of prolonging agents, was shorter than other carriers such as polymers that remain longer at the injection site [46].

6. Poly(lactic-*co*-glycolic acid) microspheres and nanospheres

The most commonly used microscale and nanoscale vehicles for drug encapsulation and delivery are microspheres and nanospheres. Microspheres or nanospheres are usually prepared from biodegradable synthetic hydrophobic materials such as homo or copolymers of polylactic and polyglycolic acids [47].

Microparticles are perfect for drug delivery as they remain at the depot site for long periods, allowing slow prolonged release of the encapsulated drug. This lends itself to prolonged local anesthetic drug release. The ultimate destination of the loaded microparticles was investigated by Kohane *et al.* [44], who demonstrated that following local sciatic nerve injection, polymeric particles of 60 µm remained at the injection site for 8 weeks. However, smaller particles dispersed after 2 weeks.

6.1 Particle size

Particle size can influence drug load and drug release kinetics. The drug load into the particle increases with particle size. The large particles with a high loading (close to 30%) showed under *in vitro* conditions a slow release over 24 – 30 h, the medium-sized carriers (loading similar to 13%) released the drug over ~ 15 h, whereas the small particles with small loading (similar to 7%) showed a rapid release over a couple of hours [48]. Larger particles should achieve longer block duration owing to the increased drug loading and longer degradation time. However, the advantage of smaller particles is improved drug binding, which slows the release of the incorporated drug. Conversely, smaller particles may demonstrate more 'burst release' with a rapid initial release of the drug [49]. However, particle size is not the only factor in drug release. For example, when PLGA microparticles were loaded with lignocaine the particle size did not affect release of the drug, rather the thickness of thin, free films [50]. Addition of dexamethasone has a greater effect on block duration [32] than particle size [51].

6.2 Limitations of microspheres

When low-molecular-mass poly(lactic acid-*co*-glycolic acid) was used, the microspheres fused together, however this was solved by increasing the molecular mass, which increased stability and eliminated fusion [15]. A further increase in the duration of analgesic efficacy was achieved by combining microsphere and hydrogel approaches. When poly (DL-lactic acid) microspheres were embedded into poloxamer 407-based hydrogel, this microsphere–gel system containing lignocaine was easy to inject (**Figure 3**). In addition it is degradable; however, analgesia was prolonged for only 8.5 h [45].

7. Hydrogels

7.1 Hyaluronic acid-based hydrogels

Hyaluronic acid (HA) is a non-immunogenic naturally occurring mucopolysaccharide. It has been investigated as a viscous carrier solution, and has been shown to prolong LA action. Jia *et al.* [52] investigated the use of both regular HA and crosslinked HA to compare their prolongation action of local anesthetic agent. The theory behind this was that HA can be used to turn a substance into a liquid in a syringe, which then reconstitutes into gel. They used a sciatic nerve block of HA-bupivacaine, HA-crosslinked-bupivacaine and plain bupivacaine. Plain bupivacaine provided only a 1.5 h block [52]. However, addition of crosslinked HA doubled the length of action of bupivacaine compared with the non-crosslinked HA. Clearly this prolonged effect is not anywhere near the prolongation seen with other delivery systems; however, doubling of action of LA drug may be desirable for dental situations, and there are no sequelae of microparticle injection. Also, this HA formulation is appealing as it is in an easily injectable liquid form.

Table 3. Summary of duration of sciatic nerve blockade following injection of encapsulated local anesthetic agent.

