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SINOMENINE AS A NOVEL ANALGESIC: MECHANISMS AND APPLICATIONS

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Sinomenine as a novel analgesic: mechanisms and applications
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This thesis is dedicated to my family

ABSTRACT

Chronic pain of various origins is a major health care issue affecting a large patient population, also bring significant social and economic cost on the society. Work presented in this thesis concerns novel methods of treatments for chronic pain using experimental models. Sinomenine is a chemical compound isolated originally from the root of the plant Sinomenium Acutum native to China and Japan. It is an alkaloid, structurally belongs to the morphine family. The root of Sinomenium Acutum, known as Qingteng, has been traditionally used in China as a medical remedy for condition likely to be rheumatism. Sinomenine is currently approved in China as an anti-rheumatic agent for clinical sue.

In first part of the thesis, we studied the analgesic effect of sinomenine in chronic experimental pain models of neuropathic and arthritic pain. We showed that sinomenine has significant analgesic effects in rat and mouse models of neuropathic pain as well as in a mouse model (collagen antibody-induced arthritis model, CAIA) of arthritic pain. More importantly, the effect of sinomenine on neuropathic and arthritic pain is maintained upon repeated chronic administration without signs of tolerance or dependence.

In the second part of the thesis, we examined the possible application of sinomenine as an analgesic, we showed that combination with sinomenine with gabapentin, a clinically used drug treating neuropathic pain, produced marked synergistic interaction in rat and mouse models of neuropathic pain and such synergism can still be observed upon repeated administration without signs of tolerance and dependence. We can also show a similar synergistic interaction between gabapentin and dextromethorphan, a low affinity non-competitive NMDA antagonist. The work presented in this thesis suggested that sinomenine could be explored as a novel analgesic in treating neuropathic and arthritic pain. The results also showed combination therapy involving sinomenine, gabapentin and dextromethorphan might be useful in the clinic. The potential mechanisms for the effect of sinomenine and its interaction with other analgesics need to be further studied.

Key words: Sinomenine, Neuropathic Pain, Arthritic Pain, Gabapentin, Dextromethorphan, Tolerance

LIST OF SCIENTIFIC PAPERS

I. Gao T, **Shi T**, Wang D-Q, Wiesenfeld-Hallin Z, Xu X-J.
Repeated sinomenine administration alleviates chronic neuropathic pain-like behaviors in rodents without producing tolerance.
Scand J Pain 2014; 5(4): 249-255.

II. Gao T, **Shi T**, Wiesenfeld-Hallin Z, Svensson CI, Xu X-J.
Sinomenine alleviates mechanical hypersensitivity in mice with experimentally induced rheumatoid arthritis.
Scand J Pain 2015; 7: 9-14.

III. **Shi T**, Gao T, Wang D-Q, Wiesenfeld-Hallin Z, Hao J-X, Xu X-J.
Synergistic interaction between sinomenine and gabapentin in treating neuropathic pain.
Manuscript.

IV. **Shi T,** Hao J-X Wiesenfeld-Hallin Z, Xu X-J.
Gabapentin and NMDA receptor antagonists interacts synergistically to alleviate allodynia in two rat models of neuropathic pain.
Scand J Pain 2018; Epub.

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
AA	Adjuvant arthriti
ANOVA	Analysis of variance
C II	Collagen type II
CAIA	Collagen antibody-induced arthritis
CIA	Collagen-induced arthritis
CNS	Central nervous system
COX	Cyclooxygenase
CRPS	Complex regional pain syndrome
DA	Dopamine
DMARDs	Disease-modifying antirheumatic drugs
DMSO	Dimethyl sulfoxide
DOPAC	Dihydroxyphenylacetic acid
DRG	Dorsal root ganglion
GABA	γ -Aminobutyric acid
HLA	Human leukocyte antigen
i.p.	Intraperitoneally
i.v.	Intravenous
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
LSD	Least Significant Difference
MAD	Median absolute deviation
MMPs	Metalloproteinases
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NSAIDs	Non-steroidal anti-inflammatory drugs
p.o.	Per os

p38MAPK	P38 mitogen-activated protein kinases
PGE2	Prostaglandin E2
PNS	Peripheral nervous system
RA	Rheumatoid arthritis
s.c.	Subcutaneously
SCI	Spinal cord injury
SD	Sprague-Dawley
SEM	Standard error of the mean
SIN	Sinomenine
TNF	Tumor necrosis factor
VDCC	Voltage-dependent calcium channels

1 INTRODUCTION

1.1 CLASSIFICATION OF PAIN

Pain has been known to mankind from the very beginning and is perhaps the oldest medical problem. Hippocrates, the great Greek physician, suggested that pain may cause by disturbance of the four-body humor's (blood, phlegm, yellow bile and black bile). He even wrote that willow leaves and bark can relieve pain which we know today contain salicylic acid. Yet, until very recently, little is known about the physiology of pain and the underlying mechanisms of pain management. Such advancement has been helped in large by our improved understanding of the classification of pain.

There are many ways to classify pain which can be based on, for example, location reference, and type of tissue involved or duration of pain. The most important classification of pain, one that bares major implication for mechanisms and treatments, is based on the causes of pain which can be divided into three major categories: nociceptive pain, inflammatory pain and neuropathic pain (Woolf, 2004).

Nociceptive pain is an unpleasant sensory experience generated by noxious stimuli that is potentially tissue damaging. Noxious stimuli can have various qualities (i.e. mechanical, heat, cold stimuli or chemical). The ability of the organisms to sense nociceptive stimuli is essential for its survival to prevent imminent tissue damage in the environment. Therefore, nociceptive pain plays a significant role as alarm system. In most cases, nociceptive pain is acute because once noxious stimuli was removed, the pain stops (Scholz & Woolf, 2002).

Inflammatory pain is a pain associated with inflammatory process such as tissue trauma, ischemia, infections, tumor growth and autoimmune diseases. A variety of chemical mediators such as cytokines, growth factors, kinase, purines, amines, prostanoid and protons release from damaged/inflammatory cells (Boddeke, 2001; Mantyh et al., 2002) that either directly activate nociceptors or increase the sensitivity of sensory nervous system to facilitate pain perception (Scholz & Woolf, 2002). Duration of inflammatory pain can be either acute, intermediate or chronic.

Neuropathic pain is a pain initiated or caused by a primary lesion or dysfunction of the nervous system (Costigan et al., 2009; Merskey & Bogduk, 1994). Such lesion or dysfunction can occur in the peripheral nerve system such as in the cases of metabolic disorders, mechanical trauma, neurotoxic chemicals, infection or tumor invasion (Decosterd et al., 2002). On the other hand, in situations such as spinal cord injury, stoke, or multiple sclerosis, central neuropathic pain

may occur (Ducreux et al., 2006). In the majority of cases, neuropathic pain is chronic, difficult to manage and inevitably associated with plastic changes of the nervous system (Hokfelt et al., 1994). The characteristic of neuropathic pain may include spontaneous pain, allodynia and hyperalgesia (Woolf & Mannion, 1999).

1.2 CHRONIC PAIN

Any pain that lasts more than three months is usually considered as chronic pain. Chronic pain is a major health problem accompany a large number of diseases involving a board patient population. Common conditions, such as rheumatoid arthritis (RA), diabetes and cancer are often associated with severe pain that may last a large part of patient's life span. The same is also true for chronic neuropathic pain (Andersson, 2004; Breivik et al., 2006). Chronic pain brings much suffering because of its severity and persistence. In European countries, 19% of adults suffer from moderate to severe intensity chronic pain, which seriously reduce their life quality of patients' affected (Breivik et al., 2006). The treatment of chronic pain, especially the origin of neuropathic pain, remains an urgent challenge. Although research in animal models have been developed for long term, few analgesics are available to treat the neuropathic pain, and do not reach the effective level of pain relief in many patients. Further, long-term application of analgesics may lead to drug tolerance, dependence and abuse.

1.2.1 Neuropathic pain

Nerve damage at any level, be it central or peripheral, could cause the neuropathic pain. Neuropathic pain also arises from any numbers of particular conditions (Jensen et al., 2001) , such as post-herpetic neuralgia, nerve root avulsion, diabetic neuropathy, central neuropathic pain syndrome, post-operative pain syndrome (such as post-mastectomy syndrome, phantom limb pain) and complex regional pain syndrome (CRPS) (Colloca et al., 2017). In addition to ongoing pain which can have various qualities, typical symptom of neuropathic pain may also include sensory loss, sensory disturbances as well as other paresthesia such as hyperesthesia, hyperalgesia and allodynia. Pain and other sensory symptoms in neuropathic pain generally last a long time and often persist even after primary lesions have recovered.

1.2.2 Chronic pain in Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory, autoimmune disease in which the patient's immune system attacks its own (mostly in this case) joint tissues (Smolen & Steiner, 2003). Smaller joints of the hands and feet are often first affected and as disease progresses, other larger joints or other tissues may also be affected. Symptoms usually include pain, swelling, and stiffness with various dysfunctions. Rheumatoid arthritis affects 0.5-1.0% world

population and most common prevalent in patients between 20-50 years old. A large portion of patients with rheumatoid arthritis (RA) patients consider pain as one of their top three priorities (Heiberg et al., 2005). Gender is an important factor with female patients are about three times as many as male patients (Scott et al., 2010). Genetic inheritance also play an important role in rheumatoid arthritis with the HLA-DRB1 genotype identified in the pathogenesis of rheumatoid arthritis (Liu et al., 2016; Scott et al., 2010; Wolfe & Michaud, 2007). The joint dysfunction and pain is RA patients are often not due to the direct damage of cartilage or bone, but rather indirect effect of the autoimmune reaction affecting the inner layer of the joint capsule by inflammatory mediators such as prostaglandins and cytokines. It will activate and sensitize nociceptors in the joints, leading to spontaneous joint pain, pain associated with movement or on palpation and hyperalgesia (Bas et al., 2012). With the progression of diseases, it will eventually damage the structure of joint cartilage, bone and nerves, producing chronic regional and/or wide spread pain in a significant percentage of these patients (Andersson et al., 2013). Pain in many patients is neuropathic pain in nature. Fibromyalgia is particular type of chronic widespread pain and In RA patients, it has significantly higher prevalence than general population (Lee, 2013).

In order to achieve effective relief of symptoms or reduce the disease process, rheumatoid arthritis treatment usually begins in the early stage. The primary goal is to improve the quality of life by reducing pain and inflammation, while slowing or preventing the development of permanent damage. Many drugs, particularly the new biologic drugs, have shown significant anti-inflammatory effect, preventing the development of inflammation by blocking the cytokines pathway or action of immune cells. However, these drugs have unintended side effects, such as an increased risk of infection. Despite this, pain management, especially for chronic pain, is still a challenge in many RA patients (Andersson et al., 2013; Lee, 2013).

1.3 ANIMAL MODELS OF CHRONIC PAIN

The development and continuous improvement of the animal pain models is important for study of the mechanisms and treatments of chronic pain. In the field of neuropathic pain, the chronic nerve constriction model as reported by Bennett and Xie (1988) (Bennett & Xie, 1988) was very important as they showed that it is possible to produce a partial injury to the sciatic nerve, enabling studies of stimulation induced behaviors similar to human conditions of hyperalgesia and allodynia. As a result, a large number of animal models has been developed using a variety of methods to induce injury in different nerves, greatly facilitating the experimental research on neuropathic pain (Xu & Wiesenfeld-Hallin, 2003).

Our laboratory has developed several rodent models of neuropathic pain after ischemic injury to the spinal cord, sciatic nerve and infraorbital nerve and many features of these models are similar to clinical pain conditions in patients with neuropathic pain conditions, such as spinal cord injury pain, diabetic neuropathy and trigeminal neuralgia. The ischemic injury is produced by an intravascular photochemical interaction between a photosensitizing dye and a laser beam, leading to the generation of singlet oxygen radicals at the endothelial cells of capillaries and subsequent platelet aggregation within the blood vessels in the irradiated tissues (Kupers et al., 1998; Watson et al., 1986). Using this technique, our laboratory developed one of the first animal model of central neuropathic pain after spinal cord injury (SCI) (Xu et al., 1992). Thus, after spinal ischemic injury rats developed marked pain-like behaviors to mechanical and cold stimulation in the dermatomes corresponding to injured spinal segments which is similar to SCI patients(Hao et al., 1991, 1992; Xu et al., 1992; Xu et al., 1994). This model has been used to test the efficacy of a large number of analgesics against central neuropathic pain. The photochemical technique has also been used in producing partial sciatic nerve injury in rats and mice (Hao et al., 2000a; Kupers et al., 1998) and infraorbital nerve injury in rats (Eriksson et al., 2005). We have used these models in the work presented in this thesis.

Several rodent models of RA are available primarily based on the pathophysiological mechanisms of RA. The adjuvant arthritis (AA) model is produced by injecting Freund's complete adjuvant into susceptible strains of rats resulting in a T cell-mediated autoimmune arthritis. In contrast, the collagen-induced arthritis (CIA) model is produced in some strains of mice by immunization with an emulsion of complete Freund's adjuvant and type II collagen (CII), resulting in autoimmune arthritis. In the work presented in this thesis, we have used a novel mouse model of RA, the collagen antibody-induced arthritis (CAIA) model. The CAIA model is produced by injecting a cocktail of monoclonal antibodies targeted against type II collagen followed by lipopolysaccharide (LPS) immunization (Nandakumar & Holmdahl, 2007). With this model, the local joint pathology resembles that observed in RA patients, and there is a robust development of pain-like behaviors in these mice (Bas et al., 2012). There are several important advantages of the CAIA model comparing to others. The mice in the CAIA model is usually in good overall health. There is a shortened disease duration as it bypasses the natural development of anti-collagen antibodies. The CAIA can also be generated in many strains of mice that are resistant in other models (Nandakumar & Holmdahl, 2007).

1.4 ANALGESICS IN NEUROPATHIC AND INFLAMMATORY PAIN

Although multimodal treatments are often used to manage pain in the clinics, such as analgesics, physical therapy and psychotherapy. The clinical management of pain still relies largely on the use of analgesics of different classes. Neuropathic pain is often shown to be resistant to available pharmacological agents. The non-steroidal anti-inflammatory drugs (NSAIDs), being the most commonly used pain medicines in the world, have no significant effect in neuropathic pain. Opiates, the ultimate strong analgesic, have limited effects against neuropathic pain in patients (Arner & Meyerson, 1988) while also producing significant side effects, such as respiratory depression and constipation. Long term administration of opiates is known to be associated with the development of tolerance, dependence and abuse.

The first line of analgesics prescribed today to treat neuropathic pain is gabapentin and pregabalin (Attal et al., 2010). They are structurally related to the neurotransmitter gamma-aminobutyric acid (GABA), but effects of these two drugs are not mediated by the GABAergic system. It is generally believe that a significant part of their action is mediated by an interaction with the α2δ subunit of the voltage-dependent L-type calcium channel in the CNS, thereby suppressing neuronal excitability and decreasing the release of neurotransmitters. The approved clinical indication for gabapentin and/or pregabalin in neuropathic pain include diabetic neuropathy, post-herpetic neuralgia, central pain and fibromyalgia. However, it is worth noting that these drugs can only achieve partial relief of neuropathic pain in a not too large proportion of patients (Gordh et al., 2008; Serpell, 2002).

Another class of analgesics that has been suggested to be useful in neuropathic pain are antagonists of the NMDA receptors, particularly those of non-competitive and low affinity nature such as the over-the-counter antitussive dextromethorphan (Hao & Xu, 1996). However, despite positive results from a large number of preclinical studies, there is still a lack of convincing clinical evidence for analgesic effect of this type of drugs in neuropathic pain(Aiyer et al., 2018).

Chronic RA pain is also known to be difficult to manage. As at present, there is no cure for RA, most treatments can only alleviate symptoms, prevent further joint damage, restore muscle capacity and increase the mobility of the RA patients. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as diclofenac acid or cyclooxygenase-2 inhibitors (COX-2 inhibitors) can be used to alleviate pain, swelling and stiffness caused by rheumatoid arthritis. However, the effectiveness of these drugs in chronic and severe RA pain is questionable (Andersson et al., 2013; Steiman et al., 2013; Taylor et al., 2010). Depending on the severity and progression of

the disease, today's doctors often applied disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate, sulphasalazine, and leflunomide (Lee, 2013). However, these drugs do not provide adequate pain relive against chronic pain in the RA patients (Andersson et al., 2013). In addition, because of significant side effects, most drugs of this type are not suitable for large doses or long-term use (Andersson et al., 2013; Lee, 2013; Whittle et al., 2012).

1.5 SINOMENINE

Sinomenine is an alkaloid (Fig. 1) isolated from the root of Sinomenium acutum (It is a climbing plant native to China and Japan, known as Fang-ji or Qing-teng in Chinese. The entire Sinomenium acutum plant has been used as traditional Chinese medicine to treat rheumatoid arthritis, arrhythmia and neuralgia (Yamasaki, 1976). The usage of sinomenine as a medicine was first recorded in a 16th century book called Ben-Cao-Gang-Mu (Compendium of Materia Medica). Structurally, sinomenine is a morphine derivative and related to dextromethorphan (Plesan et al., 1998).

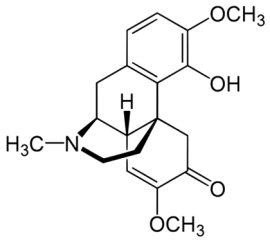

Fig1. Chemical structure of sinomenine

1.5.1 Sinomenine and pain

Our laboratory and others have reported in recent years that sinomenine has board-spectrum analgesic effect to relieve nociceptive, inflammatory and neuropathic pain in rodent models (Gao et al., 2013a). It has been reported that long-term pretreatment with sinomenine reduces the analgesic tolerance to morphine (Wang et al., 2008). In addition, sinomenine could significantly decrease the level of 5-HT, dopamine(DA), DOPAC, 5-HIAA, noradrenaline content in striatal extracellular fluid in neuropathic pain rat model induced by sciatic nerve ligation (Zhang et al., 2013).

In collagen induced arthritis (CIA) mice, treatment with sinomenine decreased the incidence and severity of disease (Feng et al., 2007). In comparison with NSAIDs, sinomenine significantly improve rheumatoid factor in patients, especially against morning stiffness, painful joints and erythrocyte sedimentation rate (Xu et al., 2008). Clinical studies also

demonstrated that sinomenine administered by subcutaneous injection or oral administration was effective in relieving pain in acute and chronic rheumatoid arthritis (Yamasaki, 1976).

1.5.2 The immunoregulatory effect of sinomenine

Sinomenine has distinct immunoregulatory function involving histamine, pro-inflammatory cytokines, COX2 dependent prostaglandin E2, INF-γ, reactive oxygen species (ROS), NO and the activity of NF-κB, p38MAPK and metalloproteinases (MMPs) (Huang et al., 2017; Yang et al., 2016; Yuan et al., 2018). Tumor necrosis factor (TNF) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that involved in the development of chronic pain. Sinomenine can effectively reduce the TNF level in activated microglia and microphages (Huang et al., 2008; Liu et al., 1994). Sinomenine can also suppress the generation of COX2 / PGE2, i.e. it can reduce synthesis of PGE2 by lipopolysaccharide (LPS) treated macrophages both in vitro and in vivo (Liu et al., 1994). In enriched microglia, Sub-picomolar concentrations of Sinomenine significantly inhibited PGE2 production and COX-2 mRNA expression (Qian et al., 2007).

In CIA mice, treatment with sinomenine reduces the levels of anti-CII and IgG and suppresses the antigen–specific splenocyte proliferation (Huang et al., 2007). In addition, Sinomenine inhibits transforming growth factor beta (TGF-β) or IL-1β induced proliferation of synovial fibroblasts in rats with experimental arthritis and reduces the expression of IL-2 receptor (Liu et al., 1996). Sinomenine also inhibit the proliferation of human CD4+T cell (Cheng et al., 2009) and murine macrophages RAW 264.7 by inducing apoptosis in a dose and time-dependent manner through activation of ERK (He et al., 2005). Finally, sinomenine inhibits lymphocytes proliferation and antibody production by B-cells (He et al., 2005). The potent anti-inflammatory and neuroprotective effect of sinomenine is thought to be mediated through inhibition of microglial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-generated superoxide (Qian et al., 2007). These immunoregulatory properties of sinomenine may account for its efficacy in treating RA. In a microarray study, sinomenine was found to be able to suppress expressions of 17 genes including IL-6, IL-13, IFITMI, TNFRII, TNF-A, PIGF, Daxx, and HSP27 in IL-1β activated T cells (Li et al., 2006).

