A novel approach for detection of functional expression of TRPV1 channels on regenerated neurons following nerve injury

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Introduction

The TRPV1 channel is a non-specific cation channel [1] expressed in the peripheral and central nervous system [2-5]. In the peripheral nervous system, TRPV1 is mostly expressed in C-fibers, and, to a lesser extent, in Aδ-fibers [2-5]. This polymodal receptor channel can be activated by varieties of exogenous and endogenous stimuli such as capsaicin (found in hot chili peppers), low pH (pH < 5.9), hot temperature, inflammatory mediators (e.g., bradykinin, prostaglandins), anandamide, arachidonic acid metabolites, lipooxygenase products, adenosine, and ATP [6-10]. TRPV1 plays an important role in transduction of pain under physiological and pathological conditions including neuropathic pain (NP) conditions [11-21]. Immunohistochemical studies revealed that the expression profile of TRPV1 changes under NP conditions [12-15,17,19]. Recently, a novel approach was developed to investigate the dynamics of functional expression of TRPV1 channel under NP conditions following trigeminal nerve injury [19]. It is important to understand the dynamics of functional expression of a channel under pathological conditions to elucidate potential uses of that channel for the development of therapeutics. The combination of a quaternary lidocaine derivative N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314) and capsaicin (a TRPV1 agonist) was used to investigate the dynamics of functional expression of TRPV1 channel on regenerated neurons following transection of the inferior alveolar nerve (IAN, a branch of the trigeminal nerve) in rats [19]. This combination of QX-314 and capsaicin (QX-CAP) was first introduced by Binshtok et al. as a pain-specific local anesthetic (LA) [22].

QX-314 as a local anesthetic

LAs are widely used for blocking pain while retaining consciousness [23-25]. LAs block the generation and propagation of action potentials by blocking voltage-gated sodium channels at the intraneuronal site [23-25]. Commercially available LAs (e.g. lidocaine) have little or no selectivity to nerve fibers [26-29]; therefore, although the goal of LAs is to block pain, the administration of LAs also produces numbness due to the blocking of pressure and touch receptors, immobility due to the blocking of motor axons, and low blood pressure due to the blocking of autonomic (sympathetic) nerve fibers [30]. During dental surgery procedures, LAs may cause numbness of the lips, cheeks, and tongue and partial immobility of the tongue [31]. Though this may be acceptable during surgery, specific blocking of only the pain sensation would be desirable, so sensory-selective local anesthesia has long been an important goal for LA development [22,32]. A strategy has been developed in preclinical research, in which the permanently charged sodium channel blocker QX-314 is allowed to enter into nociceptive nerve fibers but not into other sensory or motor nerve fibers. QX-314 is a quaternary derivative of lidocaine and is unlikely to cross neuronal membranes when applied at low concentration external to the neuron [22]. Because LAs need to block the intraneuronal domains of voltage-gated sodium channels [23-25], extraneuronal application of low concentrations of QX-314 produces no or little effect on sodium channels [22,32]. When QX-314 combines with a TRPV1 activator, it opens the TRPV1 channel, which allows QX-314 to enter nociceptive neurons. TRPV1 channels are expressed predominantly by nociceptors [22]. TRPV1 channels have been reported to undergo pore dilation upon activation, allowing the permeation of large cations such as styryl dye FM1-43, gentamicin, and QX-314 through open pores [22,33,34]. As a result, QX-314 blocks sodium channels and transmission of pain signals from peripheral nociceptors to the central nervous system, thereby producing sensory blockade without blocking motor functions [22]. Binshtok et al. observed that extraneuronal application of a combination of a low concentration of QX-314 (5 mM) and capsaicin (1 μM) rapidly and selectively blocks sodium currents in capsaicin-responsive dorsal root ganglion (DRG) neurons, whereas QX-314 alone did not block the current [22]. Injection of QX-CAP into the hind paw and adjacent to the sciatic nerve caused prolonged local anesthesia to mechanical and thermal noxious stimuli [22]. This combination was used in various subsequent studies, which revealed that the combination was effective for blocking nociceptive signals (Table 1). One study reported that application of QX-CAP adjacent to IAN reduced the amplitude of the dental pulp stimulation–induced jaw-opening reflex in the orofacial regions [35]. Additionally, when QX-CAP was injected into the rat vibrissa pad, the latency of head-withdrawal responses to thermal stimulation significantly increased, whereas QX-314 or capsaicin alone had no effect [35]. The QX-CAP was also tested under NP conditions. In a NP model induced by sciatic nerve injury, QX-CAP injected at a close proximity to the sciatic nerve was effective for reducing the sensitivity of rats to mechanical and thermal stimuli [36]. An orofacial NP model induced by injury to the inferior alveolar nerve, demonstrated that subcutaneous injection of QX-CAP into the chin area was effective for attenuating mechanical allodynia [19].