First author, year	Carrier encapsulating agent	LA agent encapsulated	Application	Maximum reported duration of sensory block	Maximum reported duration of motor block	Control
Masters, 1993 [10]	Bioerodible polymer matrices (copolymer 1,3-bis(*p*-carboxyphenoxy) propane-sebacic acid anhydride (1:4)	Bupivacaine 20%	Implant	4 – 5 days	3 – 4 days	Polymer implants
Curley, 1996 [15]	PLGA	Bupivacaine 150 mg/(kg animal)	Injection	Bupivacaine plus 0.5% dexamethasone: 170 h	Motor score recovered: 0.5% dexamethasone: 190 h	Placebo microspheres or microspheres with 0.05% dexamethasone
Castillo, 1996 [63]	PLGA	Bupivacaine (75%) 150 mg/(kg animal) with 0.05% dexamethasone, or bupivacaine alone	Injection	Bupivacaine and dexamethasone: 134 h	Bupivacaine and dexamethasone: 39 h	PLGA + bupivacaine 75% without dexamethasone, PLGA alone
Masters, 1998 [99]	Lipospheres	Bupivacaine 1.6, 3.6, 5.6%	Implant	1 – 3 days	3 days	No drug lipospheres
Kohane, 2000 [16]	LPSPs, PLGA	Bupivacaine 10% in LPSPs and 50% in PLGA microspheres	Injection	LPSPs: 7.8 h PLGA: 11.8 h	LPSPs: 8.5 h PLGA microspheres: 17.7 h	Microspheres or LPSPs without bupivacaine
Kohane, 2003 [62]	PLGA	Bupivacaine 50%, TTX 0.1%, bupivacaine 50% and TTX 0.05% with/without dexamethasone	Injection	Bupivacaine and dexamethasone and TTX: 221.7 h	Bupivacaine and dexamethasone and TTX: 197 h	None
Colombo, 2004 [46]	LPSPs 3% w/w and 60% w/w	Bupivacaine 10%	Injection	5 h (both formulations)	60% w/w DPPC: 8.3 h	3 and 60% (w/w) DPPC particles without bupivacaine
de Araujo, 2004 [100]	LUVs composed of egg phosphatidylcholine, cholesterol and α-tocopherol (4:3:0.07, mol%)	Bupivacaine 0.125, 0.25, 0.5% and mepivacaine 0.5, 1, 2%	Injection	Mepivacaine: 5 h Bupivacaine: 3 h	Ropivacaine and bupivacaine < 1 h	Plain bupivacaine, mepivacaine
Jia, 2004 [52]	Crosslinked hyaluronic acid 2% w/v	0.1 – 0.5% w/v bupivacaine	Injection	4 – 5 h	Within 5% of sensory block	
Chen, 2004 [72]	Poloxamer 407 gel	Lignocaine 23 mg in 4 different formulations	Injection	Microspheres in PO407 gel: 9.0 h	7.2 h microspheres in PO407 gel	Microspheres in saline, lidocaine in saline

DPPC: Dipalmitoylphosphatidylcholine; LPSPs: Lipid-protein-sugar particles; LUVs: Large unilamellar vesicles; PLGA: Polylactic-*co*-glycolic acid polymer microspheres.

Table 3. Summary of duration of sciatic nerve blockade following injection of encapsulated local anesthetic agent (continued).

First author, year	Carrier encapsulating agent	LA agent encapsulated	Application	Maximum reported duration of sensory block	Maximum reported duration of motor block	Control
Colombo, 2005 [51]	DPPC spray-dried LPSPs 3 and 60%	Bupivacaine 10% and 0, 0.05, or 0.1% (w/w) with and without dexamethasone	Injection	Bupivacaine with 3% DPPC LPSPs and dexamethasone 0.05%: 10.2 h	Same duration as sensory block	3 and 60% (w/w) DPPC particles without dexamethasone
Söderberg, 2006 [71]	Medium-chain triglyceride	Eutectic mixture of lidocaine and prilocaine 2.0, 5.0, 10, 20, 40, 60, 80, or 100%	Injection	80% formulation: 72 h	All animals still showed nerve block for at least 4 days	2% aqueous solution
Dyhre, 2006 [68]	Medium-chain triglyceride	Bupivacaine 4.2, 7.0% and lidocaine 20, 4, 8, 16, 32, or 64%	Injection/ implantation (64%)	64% implant: 17.4 h 64% injected: 19 h	64% implant: 5.9 h 64% injected: 5.25 h	None
Shikanov, 2007 [6]	Poly(fatty ester-anhydride) paste	Bupivacaine 10% (333 mg/(kg animal))	Injection	30 h	18 h	Normal saline injection
Karashima, 2007 [56]	G2-β-CD (cyclodextrin)	Levobupivacaine 0.5 or 1%	Injection	4 h	252 min	Plain 0.5, 1% 1% levobupivacaine
Padera, 2008 [75]	PLGA and LPSPs	Bupivacaine 10 – 50%	Injection	50% PGLA microspheres: 10.2 h	50% PGLA microspheres: 11.9 h	Bupivacaine 0.5% w/v
de Araujo, 2008 [101]	LUVs composed of egg phosphatidylcholine, cholesterol and α-tocopherol (4:3:0.07, mol%)	Ropivacaine 0.125, 0.25, 0.5%	Injection	0.5% formulation: 5 h	No increase over plain ropivacaine	Plain ropivacaine 0.125, 0.25, 0.5%
Sokolsky-Papkov, 2009 [7]	Polymer p(DLLA:CO) 3:7	Bupivacaine 10% (333 mg/(kg animal))	Injection	48 h	24 h	Normal saline injection
Suzuki, 2009 [102]	G2-β-CD (cyclodextrin)	Lignocaine 10 mg/kg	Injection	1.8 h	Not reported	Plain lignocaine 1% 0.3 mL ; 55 min Plain cyclodextrin 0.25-0.3 mL:
Epstein-Barash, 2009 [60]	Liposome	Saxitoxin, bupivacaine and/or dexamethasone	Injection	Solid saxitonin liposomes with dexamethasone: 180 h		Empty liposomes, dexamethasone liposomes
Sokolsky-Papkov, 2010 [49]	Polymer p(DLLA:CO) 3:7	Bupivacaine 15% (500 mg/(kg animal))	Injection	96 h	60 h	Normal saline in contralateral leg

