Prolonged nerve blockade delays the onset of neuropathic pain

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Prolonged nerve blockade delays the onset of neuropathic pain

Sahadev A. Shankarappa,a,b Jonathan H. Tsui,a,b Kristine N. Kim,b Gally Reznor,b Jenny C. Dohman,b Robert Langer,a,b,1 and Daniel S. Kohanea,b,1

*Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Division of Critical Care Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, MA 02115; and bThe David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

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Abstract

Aberrant neuronal activity in injured peripheral nerves is believed to be an important factor in the development of neuropathic pain. Pharmacological blockade of that activity has been shown to mitigate the onset of associated molecular events in the nervous system. However, results in preventing onset of pain behaviors by providing prolonged nerve blockade have been mixed. Furthermore, the experimental techniques used to date to provide that blockade were limited in clinical potential in that they would require surgical implantation. To address these issues, we have used liposomes (SDLs) containing saxitoxin (STX), a site 1 sodium channel blocker, and the glucocorticoid agonist dexamethasone to provide nerve blocks lasting ~1 wk from a single injection. This formulation is easily injected percutaneously. Animals undergoing spared nerve injury (SNI) developed mechanical allodynia in 1 wk; nerve blockade with a single dose of SDLs (duration of block 6.9 ± 1.2 d) delayed the onset of allodynia by 2 d. Treatment with three sequential SDL injections resulting in a nerve block duration of 18.1 ± 3.4 d delayed the onset of allodynia by 1 mo. This very prolonged blockade decreased activation of astrocytes in the lumbar dorsal horn of the spinal cord due to SNI. Changes in expression of injury-related genes due to SNI in the dorsal root ganglia were not affected by SDLs. These findings suggest that formulations of this kind, which could be easy to apply clinically, can mitigate the development of neuropathic pain.

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The authors declare no conflict of interest.

1To whom correspondence may be addressed. E-mail: daniel.kohane@childrens.harvard.edu or rlanger@mit.edu.

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Effect of SDLs on Nerve Conduction. Nerve conduction studies were performed in animals with intact sciatic nerves to determine the physical extent of SDL-induced conduction block along the sciatic nerve (Fig. 1A), specifically to confirm that the SDLs remained at the site of injection vs. spreading along the length of the nerve.

In the pristine hind limb (no SDL), stimulation of the sciatic nerve at the ankle (Fig. 1A) evoked a compound muscle action potential that traveled anterogradely and was recorded at the plantaris muscle (M wave, Fig. 1B). Stimulation at the hip also evoked retrograde conduction of the electrical impulse to the spinal cord, which was then transmitted back to the periphery monosynaptically via the sciatic nerve where it was measured at the plantaris muscle (H wave, Fig. 1B). Similarly, stimulation of the tibial nerve at the ankle evoked robust M and H waves, at different latencies compared with M and H wave latencies evoked by stimulation at the hip (Fig. 1B, table).

On day 4 after injection with SDL, electrical stimulation at the hip (proximal to the site of SDL injection), failed to generate the M and H wave in the plantaris muscle, suggesting a complete block of the proximal sciatic nerve segment. Stimulation of the tibial nerve at the ankle evoked a robust M wave, but not the H wave, suggesting normal nerve function in the tibial segment distal to the SDLs, but confirming block in the proximal sciatic nerve segment. Amplitude of the M wave elicited by ankle stimulation in the SDL-treated limb was similar ($P > 0.05$, $n = 5$) to the M wave elicited from the pristine limb, confirming normal axonal function in the distal tibial segment (Fig. 1B, table).