2 AIMS OF THE STUDY

The present thesis project is built on the initial finding of the analgesic effect of sinomenine and has the following specific aims:

1. To study the development of tolerance to the anti-nociceptive effect of long term application of sinomenine in rodent models of neuropathic pain.
2. To evaluate analgesic effect of sinomenine against experimental arthritic pain in mice following acute and chronic administration.
3. To study synergistic interactions between sinomenine and gabapentin in treating neuropathic pain in rodent models.
4. To compare such interaction with the effect of NMDA receptor antagonists and gabapentin in treating neruopathic pain.

3 MATERIALS AND METHODS

3.1 ANIMALS

The animal experiments that have been used in the studies presented in this thesis were conducted with the aim to model different clinical pain conditions. We have strictly followed the IASP ethical guidelines and all the experiments were approved by the local animal research ethics committee in Stockholm. All mice (C57BL/6 mice, male, Charles River, Sollentuna, Sweden; CBA mice, female, Harlan, Horst, The Netherlands) and rats (Sprague-Dawley rats, female, Harlan, Horst, The Netherlands; Möllegård, Denmark) were housed 6 or 4 per cage, respectively, and in standard laboratory condition (22℃ 12 hours' light/dark cycle) with ad libitum access to water and food.

3.2 NERVE INJURY ANIMAL MODELS

3.2.1 Photo-chemically induced sciatic nerve injury in mice and rats

Pervious papers have described the methods of producing sciatic nerve ischemic injury mice and rats model (Hao et al., 2000a; Kupers et al., 1998). Firstly, 75 mg/kg ketamine with 1 mg/kg medetomidine were used to anaesthetize animals. Then, the photosensitizing dye erythrosine B (Red N°3, Aldrich-Chemie, Steinheim, Germany) at dosage of 32.5 mg/kg was injected from intravenous. After that, left sciatic nerve was exposed and irradiated using an argon ion laser (514 nm, 160 mW, Innova model 70, Coherent Laser Product Division, Palo Alto, CA, USA) 45s for mice or 2 min for rats. A heating pad was used to keep the animals' body temperature between 37-38℃ during the period of the irradiation. After the irradiation, surgical incision was carefully sutured layer by layer and animals were returned and cared in their home cage.

3.2.2 Photo-chemically induced spinal cord injury in rats

Pervious paper has described the methods of producing spinal cord injury rat model from our lab (Hao et al., 1992). Firstly, 75 mg/kg ketamine with 1 mg/kg medetomidine were used to anaesthetize rats. Then, a midline incision was made over T12-L1 vertebral segments. After i.v. injection via tail vein of erythrosine B at dosage of 32.5mg/kg, rats were irradiated with an argon ion laser for 10 min on the vertebral segments T12-T13. The i.v. injection of dye was repeated once after 5 minutes of irradiation. A heating pad was used to keep body temperature of rats between 37-38℃ during the period of the irradiation. After the irradiation, surgical incision was carefully sutured layer by layer and animals were returned and cared in their home cage.

3.3 INFLAMMATORY PAIN MODEL

3.3.1 Collagen antibody induced arthritis model (CAIA)

CBA female mice (Harlan, Horst, Netherlands) were used in the study. The arthritis was induced by i.v. injection of 5 monoclonal Collagen type II antibodies cocktail (0.15ml, Chondrex, USA) on day 0. Lipopolysaccharide (LPS, 35 μg, Sigma) was administered by intraperitoneal (i.p.) injection on day 5 (Bas et al., 2012; Khachigian, 2006). After the antibody injection, inflammation appeared from day 6 and resolved after day 33. The control groups were injected with either saline or only with LPS did not show any signs of inflammation. Compare to the widely-used Collagen-induced arthritis (CIA) model (Brand et al., 2007), CAIA model has more rapid and synchronized onset time of joint inflammation which was similar with the pathogenesis of RA in the clinic.

3.4 BEHAVIOR ASSESSMENT

3.4.1 Paw withdrawal threshold to mechanical stimulation in sciatic nerve injury mice and rats

The withdrawal threshold of hind paw to mechanical stimulation was tested using a set of calibrated von Frey hairs (Stoelting, IL, USA). To test the sensitivity of mechanical stimulation, mice or rats were place in plastic cages with a metal mesh floor. After 30 min to 1hour habituation, the plantar surface of the hind paws was stimulated with increasing force (from 0.02g to 4g for mice and 0.02g to 10g for rats) until the animal withdrew the limb. The application of each monofilament last 1-2 seconds and was 5 times in total and threshold was determined when the animal withdrew the paw at least 3 out of 5 consecutive stimuli.

3.4.2 Measurement of spread mechanical allodynia in spinal cord injury rats

Vocalization thresholds to measured mechanical stimulation were tested with a set of von Frey hairs (Stoelting, Chicago, IL, USA) in rats. To perform the test, firstly, rats were restrained in a standing position on the table gently. Then, the Von Frey hair was pushed onto the skin until the filament became bent. The skin area was in dermatomes rostral to the irradiated spinal segment-the upper or lower back. The frequency of stimulation was about 1 time per second. In each intensity of Von Frey hair, the stimuli were applied 5 to10 times. Pain threshold was considered as intensity of Von Frey hair which induced consistent vocalization (>75% response rate). The cut-off values was 100g.

The response of rats to brushing stimulation was tested by using blunt pencil which gently stroked the skin on the trunk in a rostro-caudal direction. The frequency of the brush stimuli was about 1 time per second and responses were graded with a score as follows: 0 = no

observable response (usually normal rats exhibited no reaction); 1 = transient vocalization and moderate effort to avoid stimuli; 2 = consistent vocalization and aversive reactions and 3 = sustained and prolonged vocalization and aggressive behaviors.

3.4.3 Measurement of mechanical hypersensitivity and arthritis scoring in CAIA mice

The Paw withdrawal threshold of the ipsilateral hind paw to mechanical stimulation was tested by using a set of von Frey hairs (Stoelting, IL, USA). The method of testing was similar to that used in the sciatic nerve injury mice. Up-down method (Chaplan et al., 1994) was used to calculate the force that cause the 50% probability of paw withdrawal in CAIA mice. The cut-off value was 4 g. For measuring spread mechanical hypersensitivity in CAIA model, the mice were gently restrained in a standing position on the table. The von Frey hairs were pushed onto skin of flanks and upper back areas until the filament became bent. The frequency of stimulation was about 1/s, and in each intensity of von Frey hair, the stimuli were applied 5 to10 times. The intensity of von Frey hair which induced consistent avoiding or offensive behaviors (>60% respond rate) was considered as paw withdrawal threshold. The cut-off value was 100g on the flank and upper back areas.

The arthritis score was used to present the development of join inflammation in the fore and hind paws. The evaluation method was visual inspection as described previously (Nandakumar et al., 2003). The evaluation was scored every three days after antibody cocktail injection. The scoring was based on the inflamed joints' number. 1 point was given for each inflamed toe or knuckle, 5 points were given for each inflamed ankle or wrist, the maximum arthritis score for each paw was 15 points and for each mouse was 60 points.

3.4.4 Measurement of cold hypersensitivity in rats and mice

Ethyl chloride spray (Rönnings Europa AB, Sweden) was used to exam the responses to cold hypersensitivity in rats. The cold hypersensitivity response was graded with a score as follows: 0 = no observable response; 1 = localized response (skin twitch and contraction), no vocalization; 2 = transient vocalization, moderate struggle and 3 = sustained vocalization and aggression. Normal rats usually had response score of 0 or 1. Acetone was used to examine the cold responses which gently applied onto the plantar surface of the hind paws in mice. The response was graded with a score as follows: 0 = no responses; 1 = startle response without paw withdrawal, 2 =withdraw of the stimulated hind paw, 3 = sustained withdraw of hind paw combined with flitching and licking and/or vocalization.

Recently, a quantitative testing of responsiveness to cold sensitivity in spinal cord injury rats was developed (Gao et al., 2013b). A Peltier thermode was applied to provide cooling stimuli to the flank area. A liquid cooling, hand held Peltier thermode (active surface: 25mm×50mm, control resolution: >0.02 ◦C, calibration uncertainty: ±0.2 ◦C) was connected with Modular Sensory Analyzer Thermal Stimulator (Somedic, Sweden). The starting temperature was from 32 ℃ and temperature changing rate was 0.5 ℃ per second. Rats were held in a standing position. Then the Peltier thermode was gently pressed onto the shaved flank area. Three cooling stimuli were applied and gap between each stimuli was a least 1 min. The average temperature when the rats vocalized and withdrew were considered as cold response threshold. Cut-off temperature value was 6 ℃.

3.4.5 Open field test for motor deficits

Motor deficits were evaluated by using a combined neurological score tests including motor score (observed in an open field), righting reflex and extension withdrawal (Hao et al., 1996). Total travel distance and rearing numbers of rats and mice were also recorded during the open field observation. The neurological score for evaluation of motor function was graded as follows:

GRADE	DESCRIPTION	POINTS
Motor score		
0	Normal walking	0
1	Walks with only mild deficit	5
2	Hind limb can support weight	15
3	Frequent movement of hind limb, no weight bearing	25
4	Minor movement in hind limb, no weight bearing	40
5	No movement of hind limb, no weight bearing	45
Righting		
0	Normal righting counter to direction of the roll	0
1	Weakened attempt to right	5
2	Delayed to right itself	10
3	No attempt to right	15
Extension withdrawal		0
0	Normal	2.5
1	Weak and slow reflex to withdraw the hind limb	5
2	No withdraw reflex	

3.5 DRUGS

Sinomenine was obtained from the National Institute for Food and Drug Control (Beijing, China, purity > 99%). It was dissolved in DMSO (Sigma-Aldrich) by the volume rate 1:4. The solution was then added with Cremophor EL oil (Sigma-Aldrich) using the volume rate of 1:5. Vortex mixer (Bibby Scientific, UK) was used to mix the solution. Any further dilution was

made with saline. Gabapentin, dextromethorphan and MK-801 were obtained from Research Biochem Inc (Natick, MA, USA) and were dissolved in saline. For single dose administration, drugs was apply via oral administration (p.o.) or subcutaneous injection (s.c.) in mice and intraperitoneal injection (i.p.) in rats. For repeated administration, drugs was applied twice per day at 10AM and 16PM for 5days. For drug combination administration, different drugs were injected separately into different body parts to avoid blend.

3.6 STATISTICS

All the experiments were double-blind experiments. Data were presented as mean ± standard error of mean (SEM) or median ± median absolute deviation (MAD). The data were analyzed following paired t-test, Wilcoxon Signed rank test, Mann-Whitney U test or analysis of variance (ANOVA) with repeated measures followed by Fisher's Least Significant Difference (LSD). All data and figures were presented by Prism (GraphPad Software) and statistics were made by SPSS (IBM software), $P<0.05$ was considered to be statistically significant.

4 RESULT

4.1 REPEATED SINOMENINE ADMINISTRATION ALLEVIATES CHRONIC NEUROPATHIC PAIN-LIKE BEHAVIOURS IN RODENTS WITHOUT PRODUCING TOLERANCE (PAPER I)

4.1.1 Repeated administration of sinomenine alleviates pain-like behaviors in spinally injured rats

Rats developed chronic hypersensitivity to mechanical (von-Frey hair and brush) and cold stimulation after spinal cord injury. Saline as single or repeated injections has no effect on both mechanical and cold hypersensitivity (Fig 1A, 2A, 3A, Paper I). A single dose of i.p. administration of sinomenine at 10 or 20 mg/kg also had no analgesic effects on responses to mechanical or cold stimulation in SCI rats (Figs. 1-3, Paper I). This is similar as being reported in previous study (Gao et al., 2013a). On the other hand, repeated administration of sinomenine twice per day at 10 mg/kg increased vocalization threshold to mechanical stimulations and reduced response score to brushing from day 2 to day 5 of treatment (Fig 1B, 2B, Paper I). Repeated administration of sinomenine twice per day at 10 mg/kg had no effect on cold hypersensitivity (Fig 1 C, Paper I).

Twice a day repeated administration of sinomenine at 20 mg/kg markedly reduced mechanical hypersensitivity (Fig.1 C, Paper I) and brushing (Fig.2 C, Paper I) from day 2 to day 5 during repeated administration. Moreover, pre-treatment vocalization threshold to mechanical stimuli was significantly increased from day 2 (Fig.1 C, Paper I) which remained significantly elevated at least another 4 days after suspension of sinomenine administration (Fig.1C, Paper I). The pre-treatment brush score was also significantly decreased from day 4 to day 6 after sinomenine administration (Fig. 2C, Paper I). Repeated sinomenine at 20mg /kg did not effect on cold hypersensitivity (Fig.3 C, Paper I).

Repeated administration of sinomenine at 40mg/kg twice a day could effectively alleviates mechanical hypersensitivity to stimuli with von Frey hair and brushing (Fig.1 D, Fig.2 D, Paper I). Pre-treatment vocalization threshold was significantly increased from day 2 to day 9, which is again 4 days after the last sinomenine injection (Fig.1 D, Paper I). The vocalization threshold retuned pre-treatment baseline on day 12, 7 days after termination of injection (Fig.1 D, Paper I). The hypersensitivity to brush stimuli was also reversed on days 2, 4 and 5 by repeated sinomenine application (Fig.2 D, Paper I). The threshold of cold temperature was significantly decreased on day 1 and 2, and pre-treatment cold was significantly decreased compared to baseline level from day 2 to day 9 (Fig.3 D, Paper I), indicating a reduction in cold allodynia at this dose.

4.1.2 Repeated administration of sinomenine sciatic nerve injury in mice.

Saline had no effect on paw withdraw threshold (Fig.4 A, Paper I) or cold response (Fig.4 C, Paper I). Repeated administration of sinomenine orally at 80 mg/kg twice a day for 5 days in mice could significantly increase paw withdraw threshold from day 1 to day 5 (Fig. 4 B, Paper I) and reduced cold hypersensitivity (Fig. 4D, Paper I). The baseline of paw withdrawal threshold before drug administration was also significantly increased from day 2 to day 12, therefore maintaining for 7 days after the last drug application (Fig.4 B, Paper I).

4.1.3 Other behavioral effects of sinomenine

We did not observe any significant motor or other behavioral effects of sinomenine at these doses following single or repeated administration. There are no withdrawal symptoms in rats or mice following the termination of chronic sinomenine administration, including irritation, hypersensitivity or decrease in body weight, suggesting that sinomenine did not produce physical dependence.

4.2 SINOMENINE ALLEVIATES MECHANICAL HYPERSENSITIVITY IN MICE WITH EXPERIMENTALLY INDUCED RHEUMATOID ARTHRITIS (PAPER II)

4.2.1 The effect of sinomenine against mechanical hypersensitivity of the hind paw

CAIA mice developed mechanical hypersensitivity of the hind paw in association with the development of joint inflammation, both at the acute inflammatory phase (days 11-19 after CII antibody injection) and at the post inflammatory phase (days 35-54 after CII antibody injection). During the acute and post- inflammatory phase of CAIA mice, sinomenine dose-dependently reduced mechanical hypersensitivity in the hind paws via subcutaneous injection for up to 3 hours after drug administration (Fig.1 A, B, Paper II). The effects were significant at doses of 40 or 80 mg/kg.

4.2.2 The effect of sinomenine against spread mechanical hypersensitivity

In addition to localized hypersensitivity at the hind paw, a spread mechanical hypersensitivity could also observed at the neck and flanks areas in CAIA mice at both acute-inflammatory and post-inflammatory phase. Sinomenine dose-dependently alleviate the spread mechanical hypersensitivity during the first 3–4 hours after drug administration with effective doses of 40 or 80 mg/kg (Fig.2 A&B, Paper II).

4.2.3 No side effects were observed following single-dose sinomenine administration

In order to investigate the potential side effects produced by single-dose sinomenine administration, we applied open field test among three groups of mice: naive mice group, naive mice injected with saline (1h before test), and naive mice injected with 80 mg/kg sinomenine (1h before test). Duration of passivity, moving distance, rearing time and rectal temperature were observed during this experiment.

There were no significant changes of the passivity duration which indicated no obvious allergy or sedation after sinomenine application (Fig.3 A, Paper II). In addition, locomotor activities quantified by passed grid numbers and rearing time (Fig.3 B & C, Paper II) were also not significantly changed after sinomenine administration. Furthermore, rectal temperature was also not significantly changed after sinomenine treatment compared to naive or saline treatment group, suggesting that there was no severe allergy (Fig.3 D, Paper II).

4.2.4 Effect of repeated administration of sinomenine

During acute-inflammatory phase (days 11–15 after CII antibody injection), repeated sinomenine administration twice a day at dose of 80 mg/kg for 5 days, had no significant effect to alleviate the arthritis scores in comparison to saline treated CAIA mice (Fig.4 A, Paper II). After peak-inflammatory phase, the arthritis scores was slowly dissipated in the mice from day 30 to day 54. During post-inflammatory phase (days 49–53), repeated sinomenine administration twice a day at 80mg/kg for 5 days had no effect on the arthritis scores (Fig.4 A, Paper II).

During the peak of inflammation (days 11-15 after CII antibody administration), repeated administration of sinomenine at 80 mg/kg twice a day last for 5 days significantly alleviate the localized mechanical hypersensitivities in the hind paws (Fig.4 B, Paper II) and the spread mechanical hypersensitivity in back and neck regions (Fig. 4 C, Paper II) compared to corresponding pre-drug baseline on each day. After start of repeated sinomenine application, the baseline of mechanical hypersensitivity was significantly increased from day 2 and remained significantly for another 3 days after the suspension of drug application (Fig. 4B, C, Paper II).

During the post-inflammatory phase (days 49-53 after CII antibody administration), repeated sinomenine administration at 80 mg/kg twice a day significantly alleviated localized and spread mechanical hypersensitivity on the hind paws (Fig.4 B, Paper II). After start of repeated sinomenine application, the baseline of mechanical hypersensitivity of hind paw

was significantly increased from day 2, but baseline of spread hypersensitivity significantly increased only from day 5 (Fig.4 B, C, Paper II). The analgesic effect persisted for another day after the suspension of drug application. The experiments were terminated on day 54 according to a pre-determined schedule (Fig. 4 B, C, Paper II). No side effects were recorded both on acute inflammatory and post-inflammatory phases during repeated drug application.

4.3 SYNERGISTIC INTERACTION BETWEEN SINOMENINE AND GABAPENTIN IN TREAT-ING NEUROPATHIC PAIN (PAPER III)

4.3.1 Synergistic analgesia effect of sinomenine and gabapentin in a mouse model of peripheral nerve injury.

The response of paw-withdraw threshold was monitored up to 240 min and tested in every 30-60 mins after single or combination of drug administration (Fig. 1A, Paper III). A single dose of p.o. administration of sinomenine at 20 mg/kg has no significant effect to mechanical stimulation in mice with sciatic nerve injury. A single dose of i.p. administration gabapentin at 30 mg/kg has also no significant effect.

In low dose combination, when gabapentin was first injected at dosage of 7.5mg/kg, with sinomenine administrated at dosage of 10 mg/kg 30-60 min later, no analgesic effect was observed up to 4 hours (Fig. 1B, paper III). However, when sinomenine was applied first and then gabapentin was administrated 30-60 min later, there different combinations produced significant analgesic effect up to 3 hours (Fig. 1C, paper III). These dosages of combination were: sinomenine 10mg/kg + gabapentin 7.5mg/kg, sinomenine 20mg/kg + gabapentin 7.5mg/kg and sinomenine 20mg/kg + gabapentin 15mg/kg (Fig. 1C, paper III). No side effect was observed in all combination groups at these dosages. Further, the synergistic interaction effect was dose-dependent.