In the QX-CAP formulation, using capsaicin as an activator of TRPV1 channels may limit its utility in clinical applications because capsaicin is a pain inducer [37] and may cause irritation before QX-314 enters the noc-
Table 1  QX314 as a local anesthetic in *in vivo* studies

| Formulations                  | Sites of injection | Dose                        | Effectiveness to block pain in normal condition | Effectiveness to block pain under NP condition | Reference |
|-------------------------------|-------------------|-----------------------------|-------------------------------------------------|-----------------------------------------------|-----------|
| QX-314 + Capsaicin (QX-CAP)   | Intraplantar      | QX-314: 2% (approximately 60 mM) + CAP: 0.05% (approximately 1.62 mM) | Effective                                      | N/A                                           | 22        |
|                               | Perineural injection to the sciatic nerve | QX-314: 0.2% (approximately 6 mM) + CAP: 0.05% (approximately 1.62 mM) | Effective                                      | N/A                                           | 22        |
|                               | Subcutaneous injection into the vibrissa pad | QX-314: 1% (approximately 30 mM) + CAP: 0.1% (approximately 3.25 mM) | Effective                                      | N/A                                           | 36        |
|                               | Subcutaneous injection into the mental skin | QX-314: 2% (approximately 60 mM) + CAP: 0.1% (approximately 3.25 mM) | Effective                                      | N/A                                           | 19        |
|                               | Tail injection (for mouse tail-flick test) | QX-314: 2.5% (approximately 70 mM) + CAP: 0.1% (approximately 3.25 mM) | Effective                                      | N/A                                           | 45        |
|                               | Intrathecal injection | QX-314: 0.2% (approximately 6 mM) + CAP: 0.05% (approximately 1.62 mM) | N/A                                            | Partially effective with severe irritation    | 36        |
| QX314 + Lidocaine             | Intraplantar injection | QX-314: 0.2% (approximately 6 mM) + Lidocaine: 1% | Effective                                      | N/A                                           | 32        |
|                               | Perineural injection to the sciatic nerve | QX-314: 0.2% + Lidocaine: 1% or 2% | Effective                                      | N/A                                           | 32        |
|                               | Subcutaneous injection (for pinprick-evoked cutaneous trunci muscle reflex) | QX-314: 0.2% (approximately 6 mM) + Lidocaine: 1% | Effective                                      | N/A                                           | 32        |
| QX314 + Bupivacaine           | Perineural injection to the sciatic nerve | QX-314: 0.5% (approximately 15 mM) + Bupivacaine: 0.5% | Effective                                      | N/A                                           | 40        |
|                               | Perineural injection to the sciatic nerve | QX-314: 0.2-1.5% + Bupivacaine: 0.03-0.5% | Effective                                      | N/A                                           | 41        |
| QX314 in acidic solution      | Intraplantar injection | QX-314: 2% (pH 5.0) | Effective                                      | N/A                                           | 42        |
|                               | Perineural injection to the sciatic nerve | QX-314: 2% (pH 5.0) | Effective                                      | N/A                                           | 42        |
| QX314 + surfactants           | Perineural injection to the sciatic nerve | QX-314: 25 mM + Octytrimethylammonium bromide (OTAB): 30-120 mM | Effective                                      | N/A                                           | 43        |
|                               | Intrathecal injection | QX-314: 25 mM + Sodium octyl sulfate (SOS): 20-30 mM | Effective                                      | N/A                                           | 43        |
| QX314 alone                   | Intraplantar injection | QX-314: 2% (approximately 60 mM) | Non-effective                                   | N/A                                           | 22        |
|                               | Perineural injection to the sciatic nerve | QX-314: 0.2% (approximately 6 mM) | Non-effective                                   | N/A                                           | 32        |
|                               | Subcutaneous injection (for pinprick-evoked cutaneous trunci muscle reflex) | QX-314: 0.2% (approximately 6 mM) | Effective                                      | N/A                                           | 39        |
|                               | Intrathecal injection | QX-314: 3-10 mM (approximately 0.1-0.4%) | Effective but produced marked irritation.      | N/A                                           | 46        |
|                               | Intradermal injection (for intradermal wheal assay) | QX-314: 70 mM or approximately 2.5% | Effective with slower onset of action | N/A                                           | 44        |
|                               | Tail injection (for mouse tail-flick test) | QX-314: 70 mM or approximately 2.5% | Effective with slower onset of action | N/A                                           | 44,45     |

