# Site-specific analgesia with sustained release liposomes

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Prevention or relief of localized pain is a challenging problem. The conventional local anesthetic agents used clinically are characterized by limited duration of analgesia and may result in both systemic and local toxicity (1). Thus, an unmet need for longer acting and safer pain control therapeutics exists. In a search for compounds providing prolonged anesthesia without myo- and neurotoxic effects, a class of nonterpene alkaloids represented by saxitoxin (STX) have evolved as promising candidates (2). A highly potent blocker of nerve conduction, STX was identified early last century as the cause of paralytic shellfish poisoning (3). Further investigations of its mode of action led to the discovery of its primary target, the voltage-gated sodium channel (4). Since then STX, as a member of the "site 1 sodium channel blocker" family of compounds, has become an invaluable tool in biomedical research. Although the therapeutic potential of STX as a potent, long-acting blocker of nerve conduction was recognized as early as in 1975 (5), its introduction into clinical practice has been hampered by its innate systemic toxicity (6). In attempts to address the narrow therapeutic window of STX as a monotherapy, its biocompatibility and effects in combination with other drug compounds have been extensively investigated (2, 7, 8). The results of these studies suggested that the potency and duration of the anesthesia mediated by site 1 sodium channel blockers may be significantly improved by the synergistic effect of adjunct agents (2, 7), and that the efficacy and safety of such combinations can be further optimized by using injectable microparticulate sustained action preparations (9).

## Controlled Release STX Challenges

In this issue of PNAS Epstein-Barash et al. (10) describe the design and characterization of a novel controlled release system for site-specific delivery of STX either as a sole active ingredient or in combination with dexamethasone or bupivacaine. The development of a therapeutically adequate particulate carrier-based formulation enabling sustained release of STX is not trivial because of the polar nature of this molecule. STX is a trialkyl tetrahydropurine (11) possessing two charged guanidinium groups rendering it highly hydrophilic and practically insoluble in organic solvents (12). No generally applicable solution currently exists for the encapsulation of charged, small-molecule pharmaceuticals in biodegradable micro- or nanoparticles made of the Food and Drug Administration-approved aliphatic polyesters, such as polylactide or polylactide-*co*-glycolide (13). Thus, based on their previous experience with using the double-emulsion approach for the formulation of polymeric microparticles loaded with another guanidinium toxin, tetrodotoxin (9), the authors hypothesized that the efficiency of this method would be highly limited for STX. Practically, acceptable entrapment rates would be difficult to achieve for the dicationic STX in such particles without prior chemical modifications. Considering the task of creating a family of formulations suitable for the simultaneous incorporation of pharmaceuticals with extremely distinct chemical properties, liposomes capable of accommodating both water-soluble and lipophilic substances in their interior aqueous compartment and lipid membrane, respectively, and having a history of safe clinical use obviously present an advantage (14).

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## Proof of Concept

Epstein-Barash et al. (10) reported two key liposome formulations, termed (based on their phase-transition temperatures) "solid" and "fluid" liposomes designed with stearoyl and myristoyl phospholipids, respectively. Vesicle membrane fluidity has previously been shown to be an important determinant of the liposome interactions with cells and tissues (15, 16), as well as the kinetics of drug release (17, 18). In the study by Epstein-Barash et al. (10) the difference in the membrane composition of the two types of vesicles was shown to enable control over the release kinetics of the incorporated drug substances, STX and bupivacaine; the so-called solid formulation demonstrated the more sustained release. Furthermore, the authors showed that the distinct release properties translated into different nerve blockade durations achievable with the two types of liposomal formulations allowing for extending the therapeutic effect from several hours to several days. Although experimental studies of both liposomal and polymeric sustained release preparations for local and topical anesthesia have been reported (19, 20), STX has not been investigated in sustained release preparations by others. Importantly, the effective sustained analgesia provided by STX formulations was associated with minimal to no myo- or neurotoxicity in vitro or in vivo. It is of note that liposomes prepared with coincorporation of the adjunct agents were substantially different in their properties. Dexamethasone, while prolonging analgesia even more than STX alone, resulted in mortality presumably due to systemic effects. Coincorporation of bupivacaine with STX was observed to result in even greater durations of analgesia than seen with either agent alone, and was not associated with the toxicity observed with higher doses of bupivacaine used alone.

## Potential for Clinical Use

The liposome formulations described by Epstein-Barash et al. (10) represent a number of significant advances for sustained local anesthesia, and these include: (*i*) extended local anesthesia durations in vivo, (*ii*) STX sustained release formulations created for the first time by using liposomes as carriers, (*iii*) even greater durations of sustained anesthesia than noted with STX-loaded liposomes observed when STX was combined with bupivacaine, (*iv*) absence of neuro- or myotoxicity both in vitro and in vivo, (*v*) minimal inflammatory effects of the drug carrier system, and (*vi*) absence of systemic adverse effects in vivo observed with select formulations.

What are the challenges for this approach in terms of translational directions? STX, a shellfish-derived neurotoxin, is not approved by the Food and Drug Administration for human use. Nevertheless, there is a precedent for

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this type of agent to be used clinically as an injectable via the now extensive utilization of botulinum toxin for cosmetic-related procedures. What are the possible clinical indications for the use of a long-acting local anesthetic preparation? Intractable localized pain would be the general symptom to be treated, and in this setting localized nerve block could be the therapeutic goal. Intra-articular anesthetic infusions are also currently used for severe joint pain, and the local delivery system described by Epstein-Barash et al. (10) could be equally or more effective than this approach. Thus, considering the serious limitations of the conventional local anesthetic agents and the as yet unmet need for safer and more efficient therapies, the liposome-based sustained release formulations of STX reported by Epstein-Barash et al. (10) may represent a unique advance with promise for improved site-specific analgesia.

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