DPPC: Dipalmitoylphosphatidylcholine; LPSPs: Lipid-protein-sugar particles; LUVs: Large unilamellar vesicles; PLGA: Polylactic-*co*-glycolic acid polymer microspheres.

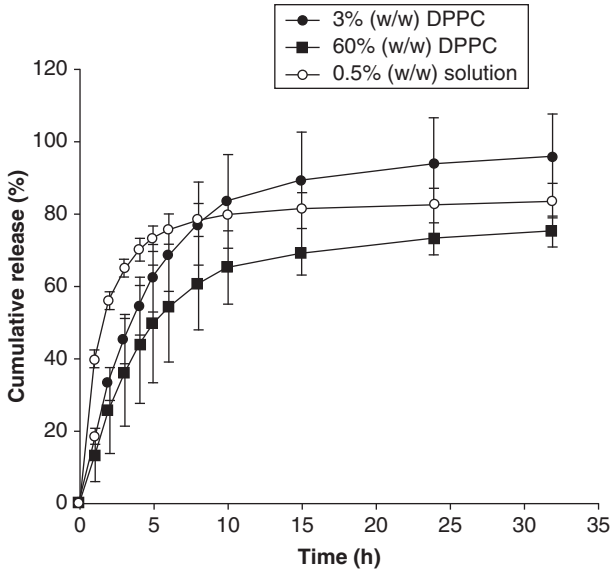

Figure 2. Cumulative release of bupivacaine from dialysis tubes, all of which contained 5 mg total initially. Data points are means ± standard deviations. n = 10 for 3% (w/w) DPPC particles, 12 for 60% (w/w) DPPC particles and 7 for 0.5% (w/w) bupivacaine solution.
Reproduced with permission from [46].

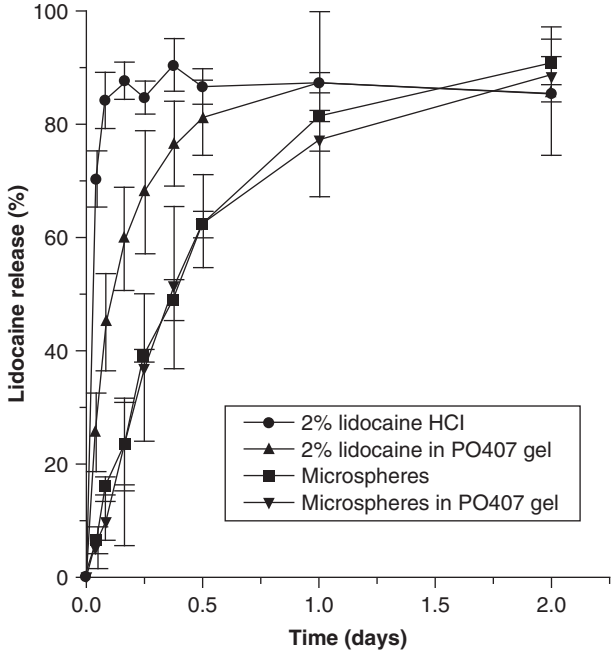

Figure 3. *In vitro* release (n = 3) of 2% lidocaine-HCl in 0.9% saline solution, 2% lidocaine-HCl in 25% (w/v) PO407 gel, 31% lidocaine microspheres in 0.9% saline solution and 31% lidocaine microspheres in 25% PO407 gel.
Reproduced with permission from [45].