The localized distribution of SDL liposomes was also confirmed by postmortem dissection of the injection sites, where localized liposomal residue was found at a segment of the sciatic nerve near the greater trochanter even 30 d after administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stimulus</th>
<th>Pristine</th>
<th>Liposome treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>M wave latency</td>
<td>Ankle</td>
<td>2.4 ± 0.1 ms</td>
<td>2.3 ± 0.1 ms</td>
</tr>
<tr>
<td></td>
<td>Hip</td>
<td>4.2 ± 0.4 ms</td>
<td>absent</td>
</tr>
<tr>
<td>M wave amplitude</td>
<td>Ankle</td>
<td>4.3 ± 1.4 mV</td>
<td>5.1 ± 1.0 mV</td>
</tr>
<tr>
<td></td>
<td>Hip</td>
<td>3.7 ± 0.9 mV</td>
<td>absent</td>
</tr>
<tr>
<td>H wave latency</td>
<td>Ankle</td>
<td>9.5 ± 0.4 ms</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td>Hip</td>
<td>7.8 ± 0.2 ms</td>
<td>absent</td>
</tr>
</tbody>
</table>

**Fig. 1.** SDLs produce focal nerve conduction deficits in the sciatic nerve. (A) Schematic showing the anatomy of the sciatic nerve in relation to the site of SDL injection and stimulating/recording sites for nerve conduction studies. (B) Evoked compound muscle action potential measurements after stimulation of the sciatic nerve at the ankle (black) and at the hip (red) were recorded from the plantaris muscle at day 4 in the SDL-treated (Right) and untreated/pristine (Left) hind limbs. M and H wave latency and M wave amplitudes are shown in the table. Data are means ± SD, $n = 5$.

**Fig. 2.** Effect of 7-d nerve blockade from SDLs on the onset of tactile hypersensitivity in the SNI model. (A) Thermal latency measurements in the ipsilateral limb. Mechanical withdrawal threshold measurements were obtained from the ipsilateral (B) and contralateral (C) hind limbs. Animals received SDLs on day 0. Shaded areas in B and C represent duration of nerve block (see A). Data are mean ± SD; *$P < 0.05$, compared with untreated SNI animals, $n = 5$ rats per group.
Effect of Prolonged Nerve Block on Onset of SNI-Induced Tactile Hypersensitivity. To determine the effect of extended nerve blockade on the onset of neuropathic pain, SNI rats received nerve block injections with SDLs in the left hind-limb, ipsilateral and rostral to the nerve injury. Rats were tested for tactile sensitivity, an indicator of neuropathic pain, over a period of 9 wk. Thermal withdrawal latencies were used to monitor SDL-induced nerve conduction block. Untreated SNI animals demonstrated a thermal withdrawal latency of under 3 s, i.e., no nerve block.

Effect of 7-d Nerve Blockade on SNI-Induced Tactile Hypersensitivity. Administration of a single dose of SDLs immediately after nerve injury achieved nerve block duration of 6.9 ± 1.2 d, as assessed by thermal latency testing (Fig. 2A). This duration was similar to those obtained in other studies on the effect of prolonged duration local anesthetics (15, 16) on neuropathic pain. Hind-limb mechanical withdrawal thresholds were determined to assess the effect of SDLs on SNI-induced tactile sensitivity (Fig. 2B and C) in the ipsilateral (nerve-injured) and the uninjured contralateral limb. (In these and all experiments, the SNI and the SDLs were in the same extremity.) In SNI animals, mechanical withdrawal thresholds dropped progressively following injury. During (and because of) nerve blockade from a single dose of SDLs, mechanical withdrawal thresholds remained elevated in the injected extremity in SNI rats ($P < 0.05$, $n = 5$) compared with animals that did not receive SDL (Fig. 2B). That difference resolved ($P > 0.05$) 2 d after recovery from block (i.e., there was a delay in the onset of hypersensitivity by 2 d). Mechanical withdrawal thresholds in the leg contralateral from the SNI decreased following SNI; this occurred irrespective of whether SDL were injected (Fig. 2C; $P > 0.05$ for the comparison of SNI vs. SNI with SDL).

A single injection of liposomes containing only dexamethasone (and citrate buffer without STX) had no effect on SNI-induced decrease in mechanical withdrawal threshold in the injured leg.