N/A, not available

ceptors. Conventional LAs, such as lidocaine or bupivacaine, have been combined with QX-314 to overcome this problem because lidocaine and bupivacaine have been reported to function as TRPV1 activators [38]. The combination of lidocaine and QX-314 produced a long-lasting, predominantly pain-selective block when injected subcutaneously and adjacent to the sciatic nerve in animals [32,39]. The coapplication of bupivacaine and QX-314 has also been found to block pain signals from the hind paws of animals when the combination is injected at a close proximity to the sciatic nerve [40,41].

QX-314 was also used in acidic formulation because acidic solutions can activate TRPV1 receptors, and the formulation was found to be effective for blocking pain signals. Intraplantar and perisciatric injection of QX-314 in acidic solutions (pH 5) produced an LA effect and was also effective for blocking pain signals under NP conditions [42].

Combining QX-314 with surfactants was also effective for blocking pain signals from the hind paw when the combination was injected near the sciatic nerve [43]. Surfactants may act as chemical permeation enhancers to increase drug flux and can result in prolonged nerve blockade [43].

Diverse results have been reported when QX-314 alone has been investigated as an LA. Binshtok et al. observed that intraplantar injection of QX-314 alone (2% or approximately 60 mM) was ineffective for blocking pain signals [22,32]. Additionally, injection of 0.2% and 0.5% QX-314 alone near the sciatic nerve was ineffective for blocking pain signals from the hind paws of animals [22,39]. However, subcutaneous injection of 0.2% QX-314 alone was found to be marginally effective for producing local anesthesia for pinprick-evoked cutaneous trunci muscle reflex [32]. Roberson et al. [39] observed that injection of 1% QX-314 near the sciatic nerve under isoflurane general anesthesia was effective for blocking pain signals from the hind paws, but the same concentration of QX-314 was ineffective without general anesthesia, indicating that isoflurane may enable entry of high concentrations of QX-314 into nerve fibers (isoflurane itself may activate TRPV1). Conversely, Lim et al. [44] observed that 70 mM or approximately 2.5% of QX-314 alone can produce effective local anesthesia with slower onset of action in the hind paws of animals when injected near the sciatic nerve. In addition, intradermal injection of 70 mM or approximately 2.5% of QX-314 alone was found to be effective with a slower onset of action to block pain signals in intradermal wheal assay [44]. Additionally, an injection of the same concentration into the
mouse tail was effective for producing local anesthesia in the tail-flick test [44,45]. In one study, when the TRPV1 activator capsaicin was combined with the same concentration of QX-314, the onset of action became faster [45]. The above findings suggest that high concentrations of QX-314 alone may be effective for producing local anesthesia with a slower onset of action. Intrathecal injection QX-314 alone produced LA action both in otherwise healthy animals and animals with NP conditions, but this injection was found to induce severe irritation and sometimes death of animals, indicating that spinal administration of QX-314 may not be safe and may produce severe side effects [36,46].

Modulation of TRPV1 channel expression under NP conditions
The modulation of expression of TRPV1 channels under NP conditions induced by nerve injury was reported in previous studies (Table 2). TRPV1 expression was found to be modulated in nerve fibers at the injury site and in the ganglia where cell bodies of injured and uninjured nerve fibers are located. In a sciatic and spinal nerve axotomy model of NP, TRPV1 mRNA was found to decrease in the DRG up to 2 weeks after injury [47]. In contrast, in a spinal nerve ligation model, TRPV1 mRNA expression increased in DRG neurons up to 4 weeks after injury [13]. A study on chronic constriction injuries of the sciatic nerve reported increased TRPV1 protein in the spinal dorsal horn and DRG 1 week after injury [48]. Another study using a chronic constrictive spinal nerve injury model reported increased TRPV1 protein in the spinal cord at 1 and 2 weeks after injury [14].