7.2 Hydrogels prepared from synthetic polymers

Keskin *et al.* [53] prepared drug depot from a combination of 2-hydroxyethyl methacrylate (PHEMA) and polyester (polyhydroxylbutyrates). In theory this system would partially degrade leaving no bio-incompatible residues. The hydrogel prepared from hydrophilic polymer (PHEMA) swells in water and helps release the LA agent. A coating of hydrophobic polymer (PHBV) would restrict the swelling of the interpenetrating network (IPN), thus decreasing the release rate of the encapsulated LA agent. The maximal measured length of analgesia was 1 – 2 days despite release of drug that continued for longer periods, although with insufficient amounts for analgesia.

Bernardo *et al.* [54] prepared hydrogels from poly(acrylamide-*co*-monomethyl itaconate) (A/MMI) crosslinked with N,N′-methylenbisacrylamide (NBA) for the controlled release of bupivacaine. The swelling behavior of these copolymers and the release rate of bupivacaine depended on the pH of the medium. However, this delivery system was not evaluated *in vivo*.

7.3 Injectable liquid polymers

Extensive reviews of polymer drug delivery describe three types of polymer for encapsulation: non-degradable polymers; synthetic and natural biodegradables that degrade to non-toxic products that are completely eliminated from the body; and drug-conjugated polymers where a drug is attached to water-soluble polymer by a cleavable bond. The drug-polymer conjugate can be directly targeted to the site of specific action.

Experiments performed using poly(ester-anhydride) from sebacic and ricinoleic acid loaded with 10% bupivacaine demonstrated *in vivo* action shorter than the *in vitro* results predicted. A new polyester-poly(lactic acid-*co*-castor oil) was synthesized that in theory would be a more hydrophobic polymer. The hydrophobic carrier could increase the degradation time of the polymer and prolong the drug release period for bupivacaine. In practice, the sensory blockade was increased from 30 to 48 h again using 10% bupivacaine [6,7]. The burst release effect was noted to vary *in vitro* according to the drug load. Lactic acid-*co*-castor oil copolymer encapsulating 20% bupivacaine was too viscous for injection despite the least burst release effect (**Figure 4**). However, 15% bupivacaine was not too viscous for injection and also showed lower burst release effect than 10% bupivacaine. Burst release *in vitro* may translate *in vivo* to excellent initial analgesia followed by sub-analgesic doses despite continued bupivacaine release [6,45]. Use of the 15% bupivacaine lactic acid-*co*-castor oil copolymer prolonged the *in vivo* effect to 96 h sensory block [49].

Injectable polymers are simple and reliable to prepare and the local anesthetic is combined by simple mixing. The main disadvantage is the prolonged time these polymer carriers dwell in the injection site, far beyond the time of the effect of the local anesthetic agent [44]. The safety and tissue compatibility of biodegradable pasty polymers have been

tested and found safe with no systemic tissue damage or polymer-related lesions [55].

8. Dextrins

Many dextrins have been used successfully for delivering prolonged LA agents. Cyclodextrins (CD) are able to form inclusion complexes easily and are popular carriers for drugs. The main types of cyclodextrin are α-, β- and γ-cyclodextrins, comprising six to eight sugar units in the ring. β-Cyclodextrin modified with a maltosyl group (maltosyl-β-cyclodextrin) was evaluated as a levobupivacaine carrier and has prolonged its effect when injected both intrathecally and directly onto the sciatic nerve [56]. Ropivacaine formed as an inclusion complex with hydroxypropyl-β-cyclodextrin increased sensory analgesia, without a prolonged motor blockade. This alkylated derivative of β-cyclodextrin is less toxic. Dollo *et al.* [14] evaluated complexes of bupivacaine with sulfobutyl ether-7-β-cyclodextrins. The advantage of this CD derivative is lower toxicity and greater water solubility. Indeed, the therapeutic index was improved compared with plain bupivacaine. Interestingly, the anesthesia (as measured by motor block to the sciatic nerve) was slower to develop, presumably as a result of the slower release of bupivacaine from the complex.