Effect of 18-d Nerve Blockade on SNI-Induced Paw Hypersensitivity in the Ipsilateral Limb. The 2-d delay in the onset of tactile hypersensitivity following 7 d of nerve blockade prompted an examination of the effect of longer durations of block. To attain very prolonged continuous nerve block, SNI rats received injections with SDLs on day 0 (immediately after injury), and on days 5 and 12 (Fig. 3A). Mechanical nociceptive block in the repeatedly injected extremities lasted $18.1 ± 3.4$ d (Fig. 3A). In the absence of SDL injection, SNI rats exhibited decreased mechanical withdrawal thresholds in the injured limb compared with preinjury levels beginning at week 2 ($P < 0.05$, $n = 5$), and continuing till week 9 (Fig. 3B, $P < 0.05$, $n = 5$, one-way ANOVA). In contrast, mechanical withdrawal thresholds in the injured limb in SNI animals treated with SDLs were similar ($P > 0.05$, $n = 5$) to preinjury threshold values until week 6 ($P > 0.05$, $n = 5$), i.e., more than 3 wk after nerve block from the SDLs had worn off. (During nerve blockade, the elevated mechanical withdrawal thresholds were likely due to effects of the SDLs, rather than reflecting a lack of hypersensitivity.) Furthermore, mechanical withdrawal thresholds were significantly higher in SDL-injected than noninjected SNI animals during weeks 2–6 ($P > 0.05$, $n = 5$), again long after nerve blockade. After week 6, mechanical withdrawal thresholds from the nerve-injured extremity of SDL-treated rats were comparable to those obtained from untreated SNI animals ($P > 0.05$, $n = 5$; Fig. 3B).

Effect of 18-d Nerve Blockade on SNI-Induced Tactile Hypersensitivity in the Contralateral Limb. Mechanical withdrawal thresholds were decreased from week 2 to week 7 in the uninjured (contralateral) limb of SNI animals ($P < 0.05$ compared with preinjury threshold values; Fig. 3C). In SNI animals administered SDLs, withdrawal thresholds in the uninjured limb were unchanged at all time points tested ($P < 0.05$, compared with untreated animals at weeks 2–7).

Effect of 18-d Nerve Block on SNI-Induced Astrocyte Response in the Lumbar Spinal Cord. Maintenance of neuropathic pain is facilitated by the activation of astrocytes in the central nervous system. To determine if prolonged nerve block ($18.1 ± 3.4$ d in these experiments) can alter astrocyte activation, we analyzed the expression activation of glial fibrillary acidic protein (GFAP, a marker for...
In all experimental groups was studied as a multiple of the expression in uninjured, unblocked animals. *Data are means ± SD of mRNA fold changes over expression levels in pristine animals (n = 5 rats per group). **P < 0.05, *P < 0.01, #P < 0.001 versus saline-treated naïve group, &P < 0.01, **P < 0.001 versus SDL-treated SNI animals at days 5 and 60. In SNI-treated rats, SDLs was similar to that from uninjured naïve animals treated with SDLs alone (in uninjured animals) did not induce an increase in the GFAP-positive area in SDL-treated animals.

Table 1. Effect of 18-d nerve block on the expression of selected genes related to nerve injury in the L4, L5 DRG