Additionally, relative expression of TRPV1 in the injured and uninjured neurons has been investigated. Following spinal nerve ligation or partial sciatic nerve transection, TRPV1 expression significantly decreased in injured DRG neurons and increased in uninjured DRG neurons compared with naïve animals [12]. In a lingual nerve transection model, 3 days after injury, TRPV1 expression increased at the injury site of damaged nerves and decreased in injured trigeminal ganglion (TG) neurons. However, 3 weeks after injury, TRPV1 expression increased significantly in the injured TG neurons compared with uninjured control groups [15]. In a trigeminal nerve (inferior alveolar and mental nerve) injury model, TRPV1 expression increased in the uninjured ipsilateral mandibular and maxillary TG neurons 3 days after injury [17]. The above findings suggest that modulation of TRPV1 channel expression varies with the type of injury, injured and uninjured neurons, and the duration that elapses following the injury.

An approach to detect the functional expression of TRPV1
QX-CAP was used to evaluate the functional expression of TRPV1 channels following IAN transection [19]. The LA effects of QX-CAP were evaluated by analyzing the head-withdrawal threshold in response to mechanical stimulation of the lateral mental skin at different time intervals following IAN transection. Simultaneously, the expression of TRPV1 on retrograde-labeled regenerated neurons in the TG was investigated. Two weeks after IAN transection, a group of rats developed allodynia (the mechanical escape threshold reduced to half of that before transection) and this allodynia-like behavior continued for up to 4 weeks following the IAN transection [19]. These rats were considered to have developed NP (NP rats). Two weeks following IAN transection, application of QX-CAP to the mental skin of rats with NP at 3 and 4 weeks following IAN transection transiently reversed the allodynia (i.e., the mechanical escape threshold did not increase). This result indicated that TRPV1 channels localized on regenerated nerves 2 weeks following IAN-transaction were not sufficiently functional to allow entry of QX-314 into neurons. This notion was supported by the immunohistochemical findings of low expression of TRPV1 channels on regenerated neurons in the TG. However, application of QX-CAP to the mental skin of rats with NP at 3 and 4 weeks following IAN transection transiently reversed the allodynia (i.e., the escape threshold increased). These rats exhibited increased expression (moderate to high) of TRPV1 channels on the regenerated TG neurons (Table 3). In sham-operated rats (i.e., without IAN transection) QX-CAP was highly effective, and TRPV1 expression on the retrograde-labeled IAN-afferent neurons in the TG was high. The effectiveness of QX-CAP in rats without NP (i.e., rats in which the escape threshold did not reduce to half of that before transection) 2, 3, and 4 weeks after the IAN transection procedure was also investigated. In these rats, there was a clear positive correlation between the extent of the QX-CAP effect and the increments of the TRPV1 expression on the regenerated TG neurons (Table 4). These observations suggest that nerve injury modulates the functional expression of TRPV1, and the effectiveness of QX-CAP in this NP model depends on the availability of functional TRPV1 channels on the regenerated neurons. These observations also revealed that the effectiveness of QX-CAP was an indirect measurement technique for

| Type of injury                              | Days after injury | TRPV1 expression at the injury site | TRPV1 expression in DRG/TG/spinal cord | References |
|--------------------------------------------|------------------|------------------------------------|----------------------------------------|------------|
| Sciatic and spinal nerves transection      | 4, 7, and 14 days after injury | N/A                                | TRPV1 mRNA expression decreased in DRG | 47         |
| Spinal nerve ligation                      | 1-28 days after injury | N/A                                | TRPV1 mRNA expression increased in the DRG neurons | 13         |
| Chronic constriction injury of sciatic nerve | 7 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 48         |
| Chronic constriction injury of spinal nerve | 7 and 14 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 14         |
| Total sciatic nerve transection            | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured DRG neurons | 12         |
| Partial sciatic nerve section              | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Spinal nerve ligation                      | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Lingual nerve transection                   | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 decreased in the injured trigeminal ganglion neurons | 15         |
| Inferior alveolar and mental nerve transection | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 increased in the injured trigeminal ganglion neurons | 17         |