9. Natural local anesthetic agents

Tetratodoxin (TTX) is a naturally occurring potent sodium channel blocker. Not only does it provide excellent local anesthesia when directly applied to a nerve, but it can also be administered in combination with commercial amide and ester LA agents. However, although it is considered not to cause neurotoxicity [57], it does cause significant systemic toxicity. Addition of epinephrine quadruples the therapeutic index [58].

Padera *et al.* [59] demonstrated that TTX in combination with bupivacaine significantly extended the duration of both motor and sensory blockade. The most prolonged block was seen with the combination of TTX and epinephrine (16 h), and minimal neurotoxicity was noted.

Saxitonin, another naturally occurring sodium channel blocker, also causes systemic toxicity, however with liposome encapsulation, nerve analgesia was safely provided for 7.5 days with only mild neurotoxicity [60].

10. Adrenergic agonists

Dexmetetomidine is an alpha agonist that provides dose-dependent potentiation of LA agent applied spinally. High-dose dexmedetomidine significantly enhanced the duration of sensory and motor blockade [61]. Adrenergic agents have been used to provide vasoconstriction, thereby reducing local anesthetic agent clearance from the injection site, which prolongs duration of action. However, the mechanism of dexmetetomidine may be synergistic LA action, or enhancement of hyperpolarization as seen with clonidine.

11. Dexamethasone

Addition of dexamethasone demonstrated prolongation of local anesthetic action for several formulations (**Table 3**). For example, addition of dexamethasone to tetradotoxin with polymer microspheres prolonged analgesia for 9 days [62]. Interestingly, the mechanism postulated was not due to altered kinetics of bupivacaine release, rather it was due to an anti-inflammatory effect causing a rise in tissue pH. This in turn gives more drug in the unionized state, which allows more drug to penetrate [63]. The effects of this formulation outlasted the anti-inflammatory effects of dexamethasone. Another theory of action was vasoconstriction of dexamethasone. Dexamethasone added to microcapsules containing bupivacaine showed longer duration of anesthesia affect compared with microcapsules without dexamethasone [64]. This was confirmed in human intercostal blockade studies [65].

Other drugs have been added to local anesthetic agents to prolong the block successfully. Calcium chloride added to 1% lignocaine or 0.1% bupivacaine prolonged the length of the block in rat sciatic nerve [66]. Amitriptyline, nortriptyline and doxepin prolonged the nerve block provided with TTX [67].

12. Limitations of available agents

12.1 Differential block

Motor block is usually shorter than sensory block [56,68]. For the prolonged LA formulations a motor block is not required for analgesia and should be as short as possible. A motor block may require prolonged unnecessary hospitalization and limit mobility for the duration of the provision of the adequate analgesia. Few formulations have provided sensory analgesia beyond 24 h. Grant *et al.* [69] achieved a maximum 26 h sensory blockade with 2% liposomal bupivacaine versus 1.58 h for plain bupivacaine. In humans, a modified 2% bupivacaine liposome formulation provided analgesia up to 48 h following local infiltration, thus the motor effects were not tested [70]. Söderberg *et al.* used a liquid injectable formulation of eutectic mixture of lignocaine and prilocaine base in medium-chain triglyceride to achieve a sensory block of 89 h and motor block of 336 h [71]. The authors attributed the long motor block to faster recovery of the less sensitive myelinated sensory C fibers, which may regain function faster than myelinated A fibers.

