Compounds of traditional Chinese medicine and neuropathic pain

LI Shun-Hua 2Δ, LI Lin 1,3Δ, YANG Ru-Nan 1,3, LIANG Shang-Dong 1,3, *

1 Neuropharmacology Laboratory of Physiology Department, Medical School of Nanchang University, Nanchang 330006, China;  
2 Undergraduate Student of Class 155 of Nanchang University Queen Mary University of London Joint Programme, Nanchang 330006, China;  
3 Jiangxi Provincial Key Laboratory of Autonomic Nervous Function and Disease, Nanchang 330006, China

Available online 20 Jan., 2020

[ABSTRACT] Neuropathic pain (NP) has become a serious global health issue and a huge clinical challenge without available effective treatment. P2 receptors family is involved in pain transmission and represents a promising target for pharmacological intervention. Traditional Chinese medicine (TCM) contains multiple components which are effective in targeting different pathological mechanisms involved in NP. Different from traditional analgesics, which target a single pathway, TCMs take the advantage of multiple components and multiple targets, and can significantly improve the efficacy of treatment and contribute to the prediction of the risks of NP. Compounds of TCM acting at nucleotide P2 receptors in neurons and glial cells could be considered as a potential research direction for moderating neuropathic pain. This review summarized the recently published data and highlighted several TCMs that relieved NP by acting at P2 receptors.

[KEY WORDS] Traditional Chinese medicine; Diabetic neuropathic pain; HIV-gp120-associated neuropathic pain; Chronic constriction injury; Nucleotide receptors

[CLC Number] R284, R965

Introduction

Neuropathic pain (NP) is defined as pain caused by a lesion or disease of the somatosensory system, belongs to chronic pain. The typical symptoms of NP include spontaneous pain, allodynia (pain response to normally innocuous stimuli), hyperpathia (abnormal pins-and-needles or electric-shock-like sensations), and hyperalgesia (aggravated pain evoked by non-noxious stimuli) [1-3]. NP has become a serious global health issue, with estimated prevalence rates ranging from 1% to 8.9% of the total population [4]. Its long duration reduces the quality of patients’ life and places a heavy burden on society. Although various drugs, including antidepressants, anti-epileptics, opioids, and topical anesthetics, are used for clinical treatment of NP, their side effects are marked, and their therapeutic effects are generally limited [5-6]. Complete freedom from NP often cannot be achieved by drugs. For all types of drugs, 20% to 40% of patients either experience a less than 30% pain relief or have intolerable side effects [5-7]. Therefore, developing novel compounds for the effective management of NP with little influence on other normal physiological functions is necessary.

Nucleotides of purinergic signaling were proposed as extracellular signaling molecules in 1972 [8]. Purinergic receptors include P1 receptors and P2 receptors, which bind to adenosine-5′-triphosphate (ATP) and its analogues [adenosine-5′-diphosphate (ADP) and adenosine] respectively [9]. Purinergic signaling was proposed and hypothesized for the initiator and modulator of pain [11]. Since then, many subsequent research studies have expanded this concept. P2X and P2Y receptors are involved in the modulation of pain transmission. After nerve injury, ATP is released from the nerve endings of primary sensory neurons. Extracellular ATP triggers the activation of different P2 receptors, which induce the production and release of bioactive factors
including cytokines and neurotrophic factors, which in turn lead to the hyperexcitability of sensory neurons and, consequently, NP developed [10-11].

Ligand-gated ion channel P2X receptor has seven subtypes, among which the P2X3, P2X4, and P2X7 subtypes are most related to pain transmission [11-12]. It has been reported that P2X antagonists were successful in relieving NP [13-14]. P2X3 receptor antagonists (A-317491, RO3 and RO4) have exhibited their potent ability to relieve NP [15-17]. An effective P2X4 receptor antagonist named paroxetine has been suggested to successfully relieve NP [18]. P2X7 receptor antagonists (A-740003, KN-62 and KN-04) produced antinociceptive effects in NP [19]. Among the G protein coupled P2Y receptor family, only the P2Y12 receptor subtype is clearly known to be involved in NP onset. Several genetic deletions of P2Y12 receptors produce analgesic effect [20-22]. These observations indicate that drugs selectively targeting specific P2 receptors can exert their analgesic activity at the site of receptor, thus limiting the occurrence of unwanted side effects [23-24].