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 d</td>
<td>60 d</td>
</tr>
<tr>
<td></td>
<td>SDLs</td>
<td>SNI + SDLs</td>
</tr>
<tr>
<td>Ppia</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.09</td>
</tr>
<tr>
<td>Cacna2d1</td>
<td>0.5 ± 0.06</td>
<td>3.2 ± 0.51</td>
</tr>
<tr>
<td>Htr3a</td>
<td>0.5 ± 0.08</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td>Scn11a</td>
<td>0.4 ± 0.13</td>
<td>38.4 ± 12.8</td>
</tr>
<tr>
<td>Npy</td>
<td>0.5 ± 0.44</td>
<td>45.3 ± 14.1</td>
</tr>
<tr>
<td>Gal</td>
<td>0.7 ± 0.11</td>
<td>144.4 ± 42.4</td>
</tr>
<tr>
<td>Vip</td>
<td>0.2 ± 0.16</td>
<td>7.0 ± 1.32</td>
</tr>
<tr>
<td>Clq</td>
<td>1.2 ± 0.33</td>
<td>28.0 ± 0.26</td>
</tr>
<tr>
<td>Jun</td>
<td>0.7 ± 0.12</td>
<td>3.7 ± 1.24</td>
</tr>
<tr>
<td>RT1Db1</td>
<td>0.6 ± 0.12</td>
<td>3.9 ± 0.64</td>
</tr>
<tr>
<td>Gadd45a</td>
<td>0.8 ± 0.13</td>
<td>37.2 ± 4.14</td>
</tr>
<tr>
<td>Atf3</td>
<td>0.8 ± 0.31</td>
<td>33.7 ± 4.14</td>
</tr>
</tbody>
</table>

Data are means ± SD of mRNA fold changes over expression levels in pristine animals (n = 5 rats per group). *P < 0.05 in comparison of SDL-treated SNI group (SDLs + SNI) and untreated SNI group by one-way ANOVA with Bonferroni post hoc comparison. Decreases in mRNA expression levels (numbers < 1, being the inverse of the fold decrease) are shaded, whereas increases are unshaded. Gene abbreviations: Ppia (control); Cyclophilin A; Cacna2d1, Calcium channel α2δ1; Htr3a, 5-hydroxytryptamine receptor 3a; Scn11a, Sodium channel IX; Npy, Neuropeptide Y; Gal, Galanin; Vip, Vasoactive intestinal peptide; Clq, Complement component 1q; Jun, Jun oncogene; RT1Db1, RT1 class II, locus Db1; Gadd45a, Growth arrest and DNA damage-inducible; Atf3, activating transcription factor 3.
several injury-related genes in ipsilateral DRG’s at 5 and 60 d, whereas treatment with SDLs did not prevent SNI-induced increase in expression of most genes at either time point.

Contralateral DRGs harvested from SNI animals showed no changes in gene expression after 5 and 60 d. However, at 60 d, DRGs harvested from SDL-treated SNI rats exhibited down-regulation of many genes tested (Table 1) (P < 0.05) compared with SNI animals that did not receive SDL.

**Discussion**

Aberrant spontaneous activity in the sensory afferents is consistently observed in animal models of painful neuropathy (37, 38). Peripheral nerve associated injury-induced discharges are thought to be responsible for irreversible changes in the central nervous system (8), leading to maintenance of chronic pain. Previous efforts to mitigate the development of neuropathic pain by prolonged neural blockade have had conflicting results. Bupivacaine–dexamethasone particles contained within a silicone tube provided nerve blockade lasting 8 d, but did not impact the development of allodynia or hyperalgesia in SNI (15) even though the same formulation was shown to prevent the rise in p38 mitogen-activated protein kinase seen in spinal microglia after SNI (17) and prevented the induction of apoptosis in the dorsal horn (16). In contrast, mitigation of pain behavior and reduced injury-induced glial cell activation in the spinal cord was seen following 1-wk block with bupivacaine free base powder or a tetrodotoxin infusion (13, 14). Different parts of our results were in agreement with both reports. Blockade with a single dose of SDL, resulting in 7-d block, did not prevent allodynia. However, 18-d blockade with repeated dosing resulted in a 4-wk delay in the onset of allodynia. Furthermore, spinal astrocyte activation, a facilitator of central sensitization in chronic neuropathic pain models (34, 39), was markedly decreased in SDL-treated SNI animals; this reduction was still in effect 60 d after SNI, 42 d after nerve block wore off. These results are consistent with the view that very prolonged nerve blockade could mitigate the development of neuropathic pain, and that such could be provided by an easily injectable sustained release system.