4.3.2 Synergistic analgesia effect of sinomenine and gabapentin in rats with spinal cord injury.

The experiments were applied 4-5 weeks after spinal cord ischemic injury in rats, the analgesic index is vocalization threshold to mechanical stimulation (grams) or cold scores to cold stimulation. A single dose administration of sinomenine at 20 mg/kg or gabapentin at 30mg/kg did not produce significant analgesia effect against mechanical or cold stimulation in SCI rats (Fig. 2A and 2B, Paper III).

In contrast, when applied in combination, sinomenine and gabapentin by produced marked antiallodynic effects in spinally injured rats. There were three dosages of combination tested in the experiment: sinomenine 5mg/kg + gabapentin 2mg/kg, sinomenine 10mg/kg + gabapentin

4mg/kg and sinomenine 20mg/kg + gabapentin 7.5mg/kg. All with sinomenine pretreated 30-60 min before gabapentin. Further, the synergistic interaction effect was dose-dependent with higher dose combination producing larger and longer effect (Fig. 2C and 2D, Paper III). Similarly, simultaneous administration of 10mg/kg sinomenine and 4mg/kg gabapentin could also produce significant synergistic analgesia effect to against mechanical hypersensitivity and cold allodynia (Fig. 3A and 3B, Paper III). No side effect was observed in all drug combination groups.

4.3.3 Synergistic analgesia effect of repeated administration of sinomenine and gabapentin in rats with spinal cord injury.

Repeated combination injection of 10mg/kg i.p. sinomenine + 4mg/kg i.p. gabapentin twice/day were used to study the analgesic effect of chronic administration of the drug combination in SCI rats. In two rounds of experiments, this combination were applied twice daily (10AM and 16PM) for 7days and 14 days. Sinomenine was applied 30-60 min before gabapentin with dosing interval of 6 hours per day. The analgesic index is vocalization threshold to mechanical stimulation (grams). The repeated combination produced significant analgesic effect against mechanical stimulation. More importantly, during the chronic administration, the pain threshold before the administration was also significantly increased, which indicates that the drug combination produced a sustained effect. Two days after termination of last drug administration, the pain threshold of rats returned to pre-treatment level (Fig. 4, Paper III). No observable side effect in rats were produced during repeated administration.

4.4 GABAPENTIN AND NMDA RECEPTOR ANTAGONISTS INTERACTS SYNERGISTICALLY TO ALLEVIATE ALLODYNIA IN TWO RAT MODELS OF NEUROPATHIC PAIN (PAPER IV)

4.4.1 Synergistic analgesia effect in spinal cord injury rats

In spinally injured rats with mechanical and cold hypersensitivity, we examined the effects of combination of gabapentin and two NMDA receptor antagonists. Single dose i.p. administration of dextromethorphan at 20 mg/kg or gabapentin at 30 mg/kg did not alleviate allodynia (Fig. 1-3, Paper IV). Increasing the dosage of dextromethorphan or gabapentin produce side effect, including sedation and motor impairment for gabapentin and hyperactivity for dextromethorphan. In contrast, the low dosage combination gabapentin with dextromethorphan: gabapentin 7.5mg/kg + dextromethorphan 5mg/kg, gabapentin 15mg/kg + dextromethorphan 10mg/kg, and gabapentin 30mg/kg + dextromethorphan 10mg/kg significantly increased the vocalization threshold to von-Frey stimulation (Fig. 1, Paper IV),

reduced and normalized brushing score (Fig. 2, Paper IV) or cold score(Fig.3, Paper IV). The synergistic effect of dextromethorphan and gabapentin is long lasting, but reversible. No side effect was observed, such as sedation, motor impairments or hyperactivity at these combinations at low doses.

Similarly, i.p. administration MK-801 at dosage of 0.05 or 0.1 mg/kg did not affect mechanical allodynia-like behavior in SCI rats. In contrast, the low dosage combination of MK-801 (0.01 or 0.05 mg/kg) together with 15 mg/kg gabapentin could significantly increase vocalization threshold to von-Frey stimulation (Fig. 4, Paper IV).

4.4.2 Synergistic analgesia effect in sciatic nerve injury rats

Sciatic nerve injury rats developed mechanical hypersensitivity of hind paw after irradiation after 1-2 weeks. Single dose of i.p. administated dextormethorphan at 4 0mg/kg or gabapentin at 100 mg/kg did not affect paw withdrawal threshold in SNI rats despite the presence of side effects (Fig. 5, Paper IV). In contrast, the combination of 30mg/kg gabapentin with 20 mg/kg dextromethorphan significantly increased paw withdrawal threshold to mechanical stimuli in rats with sciatic nerve injury (Fig. 5, Paper IV). No side effect was observed in this combination.

5 DISCUSSION

5.1 REPEATED SINOMENINE ADMINISTRATION ALLEVIATES CHRONIC NEUROPATHIC PAIN WITHOUT TOLERANCE

Previous studies by others and us showed that sinomenine exhibited antinociceptive effect in a wide range of rat and mice models (Gao et al., 2013a; Gao et al., 2014; Rao et al., 2017; Zhu et al., 2016; Zhu et al., 2014). Work presented in this thesis expanded these results by showing that repeated administration of sinomenine produced persistent effects in rat and mice models of neuropathic pain with signs of tolerance. Pre-drug response threshold was significantly increased after two injections, which also maintained for some days after the termination of drug administration. Similar to our previous results in both rats and mice (Gao et al., 2013a), sinomenine seems has less effect to against cold allodynia than mechanical hypersensitivity. Importantly, during or after repeated administration of sinomenine, there were no side effects such as sedation, motor impairment or irritation was observed. Previous study has shown that daily administration of sinomenine at dosage of 40 or 80 mg/kg in rats for two weeks did not influence growth, appetite and blood pressure (Hson-Mou Chang, 1986). No withdrawal symptoms were observed after the termination of repeated sinomenine application. These results suggest that sinomenine has great potential to treat chronic neuropathic pain with low tolerance and abuse.

Repeated administration of sinomenine produced similarly effect on neuropathic pain-like behaviors in our models compare to the anti-epileptics lacosamide and gabapentin previously studied in our laboratory (Hao et al., 2006; Hao et al., 2000b; Wu et al., 2004). Repeated administration of gabapentin could increase analgesic effect compare to the doses that were ineffective as a single injection (Hao et al., 2000b). Similarly, repeated lacosamide increased pre-drug baseline responses (Hao et al., 2006). On the other hand, systemic morphine application could not alleviate allodynia in SCI rat models. Intrathecal morphine application could have some effect to anti the allodynia, but tolerance was rapidly observed on the second day after drug application(Yu et al., 1997).

One major effect of sinomenine in our SCI rats and SNI mice models is that it reduced pre-drug baseline hypersensitivity following repeated administration. Sinomenine has a relatively short half-life in rat plasma (Liu et al., 1996). It is therefore unlikely that this effect against baseline hypersensitivity following repeated injections is due to an accumulation of the drug. The metabolites of sinomenine are known to be present in at least three forms (Cheng et al., 2007). However, it is unclear whether these metabolites are pharmacologically active or has antinociceptive effect. It is likely that the effects of repeated sinomenine administration reflect

sustained physiological changes resulting from repeated drug treatment. Such changes are, however, reversible since pain recurred within days following the last dose of sinomenine.

The mechanism of action for the effect of sinomenine in models of neuropathic pain is not clear. It is not mediated by the opioid pathway through the μ-receptor since naloxone (opioid receptor antagonist) could not reverse anti-allodynic effect of sinomenine (Gao et al., 2013a) and the profile of analgesia produced by sinomenine is different from that of systemic morphine (Bulka et al., 2002; Yamasaki, 1976). Interestingly, the effect of sinomenine is similar to a non-opioid antitussive dextromethorphan, which is also a weak noncompetitive NMDA receptor antagonist (Hao & Xu, 1996). One possible mechanism for the effect of sinomenine may be related to its ability to modulate neurotransmitter release in the central nervous system. Sinomenine upon systemic injection affects the level of monoamines in extracellular fluid in the striatum in rats with sciatic nerve injury, raising the level of noradrenaline while decreasing in level of dopamine and serotonin. These effects have been shown to be correlated with analgesic effect (Zhang et al., 2013). Chronic nerve injury may produce long lasting effects on transmitter synthesis and neuronal functions which may be altered by sinomenine. In addition, sinomenine also has distinct immunoregulatory properties, reducing the production of cyclooxygenase (COX)-2 dependent prostaglandin E2 (PGE2) (Liu et al., 1994), as well as reduce nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and p38 mitogen-activated protein kinases (p38MAPK) signal pathways (Huang et al., 2008; Wang et al., 2005). Sinomenine also has neuroprotective effect, decreasing the production of superoxide ions through inhibiting the microglial NADPH oxidase (Qian et al., 2007). Finally, previous studies have shown that some of the effects of sinomenine may be mediated through $GABA_A$ receptor (Zhu et al., 2014) or the acid-sensing ion channel and calcium channels (Wu et al., 2011). Any or some of these effects may reduce neuronal sensitization contributing to the effect of sinomenine against neuropathic pain.

5.2 THE EFFECT OF SINOMENINE ON EXPERIMENTAL RHEUMATOID ARTHRITIS

Sinomenine has long been used in China and Japan as an anti-rheumatic drug (Yamasaki, 1976). The efficacy of sinomenine against RA is well established in rodent RA models (Huang et al., 2007; Liu et al., 1996) and clinically, sinomenine has been shown in some studies to be more effective than NSAIDs in ameliorating morning stiffness, painful joints and erythrocyte sedimentation rate (Xu et al., 2008). Sinomenine reduces the production of proinflammatory cytokines by suppressing the activation of NF-κB (Cheng et al., 2009; Wang et al., 2005; Zhou et al., 2008). Sinomenine also reduces the increase of inflammatory mediators such as TNF and

IL1-β following inflammation (Wang et al., 2005). These mechanisms may contribute to the effect of sinomenine against RA.

In study II, repeated sinomenine administration twice per day for 5 days could not alter arthritic scores in the CAIA mice at the acute-inflammatory phase, which means that in our CAIA model, sinomenine did not have a direct effect against the disease progression of RA. This is likely to due to the model used. The CAIA model, by directly injecting anti-CII arthritogenic antibody cocktail to trigger arthritis, bypasses step of T-cell activation as required in the CIA model. Thus, the anti-rheumatic effect reported in the previous animal studies may suggest that the effect of sinomenine may be due to inhibition of T-cell activation. Nonetheless, single or repeated administration of sinomenine effectively and dose-dependently alleviated the localized and spread mechanical hypersensitivity during both acute- and post-inflammatory phases without producing side effects. Thus, it is likely that the analgesic mechanism of sinomenine is independent from possible anti-inflammatory action of the compound in RA and our results also suggest that some of clinical efficacy of sinomenine may in fact be due to its analgesic effect. No tolerance was observed by repeated administration of sinomenine to the analgesic effect, which was similar to neuropathic pain models (Gao et al., 2014).

The effect of sinomenine against pain-like behaviors in the CAIA model may be due to multiple factors. It has been shown previously there is a marked activation of microglia in the spinal cord of CAIA mice in comparison to control mice (Bas et al. 2012). Sinomenine can interact with neuro-immune crosstalk by suppressing microglia activation (Qian et al., 2007; Shukla & Sharma, 2011) which may be the primarily mechanism explaining the effect of sinomenine in the CAIA model. However, sinomenine produced a range of other pharmacological effects in the nervous system which may also be involved in the analgesic mechanisms in arthritic pain. These effects may include previously mentioned immunoregulative properties and actions on systems such as histamine, proinflammatory cytokines, COX2 dependent PGE2, interferon gamma (INF-γ), reactive oxygen species (ROS), nitric oxide (NO), NF-κB, p38MAPK, metalloproteinases (MMPs) and TNF. Further studies of the effects of sinomenine on neuronal and immune systems may lead to better understanding of the mechanisms of analgesia by sinomenine in mice model of RA.

5.3 SYNERGISTIC INTERACTION BETWEEN SINOMENINE AND GABAPENTIN IN TREATING NEUROPATHIC PAIN

The management of chronic pain is one of the most important health issues with limited options today. In the present study, we showed that combined administration of small doses of sinomenine and gabapentin produced marked synergistic interaction in rodent models of

neuropathic pain, far superior than each drug administered alone. Thus, in rodent models of neuropathic pain, adequate pain reduction could only be observed with the dosages at 100 mg/kg for gabapentin (Hao et al., 2000b) and 40 mg/kg (Gao et al., 2013a) for sinomenine, doses of drugs that are already producing some side effects. When two drugs were combined however, much less doses of gabapentin and sinomenine, can have strong anti-nociceptive effect without producing side effects, indicating that gabapentin and sinomenine can dramatically potentiate each other's analgesic efficacy in combination. Further, repeated administration of the combination of sinomenine and gabapentin also produced significant effect, increasing the baseline threshold before drug administration, with no observable side effects and tolerance. These findings raise the feasibility of using the combination of sinomenine and gabapentin as a novel therapy in neuropathic pain.

There are several possible mechanisms through which that sinomenine and gabapentin act synergistically to reduce neuropathic pain. Both compounds are able to modulate and/or partially block calcium channels (Striano & Striano, 2008; Wu et al., 2011) and there are some evidence that they both enhance GABAergic neural inhibition (Kuzniecky et al., 2002; Zhu et al., 2014). There are also evidences that morphine and dextromethorphan can potentiate the effect of gabapentin on chronic pain in the clinic (Gilron et al., 2005). In the last study of this thesis, we showed that this is also the case for dextromethorphan and gabapentin in our experimental model of neuropathic pain. The structure of sinomenine is related to morphine and dextromethorphan (Yamasaki, 1976), it is tempting to suggest that some of the effect of sinomenine may be mediated through pathways related to the effect of dextromethorphan..

Finally, it is worth noting that time appears to be an important factor when it comes to combined drug administration of sinomenine and gabapentin. Thus, the best effect is obtained when sinomenine was pretreated 30-60 min before gabapentin. The reason for this is unclear, maybe metabolic in nature or related to the neuronal actions of these compounds.

5.4 GABAPENTIN AND NMDA RECEPTOR ANTAGONISTS INTERACTS SYNERGISTICALLY TO ALLEVIATE PAIN-LIKE BEHAVIORS IN TWO RAT MODELS OF NEUROPATHIC PAIN

In a fashion very similar to sinomenine, the work presented in the last paper of this thesis showed that combining NMDA receptor antagonists, parimarily dextromethorpahn and gabapentin produced synergistic effect aginst pain-like behaviors in the rat models of neuropathic pain after spinal cord or sciatic nerve injury. The effect of the combination is again synergistic rather than additive in comparison to previous studies with using either drugs alone (Hao & Xu, 1996; Xu et al., 2001). At effective doses, the combination did not produce

increased side effects. The mechanisms by which synergism between dextromethorphan and gabapentin occurs are unclear. As mentioned above, the analgesic effect of gabapentin may be related to its interaction to the α2δ subunit of the voltage-dependent calcium channels (VDCCs) (Gee et al., 1996; Rose & Kam, 2002). Thus, such synergism may be derived from a reduction in calcium entry through simultaneous blockade of VDCCs and NMDA receptor/channels. It is interesting to note therefore that gabapentin per se often produced limited effect on various types of Ca^{2+} currents (Fink et al., 2000; Martin et al., 2002; Rock et al., 1993; Stefani et al., 1998). Such interaction may also occur directly at the NMDA receptor complex. The anti-hyperalgesic effect of gabapentin was blocked by D-serine which is an agonist at the glycine site of the NMDA receptor (Jun & Yaksh, 1998; Singh et al., 1996). Gabapentin is also able to reduce the release of glutamate that may also contribute to its interaction with NMDA receptor antaognists (Dooley et al., 2000; Maneuf et al., 2001). Since MK-801 also enhances the effect of gabapentin, it is unlikely that such interaction take place solely at the VDCCs.

The synergism between dextromethorphan and gabapentin appears to be larger in spinally injured rats than in rats with sciatic nerve injury. This is similar to our previous results that both drugs are less potent in the periphery vs. central model (Hao & Xu, 1996; Hao et al., 2000b). This suggest that the combination of dextromethorphan and gabapentin may be particularly useful in treating spinal cord injury pain which is a difficult clinical problem (Yezierski, 2002). In support, one earlier clinical study has shown that combination of dextromethorphan and gabapentin alleviated neuropathic pain in patients with spinal cord injury (Sang, 2002).

6 CONCLUSIONS

1. The anti-allodynic effect of sinomenine upon repeated chronic administration enhanced its effect without tolerance in two rodents model of neuropathic pain. No side effects can be observed from the persistent, reversible analgesia.
2. Sinomenine is effective in alleviating localized and spread hypersensitivities in CAIA mice both during acute inflammation and in postinflammatory phase. Repeated administration of sinomenine has increasd the pre-drug baseline threshold to mechanical stimuli without producing tolerance. The results in paper I and II may suggest potential clinical application of sinomenine as a novel analgesic in chronic neuropathic and arthritic pain management.
3. The combined therapy of sinomenine and gabapentin has promising synergic effect in alleviating neuropathic pain with less dosage and reduced side effects. The repeated administration of combined therapy also produced significant analgesic effect without introducing tolerance.
4. The combined therapy of NMDA receptor antagonists and gabapentin could provide synergic effect alleviating neuropathic pain with increased efficacy and reduced side effects. This study, together with paper III, indicate that compared to single agent, the combined therapy entails benefits including improved analgesic effectiveness and reduced adverse effects with smaller doses of individual drugs. Sinominine may have similar analgesic mechanism with NMDA receptor antagonists. However, such drug composition and the application criteria need to be further validated in clinical studies before it can be widely used.

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8 REFERENCES

Aiyer, R., Mehta, N., Gungor, S., & Gulati, A. (2018). A Systematic Review of NMDA Receptor Antagonists for Treatment of Neuropathic Pain in Clinical Practice. *Clin J Pain, 34*(5), 450-467. doi:10.1097/AJP.0000000000000547

Andersson, H. I. (2004). The course of non-malignant chronic pain: a 12-year follow-up of a cohort from the general population. *Eur J Pain, 8*(1), 47-53. doi:10.1016/S1090-3801(03)00064-8

Andersson, M. L., Svensson, B., & Bergman, S. (2013). Chronic widespread pain in patients with rheumatoid arthritis and the relation between pain and disease activity measures over the first 5 years. *Journal of Rheumatology, 40*(12), 1977-1985. doi:10.3899/jrheum.130493

Arner, S., & Meyerson, B. A. (1988). Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. *Pain, 33*(1), 11-23.

Attal, N., Cruccu, G., Baron, R., Haanpaa, M., Hansson, P., Jensen, T. S., . . . European Federation of Neurological, S. (2010). EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. *Eur J Neurol, 17*(9), 1113-e1188. doi:10.1111/j.1468-1331.2010.02999.x

Bas, D. B., Su, J., Sandor, K., Agalave, N. M., Lundberg, J., Codeluppi, S., . . . Svensson, C. I. (2012). Collagen antibody-induced arthritis evokes persistent pain with spinal glial involvement and transient prostaglandin dependency. *Arthritis Rheum, 64*(12), 3886-3896. doi:10.1002/art.37686

Bennett, G. J., & Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain, 33*(1), 87-107.

Boddeke, E. W. (2001). Involvement of chemokines in pain. *Eur J Pharmacol, 429*(1-3), 115-119.

Brand, D. D., Latham, K. A., & Rosloniec, E. F. (2007). Collagen-induced arthritis. *Nat Protoc, 2*(5), 1269-1275. doi:10.1038/nprot.2007.173

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain, 10*(4), 287-333. doi:10.1016/j.ejpain.2005.06.009

Bulka, A., Plesan, A., Xu, X. J., & Wiesenfeld-Hallin, Z. (2002). Reduced tolerance to the anti-hyperalgesic effect of methadone in comparison to morphine in a rat model of mononeuropathy. *Pain, 95*(1-2), 103-109.

Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M., & Yaksh, T. L. (1994). Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods, 53*(1), 55-63.