The advantage of traditional Chinese medicine in NP treatment

NP, a kind of chronic pain, is the focus of many research studies due to the high incidence, complex pathogenesis, and lack of efficient treatments. Trauma (surgery, amputation), metabolic disorders (diabetes, uremia), infection (herpes zoster, HIV), poisoning (chemotherapy), vascular disease (arteritis nodosa), and malnutrition are considered to be incentives for the initiation of NP [25]. NP is an area of largely unmet therapeutic need due to its complex underlying pathophysiology and heterogeneous etiology [5, 7]. The presently available treatment for NP contains non-pharmacological approaches, such as electroacupuncture [26], and drugs. On the basis of randomized clinical trials, medications are recommended as first-line treatments for NP include certain antidepressants [e.g., tricyclic antidepressants (TCAs), serotonin norepinephrine reuptake inhibitors (SNRIs)] and certain anticonvulsants (e.g., gabapentin, pregabalin, and topical lidocaine). Opioid analgesics and tramadol are recommended as second-line therapies. However, they are sometimes used as first-choice drugs [27]. But those therapies only offer marginal relief because of either a lack of efficacy or the risk of unacceptable side effects (e.g., sedation, dizziness, edema, and ataxia) [28]. Patients with NP continue to have moderately severe pain on average, despite taking prescribed medications for their condition [29]. Moreover, the development of tolerance, and the risks of addiction and abuse, significantly limit the clinical application of NP pharmacotherapy. No treatment option has been shown to reverse NP. Therefore, the discovery of novel therapeutic alternatives with superior effectiveness and minimal adverse effects would be beneficial.

Currently, more attention has been focused on herbal formulations in the field of drug discovery, especially on medicinal plants and herbal extracts from traditional Chinese medicine (TCM) (Fig. 1), which are increasingly used worldwide. TCM has unique advantages in the clinical prevention and treatment of NP because of its fewer complications and side effects compared with synthetic drugs. Moreover, the multiple components and targets of TCM avoid the shortcomings of clinical drugs targeting a single pathway [30]. Several studies have indicated that natural products could be a good source of the new specific molecules needed for the treatment of pain-related diseases through the regulation of P2 receptors [28-29]. For example, the TCM compound Emodin acts at tumor cells to inhibit their migratory ability via P2X7 blockade [31]. Recently, the antagonistic effect of bioflavonoids from the methanolic extract fractions of Rheedia logifolia on the P2X7 receptor has been found to promote analgesic action on inflammatory pain [32]. In addition, it has been reported that bioactive molecules contained in analgesic preparations from TCM (e.g., tetramethylpyrazine, sodium ferulate, and puerarin) can inhibit P2X3-mediated pain transmission in primary afferent neurons [28]. Thus, TCM may be an alterna-
Diseases such as diabetes, human immunodeficiency virus (HIV), and herpes infections, the resultant NP is often modifiable with prevention strategies. Therefore, after precise prediction of the disease risk, TCM can provide preventive treatment of diseases [50]. Compared with targeted therapy, TCM has the merit of a dispersed application point, slow effect, and relative safety, but a complex mechanism of action [45]. The difficulty in TCM-based drug development is to decrypt the scientific mechanisms responsible for the clinical effects. Several studies found that TCM inhibited spinal glial activation and the following proinflammatory cytokine production, or that eliciting anti-inflammatory effect might contribute to its capability of anti-nociception [48-50].

**P2 receptors and TCM**

*Inhibitory effects of TCM in diabetic neuropathic pain mediated by the P2 receptors in dorsal root ganglia*

Diabetes mellitus (DM) is one of the major causes of peripheral neuropathy. Diabetic neuropathic pain (DNP) is a common complication of DM with limited treatment [51]. The P2X3 receptor mediated hyperalgesia in DNP rats [52-53]. Sinomenine is the main active compound extracted from TCM Sinomenium acutum, which has anti-inflammatory and neuroprotective effects [49]. Sinomenine attenuated DNP induced by upregulated P2X3 [50]. Sinomenine reduced the thermal and mechanical hyperalgesia of DM rats, and decreased the associated overexpression of P2X3 in the dorsal root ganglia (DRG) of the DM group [53]. Phosphorylation of P38MAPK (mitogen-activated protein kinase) and the following release of pro-inflammatory cytokines are involved in inflammatory pain [29]. Sinomenine lessened pain behaviors by decreasing the activation of the DRG P38MAPK in DM rats. Furthermore, sinomenine showed a similar inhibition effect with P2X3-specific antagonist A317491 to inhibit P2X3 agonist ATP-activated currents in the transfected human embryonic kidney (HEK) 293 cells [59].