N/A, not available

| Type of injury                              | Days after injury | TRPV1 expression at the injury site | TRPV1 expression in DRG/TG/spinal cord | References |
|--------------------------------------------|------------------|------------------------------------|----------------------------------------|------------|
| Sciatic and spinal nerves transection      | 4, 7, and 14 days after injury | N/A                                | TRPV1 mRNA expression decreased in DRG | 47         |
| Spinal nerve ligation                      | 1-28 days after injury | N/A                                | TRPV1 mRNA expression increased in the DRG neurons | 13         |
| Chronic constriction injury of sciatic nerve | 7 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 48         |
| Chronic constriction injury of spinal nerve | 7 and 14 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 14         |
| Total sciatic nerve transection            | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured DRG neurons | 12         |
| Partial sciatic nerve section              | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Spinal nerve ligation                      | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Lingual nerve transection                   | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 decreased in the injured trigeminal ganglion neurons | 15         |
| Inferior alveolar and mental nerve transection | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 increased in the injured trigeminal ganglion neurons | 17         |

N/A, not available

Table 2. TRPV1 expression under neuropathic pain condition following nerve injury

| Type of injury                              | Days after injury | TRPV1 expression at the injury site | TRPV1 expression in DRG/TG/spinal cord | References |
|--------------------------------------------|------------------|------------------------------------|----------------------------------------|------------|
| Sciatic and spinal nerves transection      | 4, 7, and 14 days after injury | N/A                                | TRPV1 mRNA expression decreased in DRG | 47         |
| Spinal nerve ligation                      | 1-28 days after injury | N/A                                | TRPV1 mRNA expression increased in the DRG neurons | 13         |
| Chronic constriction injury of sciatic nerve | 7 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 48         |
| Chronic constriction injury of spinal nerve | 7 and 14 days after injury | N/A                                | TRPV1 protein levels significantly increased in the spinal cord | 14         |
| Total sciatic nerve transection            | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured DRG neurons | 12         |
| Partial sciatic nerve section              | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Spinal nerve ligation                      | 2 weeks after injury | N/A                                | Immunohistochemical expression of TRPV1 decreased in the injured and increased in the uninjured DRG neurons | 12         |
| Lingual nerve transection                   | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 decreased in the injured trigeminal ganglion neurons | 15         |
| Inferior alveolar and mental nerve transection | 3 days after injury | Immunohistochemical expression of TRPV1 increased in the injured nerves | Immunohistochemical expression of TRPV1 increased in the injured trigeminal ganglion neurons | 17         |

N/A, not available

Table 3. Effectiveness of QX-CAP under neuropathic pain condition following IAN transection and corresponding TRPV1 expression on regenerated neurons in the TG

| Time after IAN transection | Effectiveness of QX-CAP | TRPV1 expression on regenerated neurons in the TG | References |
|---------------------------|-------------------------|---------------------------------------------------|------------|
| 2 weeks                   | Non-effective           | Low                                               | 19         |
| 3 weeks                   | Effective               | Moderate to high                                  |            |
| 4 weeks                   | Effective               | High                                              |            |

Table 4. Effectiveness of QX-CAP under non-neuropathic pain condition following IAN transection and corresponding TRPV1 expression on regenerated neurons in the TG

| Time after IAN transection | Effectiveness of QX-CAP | TRPV1 expression on regenerated neurons in the TG | References |
|---------------------------|-------------------------|---------------------------------------------------|------------|
| 2 weeks                   | Effective               | Moderate                                          | 19         |
| 3 weeks                   | Effective               | Moderate to high                                  |            |
| 4 weeks                   | Effective               | High                                              |            |
the dynamic expression of TRPV1 following nerve injury. The expression pattern of TRPV1 channels in myelinated and unmyelinated neurons was also investigated and found that TRPV1 channels were more expressed in myelinated neurons following nerve injury. TRPV1 was mostly localized in small unmyelinated neurons (C-neurons) in sham-operated rats; however, TRPV1 channels were mostly localized on medium sized myelinated neurons (Aδ-neurons) following IAN transaction [19].

Nerve injury is known to alter the expression of various ion channels including the TRPV1 channel. Various studies have reported a potential role of TRPV1 in inflammatory pain and NP. It is important to know the dynamics of functional expression of TRPV1 for the development of therapeutic strategies to treat NP. A novel approach was demonstrated for detecting dynamic changes in TRPV1 expression on regenerated neurons following nerve injury using QX-CAP, which may be useful for detecting functional TRPV1 expression in other pathological conditions.

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Conflict of interest

The authors declare that they have no competing interest.

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