Cheng, W. M., Qiu, F., & Yao, X. S. (2007). Three major urinary metabolites of sinomenine in rats. *J Asian Nat Prod Res, 9*(1), 13-18. doi:10.1080/10286020500289444

Cheng, Y., Zhang, J., Hou, W., Wang, D., Li, F., Zhang, Y., & Yuan, F. (2009). Immunoregulatory effects of sinomenine on the T-bet/GATA-3 ratio and Th1/Th2 cytokine balance in the treatment of mesangial proliferative nephritis. *Int Immunopharmacol, 9*(7-8), 894-899. doi:10.1016/j.intimp.2009.03.014

Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A. H., Yarnitsky, D., . . . Raja, S. N. (2017). Neuropathic pain. *Nat Rev Dis Primers, 3*, 17002. doi:10.1038/nrdp.2017.2

Costigan, M., Scholz, J., & Woolf, C. J. (2009). Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci, 32*, 1-32. doi:10.1146/annurev.neuro.051508.135531

Decosterd, I., Ji, R. R., Abdi, S., Tate, S., & Woolf, C. J. (2002). The pattern of expression of the voltage-gated sodium channels Na(v)1.8 and Na(v)1.9 does not change in uninjured primary sensory neurons in experimental neuropathic pain models. *Pain, 96*(3), 269-277.

Dooley, D. J., Mieske, C. A., & Borosky, S. A. (2000). Inhibition of K(+)-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. *Neurosci Lett, 280*(2), 107-110.

Ducreux, D., Attal, N., Parker, F., & Bouhassira, D. (2006). Mechanisms of central neuropathic pain: a combined psychophysical and fMRI study in syringomyelia. *Brain, 129*(Pt 4), 963-976. doi:10.1093/brain/awl016

Eriksson, J., Jablonski, A., Persson, A. K., Hao, J. X., Kouya, P. F., Wiesenfeld-Hallin, Z., . . . Fried, K. (2005). Behavioral changes and trigeminal ganglion sodium channel regulation in an orofacial neuropathic pain model. *Pain, 119*(1-3), 82-94. doi:10.1016/j.pain.2005.09.019

Feng, H., Yamaki, K., Takano, H., Inoue, K., Yanagisawa, R., & Yoshino, S. (2007). Effect of sinomenine on collagen-induced arthritis in mice. *Autoimmunity, 40*(7), 532-539. doi:10.1080/08916930701615159

Fink, K., Meder, W., Dooley, D. J., & Gothert, M. (2000). Inhibition of neuronal Ca(2+) influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. *Br J Pharmacol, 130*(4), 900-906. doi:10.1038/sj.bjp.0703380

Gao, T., Hao, J., Wiesenfeld-Hallin, Z., Wang, D. Q., & Xu, X. J. (2013a). Analgesic effect of sinomenine in rodents after inflammation and nerve injury. *Eur J Pharmacol, 721*(1-3), 5-11. doi:10.1016/j.ejphar.2013.09.062

Gao, T., Hao, J. X., Wiesenfeld-Hallin, Z., & Xu, X. J. (2013b). Quantitative test of responses to thermal stimulation in spinally injured rats using a Peltier thermode: a new approach to study cold allodynia. *J Neurosci Methods, 212*(2), 317-321. doi:10.1016/j.jneumeth.2012.11.008

Gao, T., Shi, T., Wang, D.-Q., Wiesenfeld-Hallin, Z., & Xu, X.-J. (2014). Repeated sinomenine administration alleviates chronic neuropathic pain-like behaviours in rodents without producing tolerance. *Scandinavian Journal of Pain, 5*(4), 249-255. doi:https://doi.org/10.1016/j.sjpain.2014.05.006

Gee, N. S., Brown, J. P., Dissanayake, V. U., Offord, J., Thurlow, R., & Woodruff, G. N. (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J Biol Chem, 271*(10), 5768-5776.

Gilron, I., Bailey, J. M., Tu, D. S., Holden, R. R., Weaver, D. F., & Houlden, R. L. (2005). Morphine, gabapentin, or their combination for neuropathic pain. *New England Journal of Medicine, 352*(13), 1324-1334. doi:DOI 10.1056/NEJMoa042580

Gordh, T. E., Stubhaug, A., Jensen, T. S., Arner, S., Biber, B., Boivie, J., . . . Kalso, E. (2008). Gabapentin in traumatic nerve injury pain: a randomized, double-blind,

placebo-controlled, cross-over, multi-center study. *Pain, 138*(2), 255-266. doi:10.1016/j.pain.2007.12.011

Hao, J. X., Blakeman, K. H., Yu, W., Hultenby, K., Xu, X. J., & Wiesenfeld-Hallin, Z. (2000a). Development of a mouse model of neuropathic pain following photochemically induced ischemia in the sciatic nerve. *Exp Neurol, 163*(1), 231-238. doi:10.1006/exnr.2000.7373

Hao, J. X., Stohr, T., Selve, N., Wiesenfeld-Hallin, Z., & Xu, X. J. (2006). Lacosamide, a new anti-epileptic, alleviates neuropathic pain-like behaviors in rat models of spinal cord or trigeminal nerve injury. *Eur J Pharmacol, 553*(1-3), 135-140. doi:10.1016/j.ejphar.2006.09.040

Hao, J. X., & Xu, X. J. (1996). Treatment of a chronic allodynia-like response in spinally injured rats: effects of systemically administered excitatory amino acid receptor antagonists. *Pain, 66*(2-3), 279-285.

Hao, J. X., Xu, X. J., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1991). Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain, 45*(2), 175-185.

Hao, J. X., Xu, X. J., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1992). Photochemically induced transient spinal ischemia induces behavioral hypersensitivity to mechanical and cold stimuli, but not to noxious-heat stimuli, in the rat. *Exp Neurol, 118*(2), 187-194.

Hao, J. X., Xu, X. J., Urban, L., & Wiesenfeld-Hallin, Z. (2000b). Repeated administration of systemic gabapentin alleviates allodynia-like behaviors in spinally injured rats. *Neurosci Lett, 280*(3), 211-214.

Hao, J. X., Yu, W., Xu, X. J., & Wiesenfeld-Hallin, Z. (1996). Effects of intrathecal vs. systemic clonidine in treating chronic allodynia-like response in spinally injured rats. *Brain Res, 736*(1-2), 28-34.

He, X., Wang, J., Guo, Z., Liu, Q., Chen, T., Wang, X., & Cao, X. (2005). Requirement for ERK activation in sinomenine-induced apoptosis of macrophages. *Immunol Lett, 98*(1), 91-96. doi:10.1016/j.imlet.2004.10.027

Heiberg, T., Finset, A., Uhlig, T., & Kvien, T. K. (2005). Seven year changes in health status and priorities for improvement of health in patients with rheumatoid arthritis. *Ann Rheum Dis, 64*(2), 191-195. doi:10.1136/ard.2004.022699

Hokfelt, T., Zhang, X., & Wiesenfeld-Hallin, Z. (1994). Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends Neurosci, 17*(1), 22-30.

Hson-Mou Chang, P. P.-H. B. (1986). *Pharmacology and Applications of Chinese Materia Medica* (Vol. 1).

Huang, F., Yamaki, K., Tong, X., Fu, L., Zhang, R., Cai, Y., . . . Yoshino, S. (2008). Inhibition of the antigen-induced activation of RBL-2H3 cells by sinomenine. *Int Immunopharmacol, 8*(3), 502-507. doi:10.1016/j.intimp.2007.12.009

Huang, J., Lin, Z., Luo, M., Lu, C., Kim, M. H., Yu, B., & Gu, J. (2007). Sinomenine suppresses TNF-alpha-induced VCAM-1 expression in human umbilical vein endothelial cells. *J Ethnopharmacol, 114*(2), 180-185. doi:10.1016/j.jep.2007.07.036

Huang, L., Dong, Y., Wu, J., Wang, P., Zhou, H., Li, T., & Liu, L. (2017). Sinomenine-induced histamine release-like anaphylactoid reactions are blocked by tranilast via inhibiting NF-kappaB signaling. *Pharmacol Res, 125*(Pt B), 150-160. doi:10.1016/j.phrs.2017.08.014

Jensen, T. S., Gottrup, H., Sindrup, S. H., & Bach, F. W. (2001). The clinical picture of neuropathic pain. *Eur J Pharmacol, 429*(1-3), 1-11.

Jun, J. H., & Yaksh, T. L. (1998). The effect of intrathecal gabapentin and 3-isobutyl gamma-aminobutyric acid on the hyperalgesia observed after thermal injury in the rat. *Anesthesia and Analgesia, 86*(2), 348-354.

Khachigian, L. M. (2006). Collagen antibody-induced arthritis. *Nat Protoc, 1*(5), 2512-2516. doi:10.1038/nprot.2006.393

Kupers, R., Yu, W., Persson, J. K., Xu, X. J., & Wiesenfeld-Hallin, Z. (1998). Photochemically-induced ischemia of the rat sciatic nerve produces a dose-dependent and highly reproducible mechanical, heat and cold allodynia, and signs of spontaneous pain. *Pain, 76*(1-2), 45-59.

Kuzniecky, R., Ho, S., Pan, J., Martin, R., Gilliam, F., Faught, E., & Hetherington, H. (2002). Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology, 58*(3), 368-372.

Lee, Y. C. (2013). Effect and treatment of chronic pain in inflammatory arthritis. *Curr Rheumatol Rep, 15*(1), 300. doi:10.1007/s11926-012-0300-4

Li, X. J., Yue, P. Y., Ha, W. Y., Wong, D. Y., Tin, M. M., Wang, P. X., . . . Liu, L. (2006). Effect of sinomenine on gene expression of the IL-1 beta-activated human synovial sarcoma. *Life Sci, 79*(7), 665-673. doi:10.1016/j.lfs.2006.02.014

Liu, L., Buchner, E., Beitze, D., Schmidt-Weber, C. B., Kaever, V., Emmrich, F., & Kinne, R. W. (1996). Amelioration of rat experimental arthritides by treatment with the alkaloid sinomenine. *Int J Immunopharmacol, 18*(10), 529-543.

Liu, L., Riese, J., Resch, K., & Kaever, V. (1994). Impairment of macrophage eicosanoid and nitric oxide production by an alkaloid from Sinomenium acutum. *Arzneimittelforschung, 44*(11), 1223-1226.

Liu, W. X., Jiang, Y., Hu, Q. X., & You, X. B. (2016). HLA-DRB1 shared epitope allele polymorphisms and rheumatoid arthritis: a systemic review and meta-analysis. *Clin Invest Med, 39*(6), E182-E203.

Maneuf, Y. P., Hughes, J., & McKnight, A. T. (2001). Gabapentin inhibits the substance P-facilitated K(+)-evoked release of [(3)H]glutamate from rat caudial trigeminal nucleus slices. *Pain, 93*(2), 191-196.

Mantyh, P. W., Clohisy, D. R., Koltzenburg, M., & Hunt, S. P. (2002). Molecular mechanisms of cancer pain. *Nat Rev Cancer, 2*(3), 201-209. doi:10.1038/nrc747

Martin, D. J., McClelland, D., Herd, M. B., Sutton, K. G., Hall, M. D., Lee, K., . . . Scott, R. H. (2002). Gabapentin-mediated inhibition of voltage-activated Ca2+ channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. *Neuropharmacology, 42*(3), 353-366.

Merskey, H., & Bogduk, N. (1994). Classification of Chronic pain. 2nd Edition.

Nandakumar, K. S., & Holmdahl, R. (2007). Collagen antibody induced arthritis. *Methods Mol Med, 136*, 215-223.

Nandakumar, K. S., Svensson, L., & Holmdahl, R. (2003). Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. *Am J Pathol, 163*(5), 1827-1837. doi:10.1016/S0002-9440(10)63542-0

Plesan, A., Hedman, U., Xu, X. J., & Wiesenfeld-Hallin, Z. (1998). Comparison of ketamine and dextromethorphan in potentiating the antinociceptive effect of morphine in rats. *Anesthesia and Analgesia, 86*(4), 825-829.

Qian, L., Xu, Z., Zhang, W., Wilson, B., Hong, J. S., & Flood, P. M. (2007). Sinomenine, a natural dextrorotatory morphinan analog, is anti-inflammatory and neuroprotective through inhibition of microglial NADPH oxidase. *J Neuroinflammation, 4*, 23. doi:10.1186/1742-2094-4-23

Rao, S., Liu, S., Zou, L., Jia, T., Zhao, S., Wu, B., . . . Liang, S. (2017). The effect of sinomenine in diabetic neuropathic pain mediated by the P2X3 receptor in dorsal root ganglia. *Purinergic Signal, 13*(2), 227-235. doi:10.1007/s11302-016-9554-z

Rock, D. M., Kelly, K. M., & Macdonald, R. L. (1993). Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons. *Epilepsy Res, 16*(2), 89-98.

Rose, M. A., & Kam, P. C. (2002). Gabapentin: pharmacology and its use in pain management. *Anaesthesia, 57*(5), 451-462.

Sang, C. N. (2002). Glutamate receptor antagonists in central neuropathic pain following spinal cord injury. In R. P. Yezierski, Burchiel, K.J (Ed.), *Spinal Cord Injury Pain: Assessment, Mechanisms, Managements* (Vol. 23, pp. 365-377). Seattle: IASP Press.

Scholz, J., & Woolf, C. J. (2002). Can we conquer pain? *Nat Neurosci, 5 Suppl*, 1062-1067. doi:10.1038/nn942

Scott, D. L., Wolfe, F., & Huizinga, T. W. (2010). Rheumatoid arthritis. *Lancet, 376*(9746), 1094-1108. doi:10.1016/S0140-6736(10)60826-4

Serpell, M. G. (2002). Gabapentin in neuropathic pain syndromes: a randomised, double-blind, placebo-controlled trial. *Pain, 99*(3), 557-566.

Shukla, S. M., & Sharma, S. K. (2011). Sinomenine inhibits microglial activation by Abeta and confers neuroprotection. *J Neuroinflammation, 8*, 117. doi:10.1186/1742-2094-8-117

Singh, L., Field, M. J., Ferris, P., Hunter, J. C., Oles, R. J., Williams, R. G., & Woodruff, G. N. (1996). The antiepileptic agent gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by D-serine. *Psychopharmacology (Berl), 127*(1), 1-9.

Smolen, J. S., & Steiner, G. (2003). Therapeutic strategies for rheumatoid arthritis. *Nat Rev Drug Discov, 2*(6), 473-488. doi:10.1038/nrd1109

Stefani, A., Spadoni, F., & Bernardi, G. (1998). Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology, 37*(1), 83-91.

Steiman, A. J., Pope, J. E., Thiessen-Philbrook, H., Li, L., Barnabe, C., Kalache, F., . . . Bykerk, V. (2013). Non-biologic disease-modifying antirheumatic drugs (DMARDs) improve pain in inflammatory arthritis (IA): a systematic literature review of randomized controlled trials. *Rheumatol Int, 33*(5), 1105-1120. doi:10.1007/s00296-012-2619-6

Striano, P., & Striano, S. (2008). Gabapentin: a Ca2+ channel alpha 2-delta ligand far beyond epilepsy therapy. *Drugs Today (Barc), 44*(5), 353-368. doi:10.1358/dot.2008.44.5.1186403

Taylor, P., Manger, B., Alvaro-Gracia, J., Johnstone, R., Gomez-Reino, J., Eberhardt, E., . . . Kavanaugh, A. (2010). Patient perceptions concerning pain management in the treatment of rheumatoid arthritis. *J Int Med Res, 38*(4), 1213-1224. doi:10.1177/147323001003800402

Wang, M. H., Chang, C. K., Cheng, J. H., Wu, H. T., Li, Y. X., & Cheng, J. T. (2008). Activation of opioid mu-receptor by sinomenine in cell and mice. *Neurosci Lett, 443*(3), 209-212. doi:10.1016/j.neulet.2008.07.088

Wang, Y., Fang, Y., Huang, W., Zhou, X., Wang, M., Zhong, B., & Peng, D. (2005). Effect of sinomenine on cytokine expression of macrophages and synoviocytes in adjuvant arthritis rats. *J Ethnopharmacol, 98*(1-2), 37-43. doi:10.1016/j.jep.2004.12.022

Watson, B. D., Prado, R., Dietrich, W. D., Ginsberg, M. D., & Green, B. A. (1986). Photochemically induced spinal cord injury in the rat. *Brain Res, 367*(1-2), 296-300.

Whittle, S. L., Richards, B. L., van der Heijde, D. M., & Buchbinder, R. (2012). The efficacy and safety of opioids in inflammatory arthritis: a Cochrane systematic review. *J Rheumatol Suppl, 90*, 40-46. doi:10.3899/jrheum.120341

Wolfe, F., & Michaud, K. (2007). Assessment of pain in rheumatoid arthritis: minimal clinically significant difference, predictors, and the effect of anti-tumor necrosis factor therapy. *Journal of Rheumatology, 34*(8), 1674-1683.

Woolf, C. J. (2004). Pain: moving from symptom control toward mechanism-specific pharmacologic management. *Ann Intern Med, 140*(6), 441-451.

Woolf, C. J., & Mannion, R. J. (1999). Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet, 353*(9168), 1959-1964. doi:10.1016/S0140-6736(99)01307-0

Wu, W. N., Wu, P. F., Chen, X. L., Zhang, Z., Gu, J., Yang, Y. J., . . . Chen, J. G. (2011). Sinomenine protects against ischaemic brain injury: involvement of co-inhibition of acid-sensing ion channel 1a and L-type calcium channels. *Br J Pharmacol, 164*(5), 1445-1459. doi:10.1111/j.1476-5381.2011.01487.x

Wu, W. P., Hao, J. X., Ongini, E., Impagnatiello, F., Presotto, C., Wiesenfeld-Hallin, Z., & Xu, X. J. (2004). A nitric oxide (NO)-releasing derivative of gabapentin, NCX 8001, alleviates neuropathic pain-like behavior after spinal cord and peripheral nerve injury. *Br J Pharmacol, 141*(1), 65-74. doi:10.1038/sj.bjp.0705596

Xu, M., Liu, L., Qi, C., Deng, B., & Cai, X. (2008). Sinomenine versus NSAIDs for the treatment of rheumatoid arthritis: a systematic review and meta-analysis. *Planta Med, 74*(12), 1423-1429. doi:10.1055/s-2008-1081346

Xu, X. J., Alster, P., Wu, W. P., Hao, J. X., & Wiesenfeld-Hallin, Z. (2001). Increased level of cholecystokinin in cerebrospinal fluid is associated with chronic pain-like behavior in spinally injured rats. *Peptides, 22*(8), 1305-1308.

Xu, X. J., Hao, J. X., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1992). Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. *Pain, 48*(2), 279-290.

Xu, X. J., Hao, J. X., Seiger, A., Hughes, J., Hokfelt, T., & Wiesenfeld-Hallin, Z. (1994). Chronic pain-related behaviors in spinally injured rats: evidence for functional alterations of the endogenous cholecystokinin and opioid systems. *Pain, 56*(3), 271-277.

Xu, X. J., & Wiesenfeld-Hallin, Z. (2003). *Applied physiology of neuropathic pain: experimental models and their application in the study of mechanisms and treatment.* (T. S. W. Jensen, P. R. and Rice, A. S. C. Ed.). London: Arnold.

Yamasaki, H. (1976). Pharmacology of sinomenine, an anti-rheumatic alkaloid from Sinomenium acutum. *Acta Med Okayama, 30*(1), 1-20.

Yang, H., Yin, P., Shi, Z., Ma, Y., Zhao, C., Zheng, J., & Chen, T. (2016). Sinomenine, a COX-2 inhibitor, induces cell cycle arrest and inhibits growth of human colon carcinoma cells in vitro and in vivo. *Oncol Lett, 11*(1), 411-418. doi:10.3892/ol.2015.3838

Yezierski, R. P., Burchiel, K.J. (2002). *Spinal Injury Pain: Assessment, Mechanism, Management. Progress in Pain Research and Management.* (Vol. 23). Seattle: IASP Press.

Yu, W., Hao, J. X., Xu, X. J., & Wiesenfeld-Hallin, Z. (1997). The development of morphine tolerance and dependence in rats with chronic pain. *Brain Res, 756*(1-2), 141-146.

Yuan, Y., Zhang, Y., He, X., & Fan, S. (2018). Protective Effects of Sinomenine on CFA-Induced Inflammatory Pain in Rats. *Med Sci Monit, 24*, 2018-2024.