Emodin is an anthraquinone isolated from the traditional Chinese herb of Rheum. It has exhibited excellent biological activities in inflammatory diseases [56]. The targeting and bioavailability of drugs were improved by encapsulation of nanoparticles, which also lower toxic side effects. Nanoparticle-encapsulated emodin (nano emodin) can relieve DNP in diabetic rats by reducing P2X3 receptor-mediated excitatory conduction in DRG [58]. Nano emodin treatment weakened the enhanced mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) of untreated DM rats by inhibiting exaggerated P2X3 expression. The α, β-meATP-activated currents in HEK293 cells transfected with the human P2X3 receptor were restrained in nano emodin treatment. ATP released by the injured nerve acts via the facilitated P2X3 receptor to cause the phosphorylation of extracellular regulated protein kinases (ERK), inducing the release of tumor necrosis factor (TNF)-α and exacerbating NP. The attenuating effects of nano emodin on DNP were related to the blocked ERK pathway and the inhibition of TNF-α release [56].

The overexpressed P2X4 receptor in DRG is implicated...
in the initiation and maintenance of NP [67-68]. Quercetin, an abundant dietary flavonoid, possesses remarkable analgesic, antioxidative, and anti-inflammatory properties [59]. Quercetin can decrease the sensitization of P2X4 receptor-involved hyperalgesia induced by DM in rats [60]. Following nerve injury, glial cells undergo activation and exclusively increase their expression of P2X4 receptors. Quercetin could markedly reduce the co-expression of P2X4 receptor and glial fibrillary acidic protein [GFAP, a satellite glial cell (SGC) marker] in diabetic rats. Quercetin reduced P2X4 overexpression in the DRG of DM rats, increasing the threshold values of mechanical and thermal hyperalgesia. Quercetin decreased the amplitudes of currents in HEK293 cells transfected with P2X4 receptor. Those results support the notion that P2X4 receptor is the pharmacological target of Quercetin for the therapeutic treatment of DPN [60].

Osthole, an active coumarin isolated from the anti-inflammatory TCM Cnidium monnieri (L.) Cusson, has been reported to exert analgesic effect [61-63]. Osthole treatment acted on the P2X4 receptor and alleviated the mechanical and thermal hyperalgesia in DM rats [64]. The increased expression of P2X4 mRNA and protein and enhanced co-expression staining of P2X4 receptor and GFAP in the SGCs of DRG were reversed in DM + osthole treated rats, as well as the activated P2X4 receptor-p38 MAPK pathway (Fig. 2). The link between glial cells and dorsal horn neurons is established by brain-derived neurotrophic factor (BDNF) and pro-inflammatory cytokines interleukin (IL)-1β and TNF-α, through the P38 MAPK pathway [65], which was inhibited by osthole. In addition, osthole administered by intraperitoneal injection increased the downregulated expression of anti-inflammatory cytokine IL-10 in the DRG of DM rats. Therefore, osthole treatment alleviated DNP through decreasing the upregulation of P2X4 receptor and the activation of SGC, followed by the downregulation of IL-1β, TNF-α, BDNF, and p-p38MAPK and the upregulation of IL-10 in DM rats [64].