Chen *et al.* [72] using gels also found that the motor block was longer than the sensory block. The authors discussed length of motor block and proposed that after most of the lignocaine diffused away full sensation recovered but the gel still contained a level of lignocaine to cause a motor block. The more likely explanation is myotoxicity, which is possibly reversible [73]. Interestingly, epidural injection of liposomal

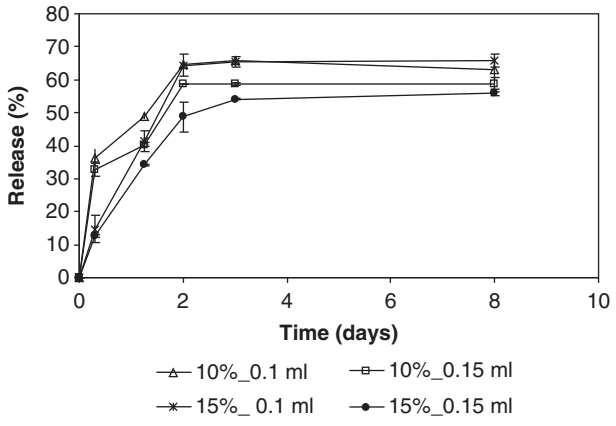

Figure 4. *In vitro* release of bupivacaine free base from poly (lactic acid-*co*-castor oil) 3:7 (10 and 15% w/w). Bupivacaine release was conducted in a 0.1 M phosphate buffer (pH 7.4) at 37°C. Bupivacaine free base content in the releasing medium was determined by HPLC.
Reproduced with permission from [7].

bupivacaine showed a twofold increase in duration of sensory block, with no motor block seen at all.

12.2 Neurotoxicity and myotoxicity

Conventional LA agents are myotoxic [74] even if the delivery agents are not [52,75]. Clinical investigations in humans demonstrate myotoxicity when using off-the-shelf LA agents [76]. Bupivacaine causes myotoxicity after 7 days as a plain single shot solution, however there is regeneration at 2 – 4 weeks [77]. Following infusion by means of a catheter in a porcine model, normal saline control caused no myotoxicity, however bupivacaine caused severe necrobiotic tissue changes. Ropivacaine caused slight damage compared with severe damage of bupivacaine. However, it is not clear that the myotoxicity seen following injection is of clinical relevance [76]. LA agents cause marked but reversible lesions to skeletal muscle tissue. However, despite fiber regeneration, there were irreversible calcific lesions [78].

Encapsulated formulations cause severe myotoxicity, therefore this injury is probably a product of a critical concentration applied over time. LA-induced myotoxicity resolved over time, often within 2 weeks [44]. Burst release of the encapsulated formulation may cause toxicity [7]. In addition, there may be later peaks of release [62]. Encapsulated agents contain significantly higher doses of LA agent and release for much prolonged periods, thus the potential for myotoxicity is greater. Nanoparticles or microparticles may enhance toxicity either by release of the LA agent, which potentiates toxicity, or alternatively by the particles themselves causing inflammation, which may worsen toxicity [44].

Burst release may also contribute to myotoxicity. However, the magnitude of the peak level of bupivacaine release is not the sole determinant of myotoxicity [75]. Other formulations caused toxicity up to 1 month [79]. Padera *et al*. administered two types of bupivacaine formulation to rat sciatic nerve; polymer and LPSPs, and also blank particles with or without bupivacaine [75]. Nerve toxicity was measured up to 3 weeks with a range of doses. They concluded that both LPSP and polymer bupivacaine formulations but not blank particles cause myotoxicity; however, there was no follow-up beyond 3 weeks, and recovery may have occurred. Similar neurotoxicity was noted after 1 week by Dyhre *et al*. using an LA lipid-depot formulation, medium-chain triglyceride, but again longer follow-up was not reported [68]. Toxicity of bupivacaine in lipospheres was evaluated [80]. Liposome consists of inert components, therefore it is expected that they are biocompatible *in vivo*. Microscopic observations demonstrated some irritation and inflammation at day 3 following injection, which reduced after 14 days following blank lipospheres, and bupivacaine 1 and 5% in lipospheres.

Myotoxicity extends with length of action of the LA agent, and although it appears inevitable that myotoxicity occurs, it appears to be reversible [6,7]. Inflammatory responses at the nerve may outlast the block length [74].