Zhang, M. Y., Li, P., Wang, D. Q., Niu, X. H., Wang, Y., Wang, Z. G., . . . Xu, X. J. (2013). [Analgesic effect of sinomenine on SSNI model rats and monoamine neurotransmitters in striatal extracellular fluid]. *Zhongguo Zhong Yao Za Zhi, 38*(4), 597-604.

Zhou, H., Wong, Y. F., Wang, J., Cai, X., & Liu, L. (2008). Sinomenine ameliorates arthritis via MMPs, TIMPs, and cytokines in rats. *Biochem Biophys Res Commun, 376*(2), 352-357. doi:10.1016/j.bbrc.2008.08.153

Zhu, Q., Sun, Y., Mao, L., Liu, C., Jiang, B., Zhang, W., & Li, J. X. (2016). Antinociceptive effects of sinomenine in a rat model of postoperative pain. *Br J Pharmacol, 173*(10), 1693-1702. doi:10.1111/bph.13470

Zhu, Q., Sun, Y., Zhu, J., Fang, T., Zhang, W., & Li, J. X. (2014). Antinociceptive effects of sinomenine in a rat model of neuropathic pain. *Sci Rep, 4*, 7270. doi:10.1038/srep07270

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Original experimental

Repeated sinomenine administration alleviates chronic neuropathic pain-like behaviours in rodents without producing tolerance

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HIGHLIGHTS

- Spinally injured rats and sciatic nerve injured mice were used to study the effect of repeatedly administered sinomenine on hypersensitivity to mechanical and cold stimuli.
- Following repeated administration, the analgesic effect of sinomenine was increased, without development of tolerance.
- Sinomenine may be explored as a novel analgesic for treating some forms of chronic neuropathic pain in patients.

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ABSTRACT

Background and aims: We have previously reported that systemic administration of sinomenine produced antinociception in various experimental pain conditions in rodents, particularly in models of neuropathic pain. In the present study we assessed the effects of repeated administration of sinomenine in two rodent models of neuropathic pain in order to study the development of tolerance.
Methods: The analgesic effect of sinomenine was tested in female Sprague-Dawley rats that exhibited mechanical and cold hypersensitivity following ischaemic injury to the spinal cord and in male C57/BL6 mice that developed mechanical hypersensitivity after ischaemic injury to the sciatic nerve. Briefly, the animals were anaesthetized and injected i.v. with the photosensitizing dye erythrosine B. Vertebral segments T12 to T13 in rats or the sciatic nerve in mice were exposed and irradiated under an argon ion laser for 10 min or 45 s, respectively. In rats, mechanical hypersensitivity to pressure with von Frey hairs, the response to brushing and decreasing cold temperature were tested in the flanks or upper back areas. In mice, mechanical hypersensitivity on the hind paw to von Frey hairs and response to cold following a drop of acetone were measured. Sinomenine was administered i.p. in rats and p.o. in mice at 10:00 and 16:00, twice a day for 5 days. Response threshold before and 2 h after drug administration at 10.00 h was recorded.
Results: Repeated administration of sinomenine at 10 or 20 mg/kg twice a day, doses that have no analgesic effect as single injection, alleviated mechanical, but not cold allodynia in spinally injured rats and the effect was maintained during the 5 day treatment period with no signs of tolerance. Furthermore, the pre-drug response threshold was significantly elevated during repeated treatment with 20 mg/kg sinomenine. Sinomenine administered at 40 mg/kg twice a day for 5 days significantly reduced mechanical and cold alldoynia, elevated pre-drug response threshold without tolerance development in spinally injured rats. Similarly, sinomenine at 80 mg/kg twice a day for 5 days significantly reduced mechanical allodynia in mice with sciatic nerve injury and increased pre-drug response threshold with no sign of tolerance. The effect of sinomenine on response threshold persisted for days after termination of the 5 day drug administration.
Conclusions: The results suggest that repeated administration of simomenine produced an enhanced anti-allodynic effect without tolerance in rodent models of neuropathic pain.
Implications: Sinomenine may be tested as a novel analgesic in treating some forms of chronic neuropathic pain in patients.

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1. Introduction

In the European Union the prevalence of chronic pain is around 20% in adults and imposes a huge burden on society [1]. Chronic neuropathic pain that occurs after injury or disease in the central or peripheral nervous system causes a great reduction of life quality in patients [2]. However, the lack of adequate treatments of neuropathic pain remains problematic. The first line drugs used to treat neuropathic pain, such as pregabalin or gabapentin, only produce partial pain relief in a subset of patients [3–5]. Opioid analgesics are ineffective against neuropathic pain in the majority of patients and are often associated with side effects including constipation, tolerance and drug abuse [6,7].

Traditional Chinese medicines (TCM) are widely used for management of various clinical pain conditions in China and may harbour a rich source of potential drug candidates, which Western drug companies are turning to with ever increasing urgency [8]. Sinomenine is a morphine derivative alkaloid purified from the root of the climbing plant Sinomenium Acutum. Sinomenine is traditionally used as a remedy for rheumatism and arthritis (RA) in Asia. Clinical research indicated that compared with NSAIDs, sinomenine was more effective in ameliorating morning stiffness, painful joints and erythrocyte sedimentation rate in RA patients [9]. In addition to possible pain relieving effect in RA, sinomenine has been suggested to be effective in some types of neuralgia, such as sciatic neuritis and lumbalgia, based mostly on anecdotal evidence [10].

In searching for effective components in TCMs for treating chronic pain, we have recently studied the effect of sinomenine in models of acute and chronic pain in rodents. Sinomenine appears to be particularly effective against neuropathic pain after injury to both the peripheral and central nervous system. In the present study, we evaluated the analgesic effect of sinomenine upon repeated administration on neuropathic pain using two rodent models, photochemically-induced spinal cord injury in rats [12], and sciatic nerve injury in mice [13].

2. Methodology

2.1. Animals

All experiments were approved by the regional research ethics committee. We used female Sprague-Dawley rats (Harlan, Horst, The Netherlands) weighing 300–350 g, and male C57BL/6 mice, (Charles River, Sollentuna, Sweden) weighing 25–30 g. The rats and mice were housed 4 or 6 per cage respectively at a constant room temperature of 22 °C in a 12:12 h light–dark cycle with ad libitum access to food and water.

2.2. Photochemically-induced spinal cord injury in rats

The method of producing photochemically induced spinal cord ischaemic injury in rats has been described in detail previously [12]. Briefly, the rats were anesthetized with 75 mg/kg ketamine + 1 mg/kg medetomidine and a midline incision was made in the skin overlying vertebral segments T12-L1. Following i.v. injection of 32.5 mg/kg of the photosensitizing dye erythrosine B (Sigma-Aldrich), vertebral segment T12 or T13 (spinal segments L3-5) was irradiated with an argon ion laser (Coherent) for 10 min. A second dose of erythrosin B was injected 5 min after the start of irradiation. During irradiation, the temperature of the animals was maintained 37–38 °C.

2.3. Behavioural tests in rats

The threshold to mechanical stimulation was tested by gently restraining the animals in a standing position and calibrated von Frey hairs (Stoelting, Chicago, IL, USA) were applied to the shaved flanks or upper back areas. The von Frey hairs were applied 5–10 times at each intensity, with the frequency of 1/s. The stimulus which induced consistent vocalization (to >75% of stimuli) was considered as vocalization threshold. The cut-off value was 100 g.

For examining the response to brush stimuli, the skin on the flanks was briskly stroked with the point of a pencil in a rostral to caudal direction [14]. The response of the animals was graded with a score of 0 = no response, 1 = moderate efforts to avoid the probe but no vocalization, 2 = clear avoiding behaviour to the stimulus with transient vocalization, and 3 = vigorous efforts to avoid the stimulus, sustained vocalization in response to the probe.

Cooling stimuli were applied with a Peltier thermode to the flank [15]. A fluid cooled, hand held Peltier thermode (active surface: 25 mm × 50 mm, control resolution: >0.02 °C, calibration uncertainty: ±0.2 °C) connected to a Modular Sensory Analyzer Thermal Stimulator (Somedic, Sweden) was used. The baseline temperature was 32 °C and the rate of temperature change was 0.5 °C/s. Rats were held gently in a standing position and the thermode was pressed against the shaved flank area. Three cooling stimuli were applied at 1 min intervals and the average temperature at which the rats vocalized was taken as cold response threshold with 6 °C as cut-off temperature.

2.4. Photochemically induced sciatic nerve injury in mice

The detailed method for producing ischaemic injury to the sciatic nerve in mice has been described previously [13]. Briefly, the animals were anaesthetized with 75 mg/kg ketamine + 1 mg/kg medetomidine and the left sciatic nerve was exposed. After i.v. injection of 32.5 mg/kg erythrosine B the sciatic nerve was irradiated under an argon ion laser for 45 s.

2.5. Behavioural test in mice

The withdrawal threshold of the ipsilateral hind paw to mechanical stimulation after sciatic nerve injury was tested using a set of calibrated von Fray hairs as described above. The response to cold after nerve injury was tested using a drop of acetone applied to the plantar surface of the hind paw ipsilateral to the nerve injury. The immediate response after acetone application was observed and scored as follows: 0 = no responses; 1 = startle response without evident paw withdrawal, 2 = withdraw of the stimulated hind paw, 3 = sustained withdraw of the simulated hind paw with flitching or licking.

2.6. Drugs

For preparation of sinomenine (obtained from The National Institute for Food and Drug Control, Beijing, China) for injection it was first dissolved with DMSO (Sigma-Aldrich), then mixed with Cremophor EL oil (Sigma-Aldrich) and saline by a vortex mixer (Bibby Scientific, UK) using the volume rate of 1:4:5. Any further dilution was made with saline. Sinomenine was administered i.p. in rats and orally in mice. To perform oral administration, the mouse was held in an upright standing position and a bulb tipped gastric gavage needle was used to deliver the sinomenine solution into the stomach by the attached syringe. Sinomenine was administered twice daily for 5 days at 10:00 h and 16:00 h. Baseline sensitivity to mechanical and cold stimuli was assessed before the administration of sinomenine at 10:00 h and two hours later, when sinomenine's effect was maximal [11]. Control groups of spinal cord injured rats and sciatic nerve injured mice were administered saline twice a day for 5 days.

2.7. Statistics

The experiments were conducted blindly wherever a control group was included. Data are presented as mean ± SEM or median ± MAD, and were analyzed by ANOVA with repeated measures and the Kruskal–Wallis test followed by Bonferroni/Dunn post hoc test, Wilcoxon signed rank test, or paired *t*-test. $P < 0.05$ is considered to be statistically significant.

3. Results

3.1. Effect of repeated administration of sinomenine on pain-like behaviours in spinally injured rats

As previously reported, rats developed chronic hypersensitivity to mechanical (von Frey hair and brushing) and cold stimuli after spinal cord injury [16,17]. The pharmacological experiments were conducted at 8 weeks following injury when hypersensitivity was maximal and stable.

Saline had no effect on either mechanical (Figs. 1A and 2A) or cold (3A) sensitivity. A single dose of i.p. sinomenine at 10 or 20 mg/kg had no effects on responses to mechanical or cold stimulation in SCI rats (Figs. 1B–D, 2B–D and 3B–D) as previously reported [11]. In contrast, repeated administration of 10 mg/kg sinomenine twice per day elevated vocalization threshold to mechanical stimulations and reduced response score to brushing from day 2 to day 5 of treatment (Figs. 1B and 2B). However, repeated 10 mg/kg sinomenine had no effect on hypersensitivity to cold (Fig. 3B).

Repeated administration of sinomenine at 20 mg/kg reduced mechanical hypersensitivity to stimulation with von Frey hairs and brushing from day 2 to day 5 (Figs. 1C and 2C). Furthermore, pre-drug response threshold to von Frey hairs was significantly elevated from day 2 of sinomenine treatment and the threshold remained significantly elevated compared to day 1 for at least 4 days after the cessation of drug application (Fig. 1C). The pretreatment response score to brushing was also significantly decreased from day 4 to day 6 after the start of drug treatment (Fig. 2C). However, 20 mg/kg sinomenine did not alleviate allodynia to cooling (Fig. 3C).

Sinomenine administered twice/day at 40 mg/kg effectively reduced mechanical hypersensitivity. Baseline thresholds to stimulation with von Frey hairs was significantly increased from day 2 of treatment and lasted until day 9, 4 days after the last administration of sinomenine (Fig. 1D). The response threshold returned to pre-sinomenine baseline level on day 12 (Fig. 1D). Hypersensitivity to brushing was also reversed on days 2, 4 and 5 following repeated sinomenine (Fig. 2D). The threshold temperature for cold stimulation was significantly decreased (indicating a decrease in cold hypersensitivity) 2 h after sinomenine during the first two days (Fig. 3D). The pre-drug cold response temperature was significantly reduced from baseline level from day 2 to day 9 (Fig. 3D), again suggesting a sustained reduction in cold hypersensitivity.

In general, sinomenine dose-dependently suppressed hypersensitivity to mechanical (Fig. 1) and cold (Fig. 3) in rats after spinal cord injury.

3.2. Effect of repeated sinomenine on neuropathic pain-like behaviours in mice following sciatic nerve injury

Saline had no effect on paw withdrawal thershold (Fig. 4A) or cold (Fig. 4C). Sinomenine at 80 mg/kg administered p.o. twice a day for 5 days produced significantly increased paw withdrawal threshold on days 1–5 (Fig. 4B). There was also a significant and persistent elevation in pre-drug baseline response threshold to simulation with von Frey hairs from day 2 and was maintained for 7 days after the termination of drug treatment (Fig. 4B). Sinomenine also significantly reduced mechanical and cold post-drug responses, in comparison with the pre-drug thresholds (Fig. 4B and D).

4. Discussion

We have previously reported that a single dose of sinomenine alleviated pain-like responses in spinal cord injured rats at or above 40 mg/kg [11]. In the present study we showed that repeated administration of sinomenine even at 10 mg/kg reduced mechanical allodynia-like responses in spinal cord injured rats after the third injection and the effect was maintained during the 5 day treatment with no signs of tolerance. Repeated administration of sinomenine at 20 or 40 mg/kg not only reduced mechanical allodynia-like responses without tolerance, but also significantly increased pre-drug baseline response threshold after two injections. The increase in pre-drug response thresholds for the response to brush were maintained during the 5 day administration of sinonemine at 10 or 40 mg/kg doses and maintained up to day 6 following the last 20 mg/kg dose.

Similar effects were observed in a mouse model of neuropathic pain after sciatic nerve injury in which 80 mg/kg sinomenine, an effective dose on its own [11], produced marked anti-allodynic response against mechanical stimulation after repeated injections for 5 days without producing tolerance. Furthermore, as in rats with spinal cord injury, we observed a significant increase in pre-drug response threshold after two injections which was maintained to day 12, 7 days after the termination of drug administration. In both rats and mice, sinomenine appears to be less effective against cold than mechanical hypersensitivity, which is similar to our previous results [11].

We found that a single i.p. administration of sinomenine at 20 and 40 mg/kg produced little or no side effects, and 80 mg/kg simonemine caused some sedation in rats [11]. In the present study no side effects (sedation, motor impairment or irritation) were observed during or after repeated sinomenine administration. Previous studies in rats have also suggested that daily administration at 40 or 80 mg/kg for two weeks did not influence growth, appetite and blood pressure [18]. There were also no apparent withdrawal symptoms following the termination of drug treatment in the present study. These observations, together with the fact that no tolerance to the anti-allodynic effects of sinomenine was observed after repeated administration, suggest that sinomenine may be useful to treat chronic neuropathic pain.

The effects of repeated sinomenine on neuropathic pain-like behaviours in our models are similar to the effect of the anti-epileptics lacosamide and gabapentin [19–21]. In particular, the analgesic effect of gabapentin was also increased following repeated administration at doses that were ineffective as a single injection [19]. Moreover, repeated lacosamide alleviated pre-drug baseline responses, similar to that of sinomenine [20]. In contrast, i.p. morphine did not alleviate allodynia in rats with spinal cord injury. Intrathecal morphine did have some anti-allodynic effect, but tolerance was observed after 2 days of twice daily treatment [22].

One of the remarkable effects of sinomenine in these two rodent models of neuropathic pain is that it reduced baseline hypersensitivity following repeated administration, resulting in persistent reduction in allodynia. Since sinomenine has a relatively short half-life in rat plasma [23,24], it is unlikely that this effect is due to an accumulation of the drug following repeated injections. Some of the anti-allodynic effects of sinomenine may be mediated by its metabolites which are known to be present in at least three forms [25]. However, it is unknown whether these metabolites are pharmacologically active. Alternatively, the effects of repeated sinomenine may reflect sustained physiological changes

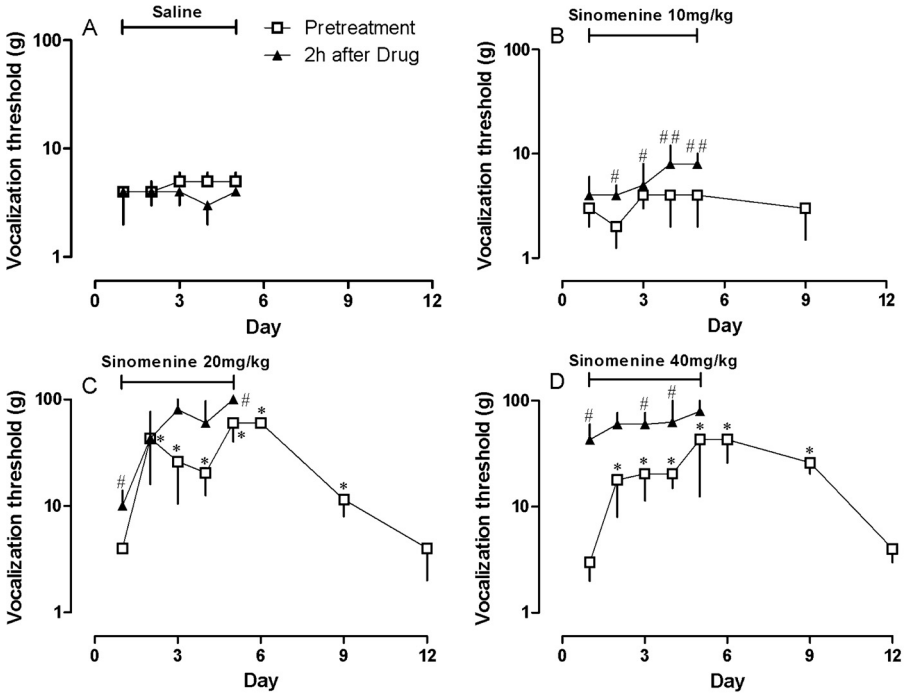

Fig. 1. Effect of i.p. saline (A), or 10 mg/mg (B), 20 mg/kg (C) and 40 mg/kg (D) sinomenine, administered twice a day for 5 days on vocalization threshold to stimulation with von Frey hairs, on the flanks of in rats with spinal cord injury. $N = 6–12$ in each group and data are presented as median ± MAD. A two-way ANOVA with repeated measures and the Kruskal–Wallis test indicated significant overall differences ($P < 0.01$) in groups with different doses of sinomenine or saline (A–D). The Bonferroni/Dunn's post hoc test revealed that pre- and 2 h post-drug responses were significantly different ($P < 0.01$) in the groups administered 20 mg/kg (C) and 40 mg/kg (D) sinonemine. Furthermore, they also had significantly higher ($P < 0.01$) pre- and post-drug thresholds than the groups who received saline (A) or 10 mg/kg sinomenine (B). Pre- and post-drug responses were compared on each day using the Wilcoxon signed rank test (B–D) and significant differences are shown as # = $P < 0.05$ and ## = $P < 0.01$. Pre-drug vocalization threshold on day 1 was compared with pre-drug thresholds following drug administration with the Wilcoxon signed rank test (C and D) and * = $P < 0.05$.