P2Y12 receptors are expressed on SGCs of DRG. The upregulation of P2Y12 receptor was found in HIV-gp120 added to combination antiretroviral therapy-induced neuropathology, and the reduction of P2Y12 receptor by short hairpin RNA (shRNA) showed resistance to HIV-associated NP [66-67]. Moreover, it has been reported that P2Y12 shRNA treatment decreased SGC activation and relieved DNP [68]. Those studies suggested that P2Y12 participated in NP can serve as a potential therapeutic target. Curcuminis extracted from turmeric rhizome, a natural medicine with anti-inflammatory and antioxidant activities, as well as the effect of relieving DNP [69]. The nanoparticle-encapsulated curcumin (nano-curcumin) treatment inhibited mechanical and thermal hyperalgesia and reduced the expression of P2Y12 receptor in the SGCs of DRG in DM rat model [70]. The activation of SGCs and the following production of IL-1β were also suppressed by nano-curcumin. Gap junctions between SGCs are involved in neuronal excitability and contribute to the induction and/or maintenance of pain. Connexin 43 (Cx43) is a gap junction subunit, whose dramatic expression level was decreased in the nano-curcumin-treated group. Protein kinase B (PKB/Akt) phosphorylation is related to the upregulation of the P2Y12 receptor and NP mechanisms. Nano-curcumin blocked this pathway and relieved DNP. Thus, the nociceptive

**Fig. 2** Multitargeted TCM inhibit NP (Take the osthole’s inhibition effect of DNP as an example). Damaged nerve triggers the release of increased extracellular ATP, which causes exaggerated expression of P2X4 receptors on DRG SGCs. The following glial cells activation induces the over-production of pro-inflammatory cytokine and decline of anti-inflammatory cytokine through p38 MAPK phosphorylation. Accordingly neuropathic pain developed, osthole decreased P2X4 expression and reversed abnormal conditions of cytokine, then relieved neuropathic pain.
signaling of DRG under the condition of DNP could be inhibited by blocking the function of P2Y12 receptor in the SGCs of DRG using curcumin [79].

**Inhibitory effects of TCMs on HIV-gp120-associated NP mediated by the P2X receptors in the DRG**

HIV-1 infection contributes to various neurological disorders. Glycoprotein 120 (gp120) is a HIV envelope glycoprotein, which has neurotoxic properties and can directly stimulate the primary sensory afferent neurons, causing hyperalgesia [71]. The P2X3 receptor in the DRG has been recognized to play an important role in the pathogenesis of gp120-induced pain [72]. Curcumin is a major bioactive component of TCM turmeric, which has the effects of alleviating NP induced by streptozotocin, sciatic nerve injury, and cisplatin [72-73]. Nano-curcumin showed a treatment effect on gp120-induced NP mediated by the P2X3 receptor in rat primary afferent neurons [74]. The significantly decreasing MWT and TWL in the gp120 model were increased by nano curcumin treatment. Upregulated P2X3 expression in gp120 rat DRGs resulted in the phosphorylation of the ERK pathway and the following pain behaviors. Nano curcumin decreased the abnormal expression levels of P2X3 mRNA and protein in a rat model to reverse the activated ERK pathway and HIV-associated NP. The exaggerated amplitudes of P2X3 agonist α, β-meATP-activated currents in DRG neurons treated with gp120 were decreased in nano-curcumin-treatment. Therefore, nano curcuminum inhibit P2X3 activation, decrease the sensitizing primary afferents in DRG, and relieve mechanical and thermal hyperalgesia in gp120-treated rats [74].

The combination of gp120 and the antiretroviral drug zalcitabine (ddC) (gp120 + ddC) results in NP characterized by mechanical allodynia and upregulates TNF-α [75-76]. The anti-inflammatory molecule IL-10 can reduce HIV-related NP [77]. The upregulated P2X7 receptor expression level in DRG is closely related to NP induced by HIV [78]. Resveratrol (RES) and andrographolide (Andro) are a natural polyphenol and an active ingredient extracted from grapes and *Andrographis (Andrographis paniculata)*, respectively. Both of them have anti-inflammatory and anti-nociceptive effects. RES or Andro relieved the hyperalgesia in the gp120 + ddC rats relating to inhibition of the P2X7 receptor in the SGCs of DRG [81-82]. In gp120 + ddC rats, the MWL and TWL were reduced, which accompanied by the overexpressed P2X7 receptor in DRG. After treatment with TCM, the hyperalgesia and the expression level of P2X7 receptor were relieved and decreased. Activating SGCs of model rats released cytokines and thus augmented NP. In RES or Andro-treated rats, the enhanced co-localization intensity of the P2X7 receptor and GFAP, and the protein levels of TNF-α receptor and IL-1β were significantly decreased. Meanwhile, the decreased IL-10 protein was increased by inhibiting the evoked P2X7-ERK pathway. Moreover, RES decreased the ATP-activated currents in the HEK293 cells that were transfected with P2X7 plasmid [81-82].

