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Letters to the editor: Nicotinic acetylcholine receptor ligands as potential targets for managing neuropathic pain induced by diabetic peripheral neuropathy

ABSTRACT

Diabetic peripheral neuropathy (DPN) is a medical condition that is progressively becoming more prevalent. The underlying cause of DPN is still unknown, although there have been several hypothesized mechanisms. There are current pharmaceutical treatments used to manage the pain, but their efficacy is largely unsatisfactory and are often associated with serious adverse effects. This review will explore the evidence of a new potential target for treating DPN, the ligands for nicotinic acetylcholine receptors (nAChRs), specifically α4β2 agonists and α9α10 antagonists.

Diabetic peripheral neuropathy (DPN) is the most common complication associated with long-term diabetes mellitus. Symptoms can vary, ranging from pain or tingling to numbness. The current hypothesis about its underlying mechanisms focuses on the interplay of metabolic, neurovascular, and immune systems producing damage to various aspects of the peripheral nerve including myelin sheath, dorsal root ganglia (DRG), and axons. Macrophages are recruited during DPN, which activates neutrophils and lymphocytes to produce proinflammatory mediators, including cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), and chemokines such as C—C motif ligand 2 (CCL2) and CCL3. These molecules sensitize the nociceptors at the primary afferents to elicit peripheral sensitization. In addition, microglia and astrocytes may also be activated to promote inflammatory responses within the spinal cord and induce central sensitization. Both mechanisms contribute to the development of neuropathic pain.

Existing treatment options are far from sufficient. We aim to examine the current evidence supporting the development of nicotinic acetylcholine receptor (nAChR) specific drugs into new efficacious therapies with less adverse side effects for DPN.

1. Nicotinic AChRs and their subtypes

Nicotinic AChRs are pentameric ligand gated non-selective cation channels found in abundance in the nervous system and immune cells such as macrophages, cochlear hair cells, or keratinocytes. Among the various subunits discovered so far, α4β2 and α9α10 are potentially useful to regulate nociception and inflammation [1,2]. The exact mechanisms of action remain elusive, but both nAChR ligands seem to play important roles in modulating inflammation and immune cell functions (Fig. 1). The results of their preclinical and clinical studies are listed in Table 1.

2. α4β2 subunit

The α4β2 subunit is one of the predominant subtypes of nAChRs in the brain. Activation of brainstem α4β2 nAChRs may engage the descending inhibitory mechanisms, such as release of serotonin (5-HT) and norepinephrine to reduce pain neurotransmission in the spinal cord [3]. It may also increase gamma aminobutyric acid (GABA) release in the rostral ventromedial medulla to promote descending inhibition. Moreover, activation of α4β2 nAChRs can suppress peripheral macrophages and possibly microglia in the CNS to decrease neuropathic pain.

Epibatidine is a toxic alkaloid and natural α4β2 agonist from the skin of the *Epipedobutes tricolor* frog. The analgesic effects of epibatidine are 200 times higher than morphine and 30 times higher than nicotine [4]. Unfortunately, it failed the clinical trial due to adverse effects such as toxicity in the CNS, gastrointestinal, respiratory and cardiovascular systems likely due to epibatidine's ability to activate ganglionic α3β4 subunits [4].