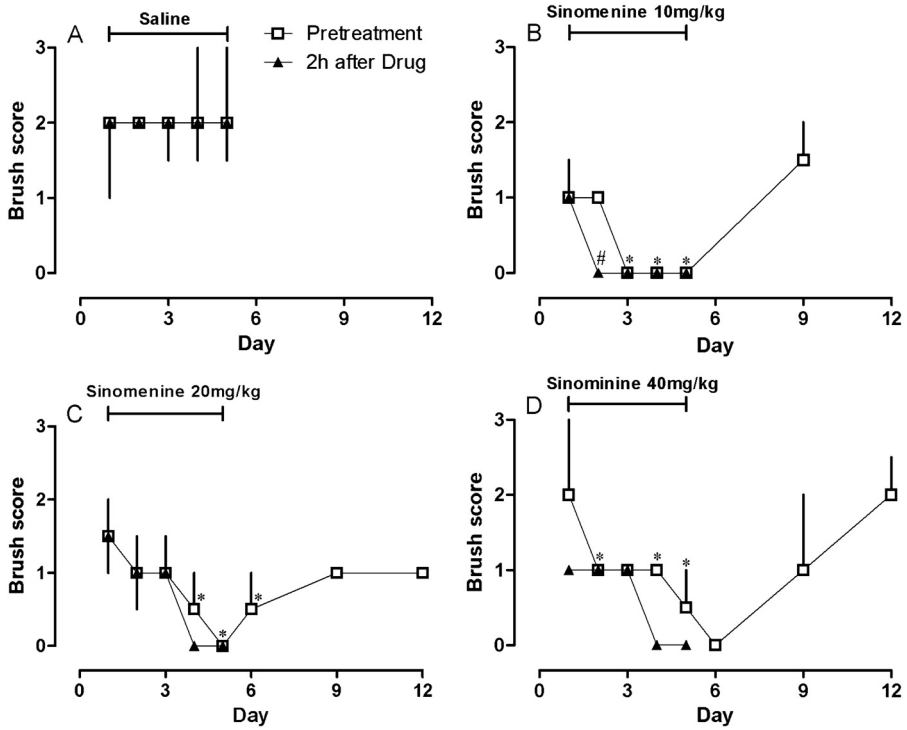

Fig. 2. Effect of i.p. saline (A) or 10 mg/mg (B), 20 mg/kg (C) and 40 mg/kg (D) sinomenine, administered twice a day for 5 days on brush score on the flanks of rats with spinal cord injury. $N = 6–8$ in each group and data are presented as median ± MAD. A two-way ANOVA with repeated measures and the Kruskal–Wallis test indicated significant overall differences ($P < 0.01$) in groups with different doses of sinomenine or saline (A–D). The Bonferroni/Dunn's post hoc test revealed that pre- and 2 h post drug responses were significantly different ($P < 0.01$) in the groups administered 10 mg/kg (B), 20 mg/kg (C) and 40 mg/kg (D) sinonemine. Furthermore, they also had significantly higher ($P < 0.01$) pre- and post-drug thresholds than the groups who received saline (A). Pre- and post-drug responses were compared on each day using the Wilcoxon signed rank test and significant differences are shown as # = $P < 0.05$. Pre-drug brush score on day 1 was compared with pre-drug scores following drug administration with the Wilcoxon signed rank test (B–D) and * = $P < 0.05$.

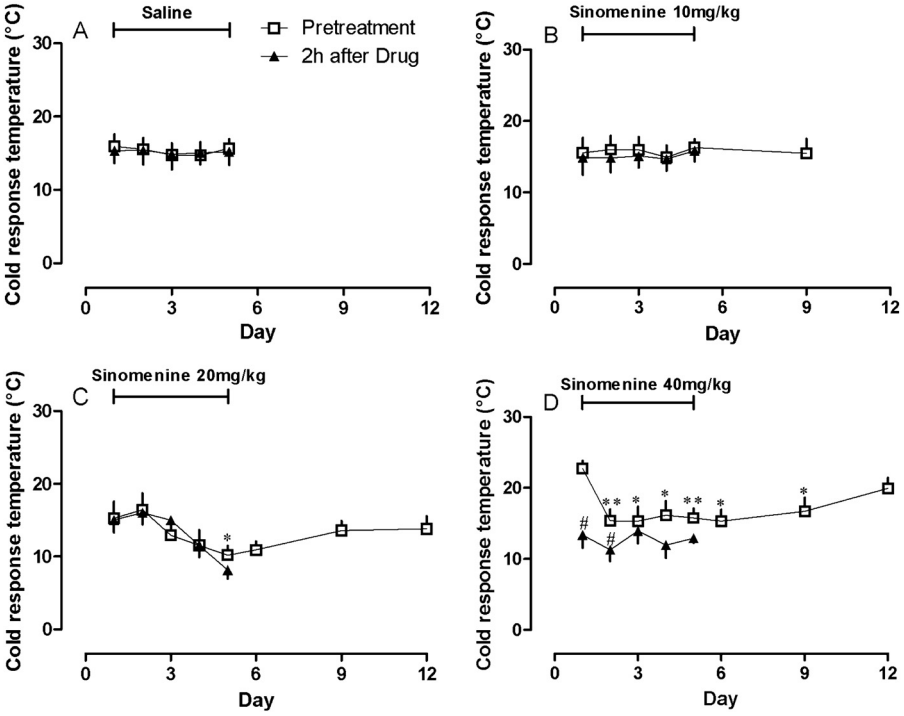

Fig. 3. Effect of i.p. saline (A) or 10 mg/mg (B), 20 mg/kg (C) and 40 mg/kg (D) sinomenine, administered twice a day for 5 days on the cold response in rats with spinal cord injury. $N = 6$ in each group and data are presented as mean ± SEM. Two way ANOVA with repeated measures and the Kruskal–Wallis test indicated significant overall differences ($P < 0.05$) in groups receiving different doses of sinomenine or saline (A–D). The Bonferroni/Dunn's post hoc test revealed that pre- and 2 h post-drug cold responses were significantly different ($P < 0.05$) only in the group receiving 40 mg/kg sinonemine. Pre- and post-drug cold responses were compared on each day using the paired *t*-test and a significant difference was found in the 40 mg/kg group (D), # = $P < 0.05$. The pre-drug cold response on day 1 was compared with pre-drug thresholds following drug administration with the paired *t*-test (C and D), *P = <0.05.

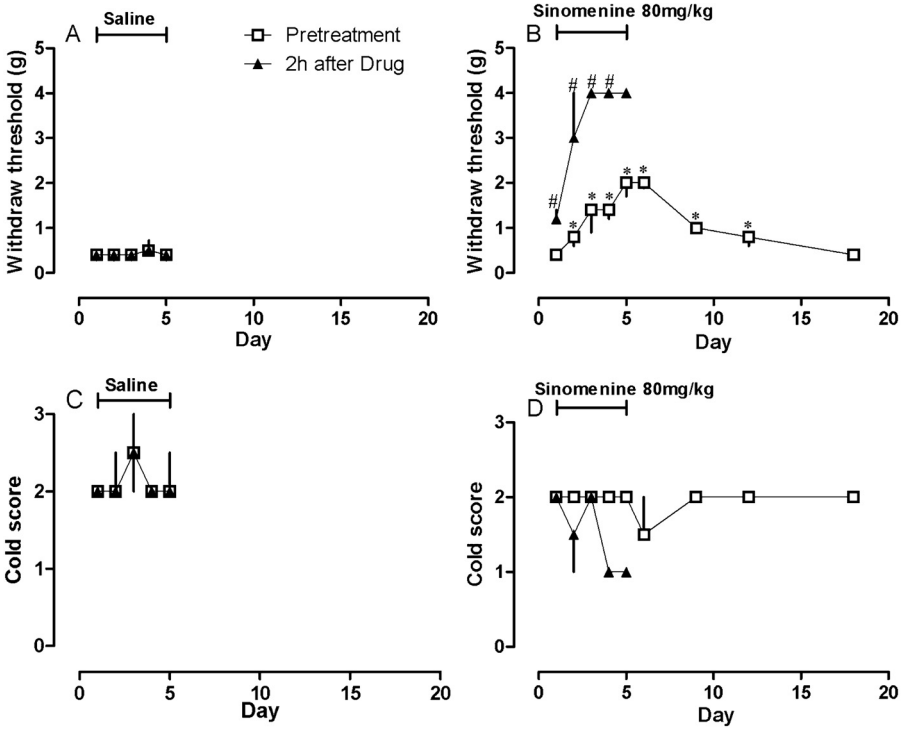

Fig. 4. Effect of p.o. saline (A and C) or 80 mg/kg sinomenine (B and D) administered twice a day for 5 days on the withdrawal threshold to stimulation with von Frey hairs (A and B) and cold score to stimulation with acetone (C and D) in mice with sciatic nerve injury. $N = 6$ animals per group. Data are presented as median ± MAD. Two-way ANOVA with repeated measures and the Kruskal–Wallis test indicated a significant overall group difference ($P < 0.01$) in mechanical (A and B) and cold (C and D) hypersensitivities. The Bonferroni/Dunn's post hoc test revealed that pre- and 2 h post-drug cold responses were significantly different ($P < 0.01$) in the group receiving 80 mg/kg sinomenine (A and C) but not saline (B and D). Pre- and post-drug withdrawal threshold (B) and cold score (D) were compared on each day using the Wilcoxon signed rank test and significant differences are shown as # = $P < 0.05$. The pre-drug withdrawal threshold and cold score on day 1 were compared with pre-drug responses following drug administration with the Wilcoxon signed rank test and *P = <0.05 (B).

resulting from repeated drug treatment. Such changes are, however, reversible and may require continuous drug treatment since allodynia recurred within days following the last dose of sinenomine.

The mechanisms for the anti-allodynic effect of sinomenine in models of neuropathic pain are not clear. We have previously shown that the anti-allodynic effect of sinomenine was not reversed by the opioid receptor antagonist naloxone [11] and the profile of analgesia produced by sinomenine is different from that of systemic morphine [22,26]. In contrast, the effect profile of sinomenine is similar to that of dextromethorphan, a non-opioid antitussive that is an weak noncompetitive NMDA receptor antagonist [11,27]. Sinomenine is structurally related to levorphanol and dextromethorphan and is also antitussive [18]. Although there is currently no evidence that sinomenine can function as an NMDA receptor antagonist, it does have a neuroprotective effect possibly mediated by blocking of acid-sensing ion channel and calcium channels [28]. Furthermore, repeated administration of sinomenine delays tolerance to morphine [29,30], which is also observed with dextromethorphan [31].

One of the possible mechanisms for the anti-allodynic effect of chronic sinomenine may be related to its ability to modulate neurotransmitter release in the spinal cord and brain. Systemic sinomenine alters the level of monoamines in extracellular fluid in the striatum in rats after sciatic nerve injury with increase in the level of noradrenaline and decrease in level of dopamine and serotonin [32]. These effects are correlated with analgesic effect of sinomenine [32]. Chronic sinomenine may produce long term effects on transmitter synthesis and neuronal functions through altered transmitter release.

Sinomenine also has distinct immunoregulatory and neuroprotective properties. It can reduce the production of COX-2 dependent Prostaglandin E2 [33], block NF-κB and p38MAPK signal pathways [34,35], and reduce microglia activation by inhibiting NADPH oxidase [36]. It is conceivable that some of these properties of sinomenine may reduce neuronal sensitization in the peripheral and central nervous system and contribute to its analgesic effects in neuropathic pain.

5. Conclusion

In conclusion, the present results showed that the anti-allodynic effect of sinomenine upon repeated chronic administration did not lead to tolerance, but rather enhanced its effect, in two rodent models of neuropathic pain, resulting in a persistent, but reversible, analgesia with no observable side effects.

6. Implications

The results from this research may suggest potential clinical application of sinomenine as a novel analgesic in treating chronic neuropathic pain.

Conflict of interest

We declare no conflicts of interests.

Acknowledgement

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References

[1] Breivik H, Collet B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain 2006;10:287–333.
[2] Ebrahimzadeh MH, Shojaei BS, Golhasani-Keshtan F, Soltani-Moghaddas SH, Fattahi AS, Mazloumi SM. Quality of life and the related factors in spouses of veterans with chronic spinal cord injury. Health Qual Life Outcomes 2013;11:48.
[3] Gordh TE, Stubhaug A, Jensen TS, Arnèr S, Biber B, Boivie J, Mannheimer C, Kalliomäki J, Kalso E. Gabapentin in traumatic nerve injury pain: a randomized, double-blind, placebo-controlled, cross-over, multi-center study. Pain 2008;138:255–66.
[4] Serpell MG. Neuropathic pain study group, Gabapentin in neuropathic pain syndromes: a randomized, double-blind, placebo-controlled trial. Pain 2002;99:557–66.
[5] Rice AS, Maton S, Postherpetic Neuralgia Study Group. Gabapentin in postherpetic neuralgia: a randomized, double blind, placebo controlled study. Pain 2001;94:215–24.
[6] Arnér S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988;33:11–23.
[7] Von Korff MR. Long-term use of opioids for complex chronic pain. Best Pract Res Clin Rheumatol 2013;27:663–72.
[8] Bloomberg News, DOI: http://www.bloomberg.com/news/2012-12-11/ancient-chinese-cures-seen-helping-drug-maker-pipelines.html, 11.12.12.
[9] Xu M, Liu L, Qi C, Deng B, Cai X. Sinomenine versus NSAIDs for the treatment of rheumatoid arthritis: a systemic review and meta-analysis. Planta Med 2008;74:1423–9.
[10] Yamasaki H. Pharmacology of sinomenine, an anti-rheumatic alkaloid from sinomenium. Acta Med Okayama 1976;30:1–19.
[11] Gao T, Hao JX, Wiesenfeld-Hallin Z, Wang DQ, Xu XJ. Analgesic effect of sinomenine in rodents after inflammation and nerve injury. Eur J Pharmacol 2013;13:726–7.
[12] Hao JX, Xu XJ, Aldskogius H, Seiger Å, Wiesenfeld-Hallin Z. Photochemically induced transient spinal ischemia induces behavioral hypersensitivity to mechanical and cold stimuli, but not to noxious-heat stimuli, in the rat. Exp Neurol 1992;118:187–94.
[13] Hao JX, Blakeman KH, Yu W, Hultenby K, Xu XJ, Wiesenfeld-Hallin Z. Development of a mouse model of neuropathic pain following photochemically induced ischemia in the sciatic nerve. Exp Neurol 2002;163:231–8.
[14] Yaksh TL. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effect of modulatory receptor systems and excitatory amino acid antagonists. Pain 1989;37:111–23.
[15] Gao T, Hao JX, Wiesenfeld-Hallin Z, Xu XJ. Quantitative test of responses to thermal stimulation in spinally injured rats using a Peltier thermode: a new approach to study cold allodynia. J Neurosci Methods 2013;212:317–21.
[16] Xu XJ, Hao JX, Aldskogius H, Seiger A, Wiesenfeld-Hallin Z. Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. Pain 1992;48:279–90.
[17] Xu XJ, Hao JX, Seiger A, Hughes J, Hökfelt T, Wiesenfeld-Hallin Z. Chronic pain-related behaviors in spinally injured rats: evidence for functional alterations of the endogenous cholecystokinin and opioid systems. Pain 1994;56:271–7.
[18] Zhu YP. Chinese materia medica chemistry, pharmacology and applications. Florida: CRC Press LLC; 1998.
[19] Hao JX, Xu XJ, Urban L, Wiesenfeld-Hallin Z. Repeated administration of systemic gabapentin alleviates allodynia-like behaviors in spinally injured rats. Neurosci Lett 2000;280:211–4.
[20] Hao JX, Stöhr T, Selve N, Wiesenfeld-Hallin Z, Xu XJ. Lacosamide, a new antiepileptic, alleviates neuropathic pain-like behaviors in rat models of spinal cord or trigeminal nerve injury. Eur J Pharmacol 2006;553:135–40.
[21] Wu WP, Hao JX, Ongini E, Impagnatiello F, Presotto C, Wiesenfeld-Hallin Z, Xu XJ. A nitric oxide (NO)-releasing derivative of gabapentin, NCX 8001, alleviates neuropathic pain-like behavior after spinal cord and peripheral nerve injury. Br J Pharmacol 2004;141:65–74.
[22] Yu W, Hao JX, Xu XJ, Wiesenfeld-Hallin Z. The development of morphine tolerance and dependence in rats with chronic pain. Brain Res 1997;756:141–6.
[23] Liu L, Buchner E, Beitze D, Schmidt-Weber CB, Kaever V, Emmrich F, Kinne RW. Amelioration of rat experimental arthritides by treatment with the alkaloid sinomenine. Int J Immunopharmacol 1996;18:529–43.
[24] Ling J, Wang Y, Xie B, Li R. Pharmacokinetic studies of sinomenine by blood microdialysis technique. J Guangzhou Univ Tradit Chin Med 2005;5:021.
[25] Cheng WM, Qiu F, Yao XS. Three major urinary metabolites of sinomenine in rats. J Asian Nat Prod Res 2007;9:13–8.
[26] Bulka A, Plesan A, Xu XJ, Wiesenfeld-Hallin Z. Reduced tolerance to the antihyperalgesic effect of methadone in comparison to morphine in a rat model of mononeuropathy. Pain 2002;95:103–9.
[27] Hao JX, Xu XJ. Treatment of a chronic allodynia-like response in spinally injured rats: effects of systemically administered excitatory amino acid receptor antagonists. Pain 1996;66:279–85.
[28] Wu WN, Wu PF, Chen XL, Zhang Z, Gu J, Yang YJ, Xiong QJ, Ni L, Wang F, Chen JG. Sinomenine protects against ischemic brain injury: involvement of co-inhibition of acid-sensing ion channel 1a and L-type calcium channels. Br J Pharmacol 2011;164:1445–59.
[29] Wang CY, Mo ZX, Liang RN. Effects of sinomenine on withdrawal syndrome in morphine-dependent mice. J Chin Med Mater 2002;25:337–9.

[30] Wang CY, Mo ZX, Shao HX. Effects of sinomenine on the psychic dependence on morphine and the brain cyclic AMP level in mice. Chin Pharmacol Bull 2003;19:575–7.
[31] Elliott K, Hynansky A, Inturrisi CE. Dextromethorphan attenuates and reverses analgesic tolerance to morphine. Pain 1994;59:361–8.
[32] Zhang MY, Li P, Wang DQ, Niu XH, Wang Y, Wang ZG, Zhang Y, Xu S, Xu XJ. Analgesic effect of sinomenine on SSNI model rats and monoamine neurotransmitters in striatal extracellular fluid. Zhongguo Zhong Yao Za Zhi 2013;38:597–604.
[33] Liu L, Riese J, Resch K, Kaever V. Impairment of macrophage eivosanoid and nitric oxide production by an alkaloid from Sinomenum acutum. Arzneimittelforschung 1994;44:1123–6.
[34] Wang Y, Fang Y, Huang W, Zhou X, Wang M, Zhong B, Peng D. Effect of sinomenine on cytokine expression of macrophages and synoviocytes in adjuvant arthritis rats. J Ethnopharmacol 2005;98:37–43.
[35] Huang F, Yamaki K, Tong XY, Fu L, Zhang RH, Yoshino S. Inhibition of the antigen-induced activation of RBL-2H3 cells by sinomenine. Int Immunol 2008;8:502–7.
[36] Qian L, Xu ZL, Zhang W, Wilson B, Hong JS, Flood MP. Sinomenine, a natural dextrorotatory morphinan analog, is anti-inflammatory and neuroprotective through inhibition of microglial NADPH oxidase. J Neuroinflamm 2007;4:23.

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Original experimental

Sinomenine alleviates mechanical hypersensitivity in mice with experimentally induced rheumatoid arthritis

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HIGHLIGHTS

- Sinomenine is effective against mechanical allodynia in mice with experimental RA.
- Sinomenine also alleviated spread pain behaviours in these mice.
- Repeated sinomenine achieved better analgesic efficacy without tolerance.
- Sinomenine may be clinically useful to treat chronic pain in RA.