12.3 Systemic toxicity

Systemic toxicity of local anesthetics may occur following inadvertent intravascular or intrathecal injection, or after administration of excessive doses. Clinically, toxicity primarily involves the central nervous system (CNS) and then the cardiovascular system. More potent agents (e.g., bupivacaine, levobupivacaine, ropivacaine) produce cardiotoxic effects at lower blood concentrations and doses than less potent LA agents (e.g., lignocaine) [81]. The (+)-(*R*)-enantiomers bind cardiac Na^+ channels with greater affinity than the (-)-(*S*)-enantiomers. Stereo-specificity of LA solution may also change the toxicity profile of an LA. Both levobupivacaine and ropivacaine demonstrate reduced toxic potential [82], allowing higher plasma concentrations before signs of systemic toxicity occur. Most studies of encapsulation of bupivacaine in massive doses have focused on bupivacaine, and have not found systemic toxicity, despite large doses of LA agent being injected. However, these data suggest that for a clinically useful drug, a stereo-specific enantiomer could be safer without compromising efficacy.

12.4 Tachyphylaxis

Tachyphylaxis to local anesthetics has been reported for more than two decades, although the molecular mechanisms remain unknown. Different pharmacokinetic factors have been proposed to explain this decrease in duration or intensity of analgesia despite repeated constant dosages. Factors may include local edema, a decrease of perineural pH, increased epidural protein concentration, changes in local anesthetic distribution in the epidural space, limiting the diffusion of the local anesthetics from the epidural space to their binding sites at the sodium channel, increased epidural blood flow, or increased local metabolism favoring clearance of local anesthetics from the epidural space [83]. However, tachyphylaxis to local

anesthetics does not result from reduced drug effectiveness at the nerve itself [84]. The recent observation that *N*-methyl-D-aspartic acid (NMDA) antagonists and nitric oxide (NO) synthase inhibitors prevent the development of tachyphylaxis suggests involvement of the NO pathway in the development of tachyphylaxis, and these may be useful in future work to facilitate the effect of slow release local anesthetic agents [85,86].

12.5 Viscosity

Toongsuwan *et al*. [87] defined injectability as the smallest needle gauge that a liposphere sample can pass through. They found that their bupivacaine liposphere formulation could all be injected through an 18G needle. The authors found that although *in vitro* release of 20% bupivacaine in castor oil showed the optimum release profile, the formulation could not be injected in through a small (22G) gauge needle as required for accurate placement by the sciatic nerve using a nerve stimulator. Others have overcome the viscosity of the encapsulated formulation, for example, by reducing viscosity through the combination with crosslinked hyaluronic acid [52].

12.6 pH

Bupivacaine rapidly accumulates within liposomes with a high pH gradient. The high proton gradient demonstrates more prolonged release compared with either free bupivacaine or bupivacaine without proton gradient, which translates to a prolonged block [8]. Importantly, acid base status alters the toxicity of LA agents; increasing acidosis lowers the convulsive threshold, and cardiotoxicity is also exacerbated in acidosis [38].

13. Developments in clinical trials and market products

One of the obstacles to overcome is the progress from animal to clinical studies. Potentially the increase dose requirement in humans could result in toxicity. The dose required for human nerve analgesia was predicted to be 200-fold higher than that required for rat analgesia [56], but in practice was only 5 – 10-fold higher. Animal studies showed no or reversible neurotoxicity and no systemic toxicity despite injection of massive doses of local anesthetic agent. This suggests that these agents are safe owing to the slow release profile with sub-toxic doses, but few human studies have described this. Biodegradable microcapsules encapsulating bupivacaine were administered as subcutaneous infiltrations in 18 volunteers. The maximum injected dose of bupivacaine was 250 mg, but weight of the adult volunteers was not reported. Safety evaluations were performed up to 6 months after the injections. Analgesia was prolonged for up to 96 h after the injections, and no serious side effects were observed [88].

In another volunteer study, skin infiltration of bupivacaine microcapsules with dexamethasone significantly prolonged local analgesia of bupivacaine microcapsules up to 7 days in adults weighing 72 – 112 kg, receiving a maximum of 125 mg bupivacaine microcapsules [64]. Clinical studies have also been performed showing prolonged anesthesia with liposomal LA agents. Grant *et al*. [70] described safe and prolonged analgesia for 48 h following local anesthetic infiltration of 2% liposomal lignocaine. Boogaerts *et al*. [73] demonstrated a twofold increase in the duration of the analgesia following epidural administration of liposomal bupivacaine in patients following abdominal surgery.