Despite failing clinical trials, epibatidine has garnered great interest because of its non-opioid superior analgesic effects. Derivatives with better selectivity have since been made from this natural compound and shown promising results. ABT-594, also known as Tebanicline, showed very potent analgesic and anxiolytic effects in rat and mouse models with little to no adverse effects at the effective antinociceptive dose [5]. It was then studied in a phase II randomized, multicenter, double-blind, placebo-controlled study in 266 patients with DPN and showed pain reduction across all doses of the treatment groups. However, the ratio of patients dropping out due to adverse effects were significantly higher than placebo. The therapeutic window is small as higher doses of ABT-594 activate the α3 nAChR as well, causing adverse autonomic effects such as decreased body temperature and impaired motor coordination, nausea, vomiting, and abnormal dreams [4].
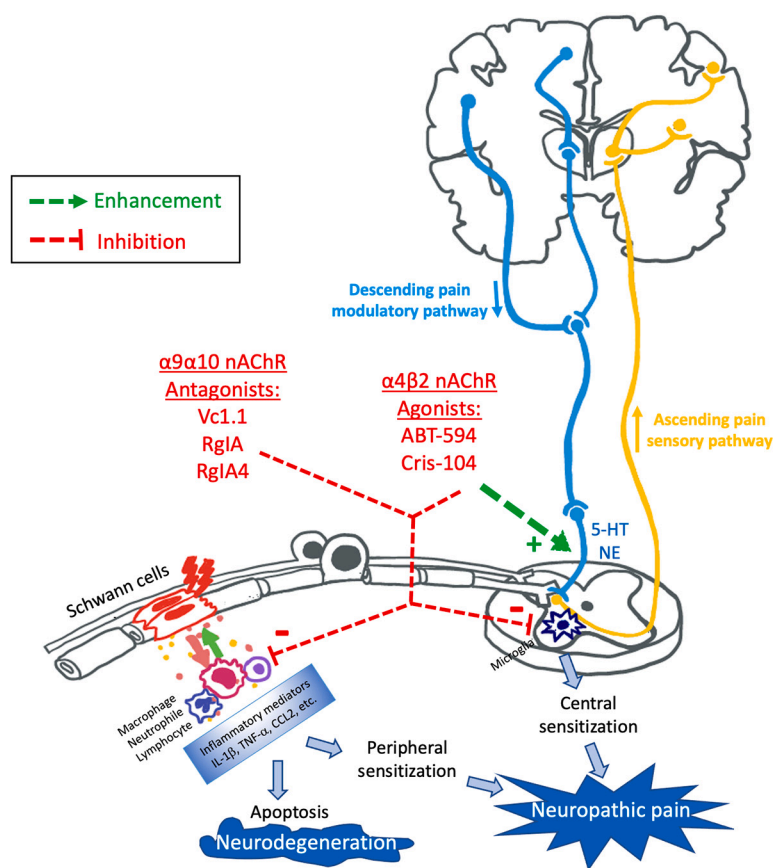
Fig. 1. Schematic illustration of the hypothesis of the pathophysiological processes leading to neuropathic pain/neurodegeneration in DPN patients and the potential mechanisms of action for nAChR ligands. Ascending (orange path) and descending (blue path) pain modulation pathways are depicted on the right panel. Serotonin (5-HT) and norepinephrine (NE) are the major neurotransmitters released to the spinal cord to suppress the ascending pain transmission. Different types of immune cells are activated including macrophages, neutrophils and lymphocytes at the periphery, and microglia at the CNS. Activation of the immunes cells leads to the release of inflammatory mediators such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, C—C motif ligand 2 (CCL2), etc. These inflammatory mediators cause peripheral and central sensitization, resulting in the development of neuropathic pain and neurodegeneration. The short red lines and green arrows indicate inhibition and promotion of the corresponding target, respectively. Agonists of α4β2 nAChRs and antagonists of α9α10 nAChRs are thought to block the immune cells and engage descending pain inhibitory mechanisms to suppress neuropathic pain and prevent neurodegeneration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Another α4β2 selective agonist, Cris-104, showed better selectivity for α4β2 subunit with a much lower affinity for other nAChR subun its [6]. In diabetic mice, it reduced mechanical allodynia and ther mal hyperalgesia, common symptoms associated with DPN [6]. Recently, 5-[(1R,5S)-3,6-Diazabicyclo[3.2.0]heptan-6-yl]nicotinonitril e (A-366833), showed even higher selectivity for the α4β2 subunit (Ki = 3.2 nM) vs. the α3 subunit. It reduced mechanical hyperalgesia and produced antinociception in rat models of diabetes and chemotherapy-induced neuropathic pain [7,8]. Although they have not been studied clinically, improved selectivity of the α4β2 subunit over the α3 subunit indicates great potential to alleviate DPN pain without concurrent side effects.

3. α9α10 subunit

The α9 subunit was first identified in the inner hair cells of rodents. Later on, α10 subunits are found to be assembled together with α9 to form the α9α10 subunit in the DRG, immune cells, and immune cell derivatives. Existing evidence indicates that they are involved in immune responses and processes including nociception and inflammation. When activated, α9α10 subunit can promote the release of IL-1β, interferon gamma (IFN-γ), and monocyte infiltration [1]. Multiple α9α10 nAChR antagonists, such as cone snail venom toxins Vc1.1, RgIA, and RgIA4, have been studied in various pain models [2].

Vc1.1 was the first compound of this class to advance to clinical trials after showing significant reduction of neuropathic pain following partial sciatic nerve ligation in rodents [7]. It blocked the development of tactile allodynia and mechanical hyperalgesia that lasted 10 days post discontinuation of treatment in streptozotocin-induced diabetic rodents

[9]. In the Phase I clinical trial no drug related adverse effects was observed with single or multiple systemic doses [2]. Unfortunately, the drug failed to show therapeutic effect in Phase II clinical trials [10]. Later on, it has been discovered that Vc1.1 has much lower affinity for human α9α10 nAChRs than their rodent counterparts [11]. Another conopeptide, RgIA, showed great efficacy in alleviating chronic neuropathic pain induced by nerve injury and chemotherapy in mice possibly via inhibition of inflammation and activation of peripheral macrophage and/or central microglia and astrocytes in the dorsal horn [9]. However, similar to Vc1.1, RgIA is about 300 fold less potent against human α9α10 nAChRs than the rodent receptors.