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Tolerance

ABSTRACT

Background and aims: We have previously reported that sinomenine, an alkaloid isolated from the root of the plant *Sinomenium acutum*, had antinociceptive effect in rodent models of acute inflammatory or neuropathic pain. As a traditional medicine, sinomenine is used in China to treat rheumatoid arthritis (RA).
Methods: In the present study, we evaluated the potential antinociceptive effect of sinomenine in a mouse model of RA, collagen type II antibody (CII Ab) induced arthritis (CAIA) after acute and chronic administration.
Results: As single administration, sinomenine at 40 or 80 mg/kg significantly reduced mechanical hypersensitivity both at the time of peak joint inflammation (days 11–19 after CII Ab injection) or during the post-inflammatory phase (days 35–54). No tolerance to the effect of 80 mg/kg sinomenine was observed during repeated injection twice a day for 5 days from day 11 to day 19 or from day 49 to day 53 after CII Ab injection in CAIA mice.
Conclusions: We have shown that sinomenine is effective in alleviating localized and spread hypersensitivities in CAIA mice both during acute inflammation and in post-inflammatory phase. Further, repeated sinomenine administration has elevated the baseline mechanical threshold without producing tolerance.
Implications: Sinomenine may be clinically useful to treat chronic pain in RA, including wide-spread pain which appears to be a difficult clinical problem despite the improvement in the acute treatment of RA by disease modifying agents.

1. Introduction

Pain is a major clinical feature of rheumatoid arthritis (RA). Not only has pain been suggested to be an important patient-reported outcome in RA [1,2], but also the severity of pain has strong impact on the quality of life of patients. Although pain is usually considered as a marker for inflammation, recent studies have shown that in many RA patients chronic pain continue to be a major problem even after the remission of inflammation [2]. Furthermore, many RA patients also suffer from chronic wide-spread pain in a fashion similar to fibromyalgia [3].

Non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are first line pain therapies in RA and weak opioids are also sometimes used [2]). In both cases, analgesia

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in a substantial portion of patients remains unsatisfactory and long-term application of these drugs is limited by side effects and in the case of opioids, the development of tolerance [4]. Disease-modifying anti-rheumatic drugs such as methotrexate are known to reduce acute symptoms in RA including pain, but their efficacy against the development of chronic pain is less clear [1,3,5].

The root of the climbing plant Qingteng (*Sinomenium acutum*) has long been used in East Asia as a remedy for disease conditions similar to rheumatism as recorded in for example the 16th century book, Bencao Gangmu (Compendium of Materia Medica) [6]. The major active component in Qingteng has been identified as sinomenine, a morphinan derivative alkaloid that is structurally similar to dextromethorphan. We have recently shown that sinomenine possesses a wide spectrum of analgesic properties towards different experimental pain conditions in rodents, including acute inflammatory pain by carrageenan or neuropathic pain after peripheral/central nervous system injury [7]. In the present study, we examined the potential antinociceptive effect of single or repeated sinomenine administration against experimental arthritic pain. The collagen antibody induced arthritis (CAIA) model was used, which is a mouse model of RA based on the injection of a cocktail of monoclonal antibodies directed against type II collagen followed by immunizing the animals with lipopolysaccharide (LPS) [8]. In this model the local joint pathology resembles that observed in RA patients and pain-like responses, mainly manifested as localized and/or spread mechanical hypersensitivity, can be detected both during the acute inflammatory phase or chronic post-inflammatory phase [9].

2. Material and methods

2.1. Animals

All experiments were approved and conducted strictly followed regulations of the regional research ethics committee. Female CBA mice (Harlan, Horst, The Netherlands), weighing 25–30 g were used. The animals were housed 6 per cage with a constant room temperature at 22 °C in a 12:12 light–dark cycle and ad libitum access to food and water.

2.2. CAIA in mice

In CBA mice, as described previously [9], CAIA was induced by intravenous (i.v.) injection of anti-CII arthritogenic antibody cocktail (0.15 ml, Chondrex, USA), which contains 5 monoclonal antibodies on day 0. Then, immune reaction was triggered by intraperitoneal (i.p.) injection of 35 μg LPS, (serotype O55:B5; Sigma) diluted in 100 μl of physiologic saline on day 5. Inflammation in the joints was examined and evaluated by visual inspection after antibody cocktail injection. The scoring was based on joint inflammation in each paw, being defined by swelling and redness [8]. Briefly, each inflamed toe gave one point, an inflamed wrist or ankle gave five points, resulting in a score of between 0 and 15 for each paw and between 0 and 60 for each mouse.

2.3. Assessments of mechanical hypersensitivity

Baseline mechanical sensitivity in the hind paw, neck and flank areas were measured five times at three day intervals before the collagen antibody injection. Animals with baseline threshold below 50% of the average value were excluded. After collagen type II antibody injection, mechanical threshold was tested for 54 days, always at the same time during the day. For testing of paw withdraw threshold, the mice were placed in plastic cages with a metal mesh floor. After habituation for 1 h the plantar surface of the hind paw was stimulated with a set of calibrated von Fray hairs (Marstock, Denmark). The up-down method [10] was used to calculate the force that caused paw withdrawal in 50% of trials. For testing spread mechanical hypersensitivity the mice were gently restrained in a standing position, and the flanks and upper back were stimulated using von Frey hairs (Stoelting, Chicago, IL, USA). Stimuli were applied 5–10 times at each intensity at 1 s^{-1}. The stimulus which induced consistent avoiding or offensive behaviours (>60% respond rate) was considered as responding threshold. The cut-off value was 4 g on the paws and 100 g on the flanks and back.

2.4. Assessments of side effects

To assess if mice develop motor deficiencies, severe allergy or sedation after sinomenine application, we performed an open field test in naïve mice. The open field arena is 50 cm × 50 cm with 25 grids (the area of one grid is 10 cm × 10 cm), in which mice (without any previous experience in the open field test) were allowed to move freely for 5 min. The total travel distance (quantified by the number of passed grids), number of rearing behaviours and duration of passivity (time when animal showed no movement) were measured. After the open field test, rectal temperature was monitored by a thermometer (FHC, Bowdoin, ME, USA).

2.5. Drugs

For preparation of injecting solutions, sinomenine (standard substance was obtained from The National Institute for Food and Drug Control, Beijing, China) was first dissolved with DMSO (Sigma–Aldrich), then mixed with Cremophor EL oil (Sigma–Aldrich) and saline by a vortex mixer (Bibby Scientific, UK) using the volume rate of 1:4:5. Any further dilution was made with saline. Sinonemine was injected subcutaneously (s.c.), into the loose skin over the neck. Dose response curves for sinomenine were acquired in the acute phase (days 11–19 after CII antibody injection) and chronic phase (days 36–54 after CII antibody injection).

2.6. Statistics

The experiments were conducted blindly. Data were presented as mean ± SEM, and were analyzed by ANOVA (with or without repeated measurements) followed by Fisher's PLSD post hoc test, Wilcoxon signed rank test, and Mann–Whitney *U* test. $P < 0.05$ was considered to be statistically significant.

3. Result

3.1. The dose-dependent effect of sinomenine against mechanical hypersensitivity of the hind paw

During the first 3 h after drug administration in the inflammatory phase of CAIA (days 11–19 after CII antibody injection), a single dose of 40 and 80 mg/kg s.c. sinomenine dose-dependently reduced mechanical hypersensitivity in the hind paws (Fig. 1A). In the post-inflammatory phase during days 35–54 post CII antibody, sinomenine also had a similar effect as during peak inflammation (Fig. 1B).

3.2. The dose-dependent effect of sinomenine against spread mechanical hypersensitivity

As we have previously observed, mice subjected to CAIA developed, in addition to localized mechanical hypersensitivity of the paws, a spread mechanical hypersensitivity primarily at the neck and flanks. A single dose of 40 or 80 mg/kg sinomenine also significantly alleviated the spread mechanical hypersensitivity during the

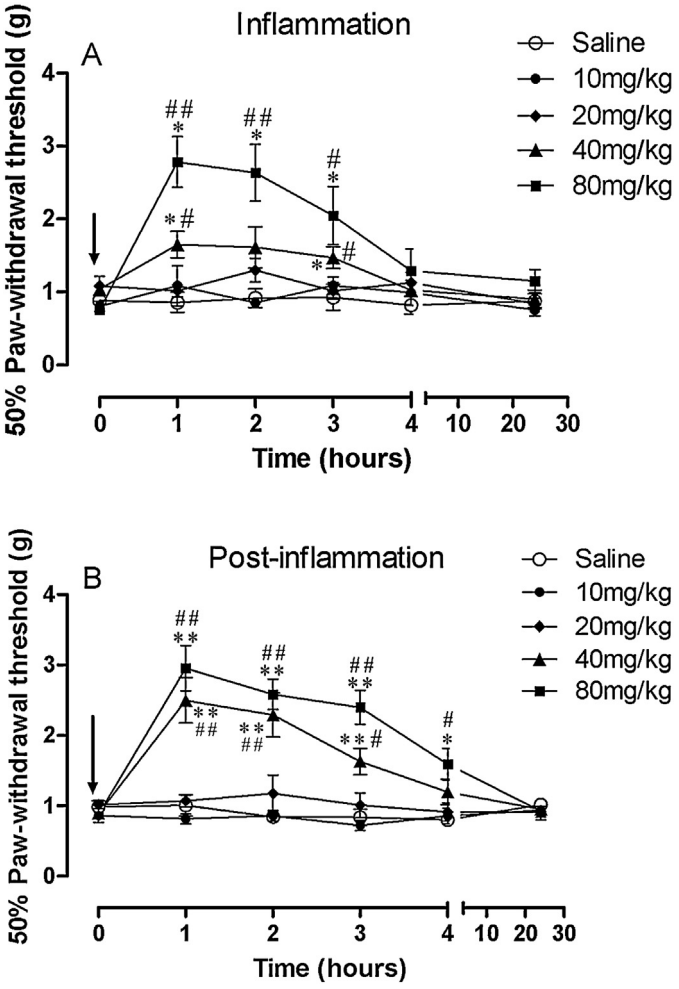

Fig. 1. The effect of saline or various doses of sinomenine injected s.c. on hind paw withdrawal threshold to mechanical stimulation during acute inflammation (at one day during days 11–19, A) and post inflammation (at one day during days 36–54, B) in CAIA mice. Arrow indicates drug application which is just prior to behavioural testing at time 0 h. The threshold at time 0 h represents hyperalgesic value. Increased threshold represents an attenuated/alleviated hyperalgesia. $N = 6–11$ mice in each group. Data are presented as mean ± SEM. ANOVA with repeated measures indicated a significant general difference between the groups ($F = 11.70$, $P < 0.0001$ for A, and $F = 14.27$, $P < 0.0001$ for B). Fisher's PLSD post hoc test indicated that the effect of sinomenine at 40 or 80 mg/kg is significantly different from the saline group. $^{*}P < 0.05$, $^{**}P < 0.01$, post-drug time points were compared with pre-drug baselines using Wilcoxon signed rank test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, post-drug time points were compared with corresponding saline controls using Mann–Whitney U test.

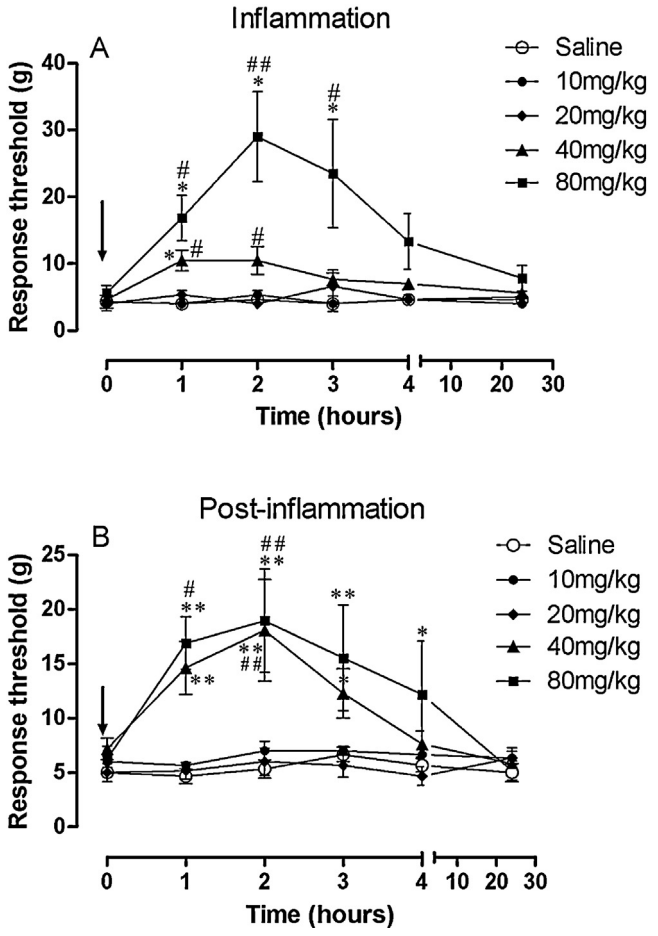

Fig. 2. The effect of saline or various doses of sinomenine on response threshold to mechanical stimulation at the neck/flank region during acute inflammation (at one day during days 11–19, A) and post inflammation (at one day during days 36–54, B) in CAIA mice. Arrow indicates drug application which is just prior to behavioural testing at time 0 h. The threshold at time 0 h represents hyperalgesic value. Increased threshold represent an attenuated/alleviated hyperalgesia. $N = 6–11$ mice in each group. Data are presented as mean ± SEM. ANOVA with repeated measures indicated a significant general difference between the groups ($F = 7.00$, $P < 0.01$ for A, and $F = 2.68$, $P < 0.05$ for B). Fisher's PLSD post hoc test indicated that the effect of sinomenine at 40 or 80 mg/kg is significantly different from the saline group. $^{*}P < 0.05$, $^{**}P < 0.01$, post-drug time points were compared with pre-drug baselines using Wilcoxon signed rank test (A, B). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, post-drug time points were compared with corresponding saline controls using Mann–Whitney U test.

first 3–4 h after drug administration both in the inflammatory and post-inflammatory phases of CAIA (Fig. 2A and B).

3.3. No side effects were observed following single-dose sinomenine administration

For detecting potential side effects produce by single-dose sinomenine, we applied open field test for 5 min in naïve mice and naïve mice injected with saline or 80 mg/kg sinomenine (1 h prior to test). There was no increase of the duration of passivity (Fig. 3A), which is the sign for allergy or sedation, after sinomenine or saline application. In addition, locomotor activities quantified by number of passed grids (Fig. 3B) and number of rearing behaviours (Fig. 3C) were also not changed following sinomenine administration. Further, rectal temperature (which can be affected by severe allergy), was also similar in the sinomenine treated group compared to naïve or saline treated animals (Fig. 3D).

3.4. Effect of repeated administration of sinomenine

Repeated injection of 80 mg/kg sinomenine 2 times/day for 5 days during days 11–15 post CII antibody administration (inflammatory phase), had no effect on the arthritis scores in the CAIA model in comparison to saline treated animals (Fig. 3A). During the post-inflammatory phase, the arthritis in CAIA animals slowly dissipated from day 30 to day 54, repeated sinomenine at days 49–53 had no effect on the arthritis scores (Fig. 3A).

Sinomenine administered 2 times/day for 5 days during the peak of inflammation significantly alleviated the mechanical hypersensitivities in the hind paws (Fig. 3B) and in the neck/flank region (Fig. 3C). Baseline mechanical hypersensitivity was significantly increased from the second day after the start of repeated sinomenine treatment, and remained significantly elevated for 3 days after the cessation of sinomenine treatment (Fig. 3B and C).

During the post-inflammatory phase, repeated sinomenine administration (at days 49–53 post CII antibody administration, 80 mg/kg, 2 times/day) significantly alleviated mechanical hypersensitivity both of the hind paws and on the neck/back regions

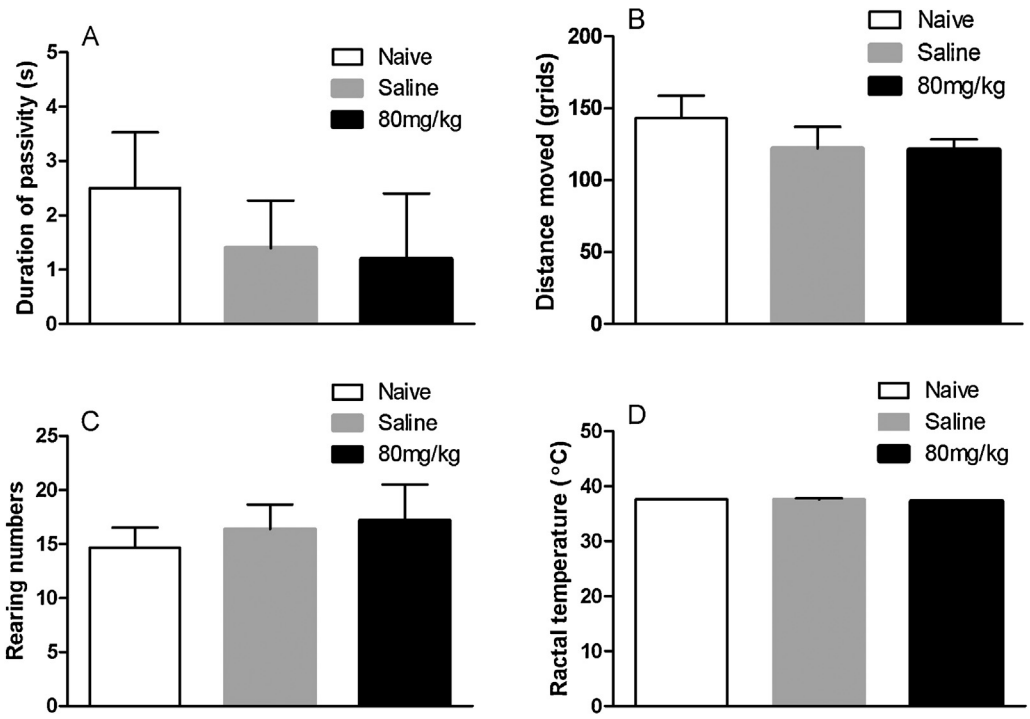

Fig. 3. Effect of saline or 80 mg/kg sinomenine on duration of passivity (A), moved distance (B), number of rearing behaviours (C) and rectal temperature (D), in naïve mice during open field test for 5 min. $N = 5–6$ mice. Data are presented as mean ± SEM. One-way ANOVA indicated there is no significant difference between groups in (A–D; $P > 0.05$).

(Fig. 3A and B). Baseline mechanical hypersensitivity was significantly increased from the second day after the onset of repeated sinomenine treatment for the hind paw, but only on day 5 for the spread hypersensitivity (Fig. 3B and C). The effect persisted for at least one day after the cessation of sinomenine treatment as the experiments were terminated on day 54 according to a predetermined schedule (Fig. 3B and C).

No side effects were observed during repeated sinomenine treatments during both the inflammatory and post-inflammatory phases (Fig. 4).

4. Discussion

As previously observed [9], experimental arthritis using the CAIA model generated hyperalgesic response (mechanical hypersensitivity to paws) both during the acute inflammatory phase and during the post-inflammatory phase. Furthermore, in CAIA mice there is a spread mechanical hypersensitivity in the neck/back region which can be observed both acutely and post inflammation. In the present study, we were able to demonstrate that administration of sinomenine effectively and dose-dependently alleviated the localized and spread mechanical hypersensitivity during both phases without producing side effects such as motor deficiency, severe allergy or sedation. This supports our previous conclusion that sinomenine is a novel analgesic with a wide spectrum of activities against different types of pain [7]. Furthermore, repeated administration of sinomenine during the peak of inflammation did not change the arthritic scores, despite producing marked analgesia. Thus, it is likely that the analgesic mechanism of sinomenine is independent from possible anti-inflammatory action of the compound.

Sinomenine is used in China and Japan as an anti-rheumatic drug [11]. It has been reported that in collagen induced arthritis (CIA) mice, treatment with sinomenine decreased the incidence and severity arthritis [12]. Clinical research has also indicated that compared with NSAIDs, sinomenine was more effective in ameliorating morning stiffness, painful joints and erythrocyte sedimentation rate in RA patients [13]. In the present study, however, we did not find that repeated sinomenine (2 times/day for 5 days) reduced acute inflammation (arthritis score) in the CAIA mice. This could be due to several factors, such as dose, timing of the treatment and models used. In contrast to the CIA model, which requires T-cell activation, the CAIA model, by directly injecting antibodies against the type II collagen to trigger arthritis, bypasses this step. Thus, the anti-rheumatic effect of sinomenine may be related to inhibition of T-cell activation. It is also possible that some of the attribution of sinomenine as an anti-rheumatic may be derived from its analgesic effect against arthritic pain.