14. Conclusion

Site-directed prolonged local anesthetic action is possible. However, the effect is more limited than would be expected according to the *in vitro* release figures, as bupivacaine is released from the formulation beyond the duration of action. Neurotoxicity and myotoxicity are a concern, although damage appears to be reversible. The few human studies have confirmed the potential safety and efficacy of prolonged LA formulations, therefore this field has established itself as having great clinical potential.

15. Expert opinion

The explosion of studies using various encapsulating agents confirms that this field has a promising future. Progress towards the ultimate goal of a reliable safe commercial clinical product is slow. Duration of analgesia reported in recent studies is similar to that seen 1993 using implants in rats [10], although advances have been made in toxicity, improving safety, and viscosity, allowing site-directed injection.

Twelve years ago, a tabulated review on long-acting local anesthetic agents summarized the available techniques [89]. Most described are still being developed and none has reached clinical use so far. Interestingly, at that time tetrodotoxin and saxitonin were classified as 'without any ongoing research' owing to the toxicity and poor penetration into the nerve, resulting in unreliable nerve blockade. In the same review, polymers and liposomes were reported as being tested in humans, although so far a paucity of such articles has reached publication.

The chief concerns for clinical development of the formulation are, correctly, nerve and muscle toxicity. At present, catheter techniques deliver these same local anesthetic agents for a prolonged period to the target nerve without resulting nerve damage.

Current studies are reporting their optimum, least myotoxic and neurotoxic anesthetic agent for prolonged exposure of the target nerve. Furthermore, the lower toxicity found with naturally occurring LA agents has prompted a renewal of research in this area.

Future studies should address several points. The first involves the design and reporting of studies. Reporting of findings is often confusing and non-standard. It is not always simple to elucidate from an in vivo experiment the precise

details of the duration of sensory and motor analgesia, and sometimes neither is reported [14]. In addition, the dose per kilogram administered may not be clearly described, and this is important when considering how this dose may be increased for larger animals or clinical trials. Reporting of the recovery of the nerve should be followed up to at least 3 months, which is the expected time of recovery in order to establish the safety. Studies using in vivo models (animals or human) do not report sample size calculations, which are necessary to avoid a statistical error in reporting block success.

Control agents are also non-standard, with some studies using blank carrier agent and others using plain LA agent, both of which may have a centralized toxic effect that may influence the results seen despite the lack of sensory blockade noted in cases when blank polymer is used as the control. So far the carrier agents have not been adequately followed through the body on their excretion pathway.

The required dose to be administered for a specific duration of effect should be established. At present, it appears from *in vitro* studies that release of LA agents continues long beyond the identified clinical effect seen *in vivo*, and although this may not provide a block it may continue to cause nerve damage at these sub-therapeutic concentrations. Establishing the correct dose and release characteristics of the LA agent should allow a sensory block to be as prolonged as possible, while achieving a short or non-existent motor block.

Translating in vitro findings in terms of the clinical effects of burst release or prolonged release is very important in order to avoid systemic and neuro/myotoxicity. Current work on modifying the authors' polymer has addressed modifying the internal polymer-LA agent bonds to slow the release [7,49]. The cost may be prohibitive, although it must be compared with the costs of inserting and maintaining a catheter and continuous infusion.

There is also a consideration regarding the possibility of home discharge. If the formulation is applied as a wound infiltration then a sensory block alone is expected and motor ability will not be affected. However, Sites *et al.* reported prolonged motor sensory block after apparent resolution [90].

Liposome formulations, liposphere systems and polymers are now being tested with the aim of providing anesthesia for days or even months [68]. Theoretically there is a risk of long-term damage, unnoticed despite potential benefits of the analgesia. This requires a system for guidelines, home assessments and detailed neurologic limb evaluation [91]. The notion that a patient may be discharged following surgery with safe, site-directed analgesia for days is promising and exciting. Laboratory studies are already being translated to clinical ones.

Non-conventional local anesthetics such as tetrodotoxin and saxitoxin show promising efficacy with potentially reduced toxicity, and if the systemic toxicity can be avoided by encapsulation, they may be superior for prolonged release to synthetic LA agents. Future work should be directed towards establishing the most advantageous properties of the encapsulating agents, optimum release of the LA agent, together with their safety. Ultimately these carriers may enable physicians to alleviate pain not just for hours but for days or even weeks with a simple injection.

Declaration of interest

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