More recently, McIntosh group created RgIA4 [12], a derivative of RgIA, that have significantly higher affinity to human α9α10 nAChRs than its predecessors. It has equal potency to both receptors with half-maximal inhibitory concentrations (IC$_{50}$) of 0.9 and 1.5 nM for rodent and human receptors, respectively [13]. RgIA4 has been shown to produce remarkable "disease-modifying" effects for the prevention and treatment of neuropathic pain in rodents without observable tolerance effects [2].

While these newer α9α10 nAChR antagonists haven't been tested directly in DPN patients, they have been shown to decrease the degeneration of the DRG and nucleolar alterations associated with DPN [10], reduce the activation of macrophages and T cell infiltration following a nerve injury, which may contribute to their anti-neuropathic properties.

In summary, DPN is a growing problem worldwide. α4β2 agonists and a9a10 antagonists have shown promising results in rodent models in alleviating several types of neuropathic pain. The selective ligands of these subtypes that have been tested in clinical trials seemed to be well tolerated. Based on the current research, α4β2 nAChR agonists and

Table 1
Results of preclinical studies and clinical trials for the efficacy of novel nAChR ligands in alleviating DPN.

Drug	Mechanism of action	Animal models	Human clinical trial results	Outcomes	Adverse effects	Reference number
Epibatidine	Non-selective agonist for α4β2, α3β4, and α7 nAChRs	Mice, rats, and pregnant goats	Not assessed	Antinociception - Decreased response to radiant heat on tail flick testing	Rhinorrhea, lacrimation, seizures, hypertension, and muscle paralysis	[4]
ABT-594	Derivative of Epibatidine with higher selectivity for α4β2, but activates a3b4 nAChRs as well	Mice and rats	Phase 2 clinical trial: randomized, double-blind, placebo-controlled	Improvement from baseline daily pain rating scales	Nausea, dizziness, vomiting, and abnormal dreams in humans	[5]
Cris-104	Derivative of Epibatidine with high affinity for α4β2 and less affinity for a3b4 nAChRs	Mice	Not assessed	Antinociception - Reduced thermal hyperalgesia and mechanical allodynia, without impairment of locomotor activity	None observed	[6]
A-366833	α4β2 nAChR agonist	Mice, rats	Not assessed	Anti-hyperalgesia in sciatic nerve ligation and chronic constriction injury. Antinociception and reduction in mechanical hyperalgesia in diabetic and chemotherapy induced neuropathic pain models. Complete attenuation of mechanical hyperalgesia in inflammatory pain models.	None observed.	[7]
Vc1.1	α9α10 nAChR antagonist, less affinity for human α9α10 nAChRs than rat counterparts	Mice and rats	Phase 2 clinical trial: a randomized, double-blind, placebo-controlled trial	Reduction of tactile allodynia and mechanical hyperalgesia in rodent models of neuropathic pain induced by diabetes and sciatic nerve injury; however, ineffective in humans likely due to lack of affinity for human α9α10 nAChRs.	None observed	[11]
RgIA	α9α10 nAChR antagonist, less affinity for human α9α10 nAChRs than rat counterparts	Rats	Not assessed	Reduced mechanical allodynia, mechanical hyperalgesia, and immune cell infiltration in chronic constriction injury of the sciatic nerve. Reduced mechanical hyperalgesia, and cold allodynia in chemotherapy induced neuropathy.	None observed	[10]
RgIA4	α9α10 nAChR antagonist, high affinity for both human and rat α9α10 nAChRs	Rats	Not assessed	Reduced mechanical hyperalgesia, and cold allodynia in chemotherapy induced neuropathy.	None observed	[12]

a9a10 nAChR antagonists hold great potentials to relieve neuropathic pain induced by DPN with less adverse side effects than the current available therapies.

Declaration of Competing Interest

None of the authors has any conflict of interest.

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Sarah Westlake[a], Matthew Jones[a], Krishna D. Sharma[b], Jennifer Yanhua Xie[a,*]

[a] Basic Sciences Department, New York Institute of Technology College of Osteopathic Medicine at Arkansas State University, Jonesboro, AR, USA
[b] Department of Biological Sciences and Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR, USA

[*] Corresponding author at: 2713 Pawnee St.(Wilson Hall), NYIT College of Osteopathic Medicine at A-State, Jonesboro, AR 72401, USA.
E-mail addresses: swestlak@nyit.edu (S. Westlake), mjones15@nyit.edu (M. Jones), krishna.sharma@smail.astate.edu (K.D. Sharma), Jennifer.xie@nyit.edu (J.Y. Xie).