There has been interest for several decades in using drug delivery technologies to affect neuronal function locally, in the central (40) and peripheral nervous systems (51), particularly in the development of prolonged duration local anesthetics. A wide range of devices have been used for the latter application (24, 26, 27, 30, 41). Few have achieved blocks lasting even a few days (24, 25). Those that did often caused severe difficulties with local inflammation and/or tissue toxicity (21), particularly myo- and neurotoxicity (21, 25). SDLs produce prolonged nerve blockade (~1 wk from a single injection) with only mild inflammation and without evidence of myo- or neurotoxicity (26).

One possible explanation for the ability of SDLs to mitigate the development of mechanical allodynia where the bupivacaine–dexamethasone particles had failed is that the latter were neurotoxic and injured the nerves during blockade. That explanation is not consistent with the reports that nerve block lasting ~1 wk did not mitigate the development of allodynia whether achieved by the bupivacaine–dexamethasone particles (15) or the SDLs, whereas bupivacaine free base powder (presumably more tissue-toxic) was not consistent with the reports that nerve block lasting 1 wk from a single injection) with only mild inflammation and without evidence of myo- or neurotoxicity (26).

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Some studies demonstrate the consistent onset of contralateral hyperalgesia in nerve-injured animal models of neuropathic pain (47–51), whereas others report a mild or no hyperalgesic response (52, 53). Here, we observed transient contralateral hyperalgesia in SNI animals that was both slower in onset and lower in intensity compared with its ipsilateral counterpart. At the spinal cord level, astrocyte activation was increased in the contralateral dorsal horn, but it was milder compared with activation in the ipsilateral dorsal horn. The mechanism for contralateral pain is largely unknown, but altered spinal processing of sensory signals has been proposed (54). Mirror image pain, a well documented feature of human causalgia, has been equated with experimental contralateral hyperalgesia (49); the findings of this study raise the possibility of using prolonged ipsilateral nerve conduction block in preventing mirrored pain syndromes.

Nerve injury-induced gene expression changes in the DRG cell soma have been well documented, with the majority of injury-related genes changing expression 3 d after nerve injury (35) and remaining altered even after 13 d (36). In the present study, both 7- and 18-d nerve block failed to prevent SNI-induced gene expression changes in the ipsilateral L4, L5 DRG, even though nerve block mitigated the development of allodynia. These findings raise the possibility that activity-dependent changes in gene expression are not by themselves sufficient to cause neuropathic pain. In the contralateral DRGs, prolonged nerve block induced a significant decrease in expression of 7 of 11 genes, 2 mo after nerve injury. The significance of that finding is unclear; as noted above, contralateral pain following nerve injury is not well understood (nor universally reported) at this time.

Ultra-long-duration nerve block obtained by repeated administration of SDLs delays nerve injury-induced tactile hypersensitivity and attenuates the central astrocyte activation response. There would appear to be many important factors with potential impact on the effectiveness of formulations in mitigating pain behaviors, including type of drug, type of formulation, and duration and intensity of nerve blockade. SDLs provide an approach that is potentially clinically practical for preventing neuropathic pain conditions.

**Materials and Methods**

**Liposome Preparation.** Liposomes were produced by the thin lipid film technique, using 1,2-distearyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearyl-sn-glycero-3-phosphatidylglycerol (DSPG) (Genzyme) as described (26). Size of liposomes was determined using a multiosizer 3 Coulter Counter (Beckman Coulter). Intraliposomal STX content was determined after separating the lipid fraction using the Bligh and Dryer method (55). Determination of STX content was based on a previously published method by Bates et al. (56). STX was received as a generous gift from Sherwood Hall (Food and Drug Administration, College Park, MD).

**Nerve Conduction Studies.** For assessing the effect of SDLs on nerve function, we measured evoked compound muscle action potential latencies and amplitudes from the sciatic nerve (57) at the peak of nerve block (day 4 after SDL treatment, as characterized by the thermal paw withdrawal test). Rats were weighed daily and their body weight monitored (Fig. S2).

Detailed protocols for nerve conduction studies, immunohistochemistry, PCR array, spared nerve injury, behavioral tests for nociception, and historical and statistical methods are described in **SI Materials and Methods**.


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