**Inhibitory effect of TCM on NP transmission mediated by P2X receptors in DRG**

NP has a long duration, and treatments for NP have been less effective [80]. The upregulated expression of P2X4 receptor in the SGCs of the DRG is a key process in NP. Artemisinin, a sesquiterpene endoperoxide lactone found in the TCM plant *Artemisia annua* L., has been used clinically as an anti-malarial and an anti-tumor drug [83]. Artemisinin inhibits the nociceptive transmission, which mediated by the P2X4 receptor in the SGCs of DRG, thus, relieves pain behaviors in rats with chronic constriction injury (CCI) [84]. Consistent with the time course of NP, a dramatic increase in the expression of P2X4 in the SGCs of DRG was detected in the hyperalgesic CCI rats. Artemisinin administration reversed abnormal hyperalgesia response to heat and mechanical stimulation by reducing the expression of P2X4 in the SGCs of DRG. The SGCs were activated under the condition of nerve injury, whereas the increased co-expression staining of P2X4 and GFAP in the DRG was decreased in artemisinin-treated rats. The results also confirmed that the effects of artemisinin on attenuating chronic NP were related to decreased the expression of P2X4 in the SGCs of DRG and reduced SGC activation [85].

P2X7 receptors are localized in the SGCs of DRG and activated by extracellular ATP, promoting to the release of inflammatory cytokines and thus resulting in NP. P2X7 levels are increased in the CCI rat model [85-86]. RES can moderate the hyperalgesia after nerve injury [87]. RES suppressed the transmission of P2X7 receptor activation in CCI rats to increase the threshold of thermal or mechanical hypersensitivity. RES blocked the activation of P2X7 receptor in SGCs to diminish the following pro-inflammatory cytokine (e.g., TNF-α and IL-6) production. Moreover, RES may alleviate NP through the ERK1/2 and p38 MAPK pathways. RES antagonizes the P2X7 receptor as the P2X7 receptor agonist BzATP-activated current was inhibited in RES-treated rats. Thus, RES could inhibit the transmission of pain in rats with NP by suppressing P2X7 expression and preventing the elevation of the pain threshold after CCI [87]. In addition, it has been reported that the stimulating P2X7 receptor triggers the P2X3-mediated pain response in DRG neurons [88], suggesting the TCM acting on P2X7 receptors may be the potent target to relieve NP through further regulating the neural P2X3 receptor.

**Conclusion**

NP is a complex and heterogeneous group of disease states with limited treatment protocols. The current existing painkillers are generally accompanied by harmful side effects. Furthermore, under the lack of effective alternatives, the major abuse of opioids has become an ongoing crisis. Recently, natural products and their constitutive compounds, mainly TCMs, have been considered as a new direction for the development of analgesics to resolve NP. Different from traditional analgesics, which target a single pathway, TCMs...
take the advantage of multiple components and targets, and can significantly improve the efficacy of treatment and contribute to the prediction of the risks of NP.

P2 receptors are promising potential targets for NP therapeutics, especially for P2X3, P2X4, P2X7, and P2Y12 receptors, which play a particularly crucial role in the initiation and maintenance of NP. Therefore, studies on the P2 receptor and associated pharmacology may provide new tools and insights into the pathological process of NP and reduction of adverse effects. Based on the recent studies, this review highlight several TCMs that relieve NP by acting at P2 receptors. The characteristics and widely application of TCMs inspire that the combination of TCM and Western medicines may improve the curative effect of many diseases. Secondly, diseases prevention provides a potential focus in the further study of TCM. Still, more investigation of the detailed mechanism of NP is needed for the advanced theoretical support of pharmacological development. In future work, it is necessary not only to describe the mechanism of action of a single herb and a single component, but also to conduct in-depth research and analysis of the interaction of the combination of substances and the interaction with chemotherapy.

References