No tolerance was seen to the analgesic effect of sinomenine following repeated administration. Rather, there was a significant increase in pre-drug response threshold which lasted beyond the duration of drug treatments. Lack of tolerance to the effect of sinomenine was similarly noted in rodent models of neuropathic pain [14]. It has also been shown that long-term pretreatment with sinomenine may delay the analgesic tolerance to morphine [15]. Sinomenine has a relatively short half-life in the plasma of rodents with no accumulation [16,17]. It is thus unlikely that this effect of repeated sinomenine administration is due to an accumulation of the drug. The metabolites of sinomenine are present in at least three forms [18]. It is unclear whether these metabolites are responsible for the effect of repeated sinomenine since pharmacological properties of these metabolites are unknown. Finally, the effects of repeated sinomenine may reflect lasting, but not permanent, changes in the nervous system resulting from repeated drug treatment.

The mechanism for the effect of sinomenine in the CAIA model is not clear. Sinomenine is not an opioid and the antinociceptive effect of sinomenine in neuropathic pain is not mediated by naloxone sensitive opioid receptors [7]. Sinomenine can interact with neuro-immune crosstalk by suppressing microglia activation

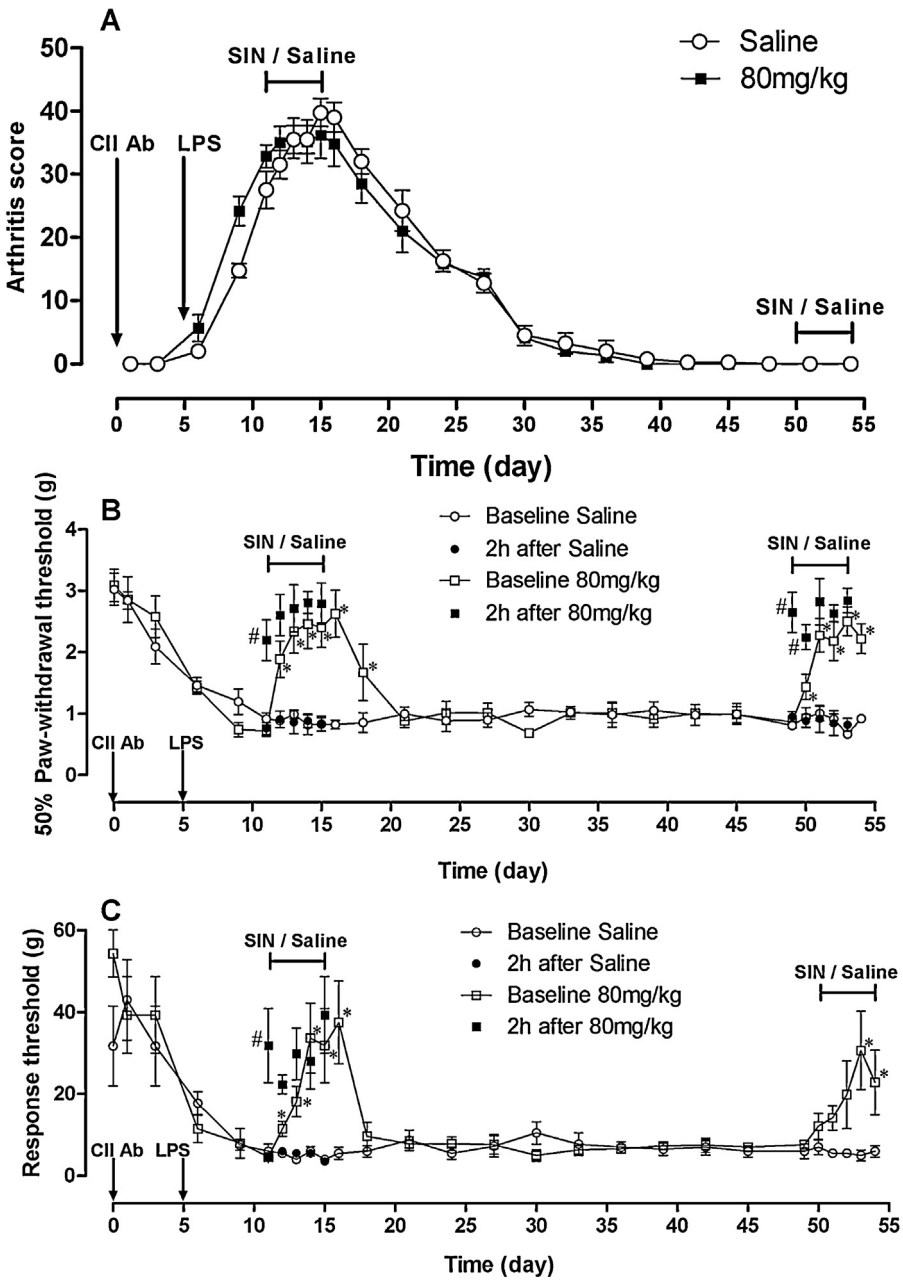

Fig. 4. Effect of 80 mg/kg sinomenine injected 2 times/day for 5 days on the development of arthritic scores (A), mechanical hypersensitivity of the hind paw (B) and spread mechanical hypersensitivity (C) in CAIA mice. $N = 6$ mice per group. Data presented as mean ± SEM. In (A) there is no significant difference between sinomenine and saline treated groups in the arthritic scores. In (B and C) sinomenine upon repeated treatment reduced mechanical hypersensitivitiy ($^{\#}P < 0.05$) compared to corresponding pre-drug value on each day of the treatment (B, C), and $^{*}P < 0.05$, compared to the baseline value of days 11 and 49 (starting time of repeated sinomenine treatment) using Wilcoxon signed rank test.

[19,20], reduce inflammation and hyperactivity in the central nervous system. Previously, microglial activation was found in the spinal cord of CAIA but not control mice [9], which suggests the presence of microglia mediated central sensitization in this model. Thus, it is possible that down-regulation of microglial activities in the spinal cord by sinomenine can be responsible for the reduction pain-related behaviour in the CAIA model. Moreover, sinomenine can modulate the synthesis of factors considered important for RA induced inflammation and pain, such as TNF, prostaglandin E2, INF-γ, reactive oxygen species, NO, NF-κB, p38MAPK and metalloproteinases [12,19,21–23]. Suggesting that, it is also conceivable that the analgesic mechanisms of sinomenine can be mediated by these factors.

5. Conclusions

In conclusion, the present results have shown that sinomenine is effective in alleviating localized and spread hypersensitivities in CAIA mice both during acute inflammation and in post-inflammatory phase. Further, repeated sinomenine administration has raised the baseline mechanical threshold without producing tolerance.

6. Implications

The present results indicate that sinomenine by itself or in combination with other established drugs, may be clinically useful in

management of chronic pain in RA conditions, including widespread pain which appears to be a difficult clinical problem despite the improvement in the acute treatment of RA by disease modifying agents.

Conflict of interest

We declare that there is no conflict of interest.

Acknowledgements

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References

[1] Taylor P, Manger B, Alvaro-Gracia J, Johnstone R, Gomez-Reino J, Eberhardt E, Wolfe F, Schwartzman S, Furfaro N, Kavanaugh A. Patient perceptions concerning pain management in the treatment of rheumatoid arthritis. J Int Med Res 2010;38:1213–24.
[2] Lee YC. Effect and treatment of chronic pain in inflammatory arthritis. Curr Rheumatol Rep 2013;15:300.
[3] Andersson ML, Svensson B, Bergman S. Chronic widespread pain in patients with rheumatoid arthritis and the relation between pain and disease activity measures over the first 5 years. J Rheumatol 2013;40:1977–85.
[4] Lang LJ, Pierer M, Stein C, Baerwald C. Opioids in rheumatic diseases. Ann N Y Acad Sci 2010;1193:111–6.
[5] Steiman AJ, Pope JE, Thiessen-Philbrook H, Li L, Barnabe C, Kalache F, Kung T, Bessette L, Flanagan C, Haraoui B, Hochman J, Leclercq S, Mosher D, Thorne C, Bykerk V. Non-biologic disease-modifying antirheumatic drugs (DMARDs) improve pain in inflammatory arthritis (IA): a systematic literature review of randomized controlled trials. Rheumatol Int 2013;33:1105–20.
[6] Luo XW. Translation, Compendium of Materia Medica, vol. 6. Peking: Foreign Languages Press; 2003. ISBN 7-119-03260-7.
[7] Gao T, Hao J, Wiesenfeld-Hallin Z, Wang DQ, Xu XJ. Analgesic effect of sinomenine in rodents after inflammation and nerve injury. Eur J Pharmacol 2013;721:5–11.
[8] Nandakumar KS, Holmdahl R. Collagen antibody induced arthritis. Methods Mol Med 2007;136:215–23.
[9] Bas DB, Su J, Sandor K, Agalave NM, Lundberg J, Codeluppi S, Baharpoor A, Nandakumar KS, Holmdahl R, Svensson CI. Collagen antibody-induced arthritis evokes persistent pain with spinal glial involvement and transient prostaglandin dependency. Arthritis Rheum 2012;64:3886–96.
[10] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.
[11] Yamasaki H. Pharmacology of sinomenine, an anti-rheumatic alkaloid from *Sinomenium acutum*. Acta Med Okayama 1976;30:1–19.
[12] Huang F, Yamaki K, Takano H, Inoue K, Yanagisawa R, Yoshino S. Effect of sinomenine on collagen-induced arthritis in mice. Autoimmunity 2007;40:532–9.
[13] Xu M, Liu L, Qi C, Deng B, Cai X. Sinomenine versus NSAIDs for the treatment of rheumatoid arthritis: a systemic review and meta-analysis. Planta Med 2008;74:1423–9.
[14] Gao T, Shi T, Wang DQ, Wiesenfeld-Hallin Z, Xu XJ. Repeated sinomenine administration alleviates chronic neuropathic pain-like behaviors in rodents without producing tolerance. Scand J Pain 2014;5:249–55.
[15] Wang HM, Chang CK, Cheng JH, Wu HT, Li YX, Cheng JT. Activation of opioid μ-receptor by sinomenine in cell and mice. Neurosci Lett 2008;443:209–12.
[16] Liu L, Buchner E, Beitze D, Schmidt-Weber CB, Kaever V, Emmrich F, Kinne RW. Amelioration of rat experimental arthritides by treatment with the alkaloid sinomenine. Int J Immunopharmacol 1996;18:529–43.
[17] Ling J, Wang Y, Xie B, Li R. Pharmacokinetic studies of sinomenine by blood microdialysis technique. J Guangzhou Univ Tradit Chin Med 2005;5:021.
[18] Cheng WM, Qiu F, Yao XS. Three major urinary metabolites of sinomenine in rats. J Asian Nat Prod Res 2007;9:13–8.
[19] Qian L, Xu Z, Zhang W, Wilson B, Hong JS, Flood PM. Sinomenine, a natural dextrorotatory morphinan analog, is anti-inflammatory and neuroprotective through inhibition of microglial NADPH oxidase. J Neuroinflamm 2007;4:23.
[20] Shukla SM, Sharma SK. Sinomenine inhibits microglial activation by Aβ and confers neuroprotection. J Neuroinflamm 2011;8:117.
[21] Huang F, Yamaki K, Tong X, Fu L, Zhang R, Cai Y, Yanagisawa R, Inoue K, Takano H, Yoshino S. Inhibition of the antigen-induced activation of RBL-2H3 cells by sinomenine. Int Immunopharmacol 2008;8:502–7.
[22] Liu L, Riese J, Resch K, Kaever V. Impairment of macrophage eivosanoid and nitric oxide production by an alkaloid from *Sinomenium acutum*. Arzneimittelforschung 1994;44:1123–36.
[23] Zhou H, Wong YF, Wang J, Cai X, Liu L. Sinomenine ameliorates arthritis via MMPs, TIMPs, and cytokines in rats. Biochem Biophys Res Commun 2008;376:352–7.

III

Synergistic interaction between sinomenine and gabapentin in treating neuropathic pain

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Abstract

Background and aims: Chronic pain is one of the biggest unmet clinical challenge, involving a broad patient population in the world. The management of chronic pain in the clinical settings is restricted by the lack of effective tools. This study aims to assess the efficacy of sinomenine and gabapentin in combination for treating peripheral and central chronic neuropathic pain using animal models.

Methods: The study was conducted in mice with photochemically induced sciatic nerve injury, and rats with photochemically induced spinal cord injury. Sinomenine was applied by oral administration (p.o.) in mice, injected subcutaneously (s.c.) in mice and intraperitoneally (i.p.) in rats. Gabapentin was applied i.p. both in mice and rats. Different treatment schedules were utilized regarding the time points of administration of the two drugs, and the efficacy of repeated treatment was tested in rats with effective combination treatment regime. The paw withdrawal threshold and the vocalization threshold to mechanical stimulation were tested in mice and rats respectively, after single and repeated drug administration.

Results: In low dose combination (in which each drug alone does not show efficacy) in mice with peripheral neuropathic pain, when gabapentin was first applied, and sinomenine administrated 30-60 min later, no analgesic effect was observed. However, when sinomenine was applied first and then gabapentin was administrated 30-60 min later, the combination produced significant analgesic effect up to 3 hours. Similarly, in rats with spinal cord injury, the drug combination produced a strong and prolonged analgesic effect at the dosages 1/10 of the effective dosages of each drug alone. Moreover, the repeated administration of low dose combination of sinomenine and gabapentin also elevated the baseline threshold before drug administration, with no notable side effects.

Conclusions: The combined therapy of sinomenine and gabapentin has synergic effect in alleviating experimental neuropathic pain after spinal cord and peripheral nerve injury in rodent models. The repeated administration of combined therapy also produced significant effect without producing tolerance. These findings suggest feasibility of applying the combination of sinomenine and gabapentin as a novel neuropathic pain therapy.

Key words
Sinomenine, Gabapentin, Neuropathic Pain, Drug Combination

1. Introduction

Chronic pain, such as neuropathic pain after injuries to the peripheral or central nervous systems is one of the biggest unmet clinical challenge, involving a broad patient population in European Union and the world (Geber et al., 2009; Treede et al., 2008). Such pain is complex, long-term, resistant to treatments, and can significantly decrease the quality of life of affiliated patients (Breivik et al., 2006; Jensen & Finnerup, 2007; van Hecke et al., 2014).

The management of chronic pain in clinical settings is restricted by the lack of effective tools and the improvement in treatment strategy is limited during recent years (Beniczky et al., 2005; Jensen & Finnerup, 2007). Current treatment strategy mainly depends on the first-line pharmaceutical drugs, such as TCA antidepressants and antiepileptic (i.e., gabapentin and pregabalin) medications, however, they do not provide adequate pain relief in majority of the patients (O'Connor & Dworkin, 2009). The latest report has shown that, only 32% patients with gabapentin had substantial benefit (at least 50% pain relief) and 46% had moderate benefit (at least 30% pain relief) in postherpetic neuralgia (Wiffen et al., 2017). Moreover, the usage of opioid analgesics, as the second-line treatments under certain clinical circumstance, are often ineffective in long run and are associated with side effects including constipation, nausea and tolerance (Benyamin et al., 2008; Von Korff, 2013). These current challenges advocate the development of novel methods for the treatment of chronic pain with increased analgesic efficacy, minimized side effects and no occurrence of drug tolerance.

Sinomenine is an alkaline substance extracted from the roots of the plant *Sinomenium acutum*, which has similar structure to morphine and dextromethorphan (Yamasaki, 1976). It has long been used in East Asia to treat conditions of rheumatism. In mouse collagen-induced arthritis (CIA) model, Sinomenine treatment can reduce the severity and frequency of arthritis (Huang et al. 2007). Compared with non-steroidal anti-inflammatory drugs (NSAIDS), sinomenine is superior on morning stiffness, joint pain and on erythrocyte sedimentation rate (Xu et al. 2008). Sinomenine is also used as an immunosuppressive agent, which inhibits lymphocyte proliferation and the synthesis of B cell antibodies (He et al. 2005). In our previous study, we found that sinomenine possessed analgesic properties in treating rodents with neuropathic pain after peripheral and central nervous system injury, without producing observable side effects (Gao et al., 2013). In addition, repeatedly administered sinomenine resulted no tolerance, but on the other hand, increased the baseline pain threshold (Gao et al., 2014), suggesting it has a promising potential to be applied in chronic pain therapy.

In clinical practice, it is very common that chronic pain patient starts with monotherapy, but ends up in using drug combinations. It has been shown that the effectiveness of combined therapy are improved compared with monotherapy in treating neuropathic pain (Vorobeychik et al., 2011). The monotherapy of gabapentin is commonly used as the first-line regimen for management of neuropathic pain. Evidence has showed

that gabapentin added with morphine can achieve better effectiveness in relieving neuropathic pain than single agent of each drug, at lower dosage (Gilron et al., 2005). What is more, our recent study has shown that gabapentin and NMDA receptor antagonist dextromethorphan can interact synergistically to alleviate allodynia in animal model of neuropathic pain (Shi et al., 2018). Since sinomenine is structurally related to morphine and dextromethorphan (Yamasaki, 1976), but do not have morphine associated addiction and tolerance, assessing the possibility if sinomenine could replace morphine to combine with gabapentin and obtain an enhanced analgesic effect is of high clinical importance.

Therefore, this study aims to assess the efficacy of sinomenine and gabapentin combination for treating peripheral and central chronic neuropathic pain using our well-established photochemically induced nerve injury animal models, and to determine the dosage effect for monotherapy as well as combination therapy of these two drugs at different application scenarios.

2. Material and methods

2.1 Animals

All the experiments were strictly following the IASP ethical guidelines and approved by the local animal research ethics committee. All mice (C57BL/6 mice, male, Charles River, Sollentuna, Sweden) were housed 6 per cage, and rats (Sprague-Dawley rats, both sexes, Harlan, Horst, The Netherlands; Möllegård, Denmark) were housed 4 per cage, both respectively and in standard laboratory condition (22 ºC 12 hours' light/dark cycle) with ad libitum access to water and food.

2.2. Photochemically induced sciatic nerve injury in mice

Detailed method for producing sciatic nerve ischemic injury in mice has been described previously (Hao et al., 2000a). Mice were anaesthetized by 75 mg/kg ketamine add with 1 mg/kg medetomidine, then the left sciatic nerve was exposed from surrounding tissue above 1cm to the trifurcation. After being injected intravenously (i.v.) with 32.5 mg/kg of the photosensitizing dye erythrosine B (Red N13, Aldrich-Chemie, Steinheim, Germany), the sciatic nerve was irradiated under an argon ion laser (514 nm, 0.16W, Innova model 70, Coherent Laser Product Division, Palo Alto, CA) for 45 seconds. During the experiment, a heating pad was applied to maintain the body temperature between 37-38 ºC. After irradiation, the wound was closed and mice were returned to their home cages.

2.3 Behavioral test in mice

The paw withdrawal threshold to mechanical stimulation was applied two weeks after sciatic nerve injury, when animal exhibited mechanical sensitivity of the hind paws. The withdrawal threshold of the ipsilateral hind paw to mechanical stimulation was tested using a set of calibrated von Frey hairs (Stoelting, IL, USA). To test the sensitivity of mechanical stimulation, mice were place in plastic cages with a metal mesh floor. The plantar surface of the hind paws was stimulated with increasing force from 0.02g to 4g until the animal withdrew the limb. Each filament was applied 5 times and threshold was taken when the animal withdrew the paw at least 3 out of 5 consecutive stimuli.

2.3. Photochemically induced spinal cord injury in rats

The method of producing photochemically induced spinal cord ischemic injury in rats has been described previously (Hao et al., 1991, 1992). Rats were anaesthetized by 75 mg/kg ketamine add with 1 mg/kg medetomidine. Then a midline incision was made in the skin overlying vertebral segments T12-L1. After i.v. injection of erythrosine B (32.5 mg/kg), vertebral segment T12 or T13 (spinal segments L3–5) was irradiated under an argon ion laser for 10 minutes. A second dosage of erythrosin B was applied 5 min after the start of irradiation. During irradiation, the body temperature of rats was maintained between 37–38 ºC. After irradiation, the wound was closed in layers and rats are returned to their home cages.

2.4 Behavioral test in rats

The vocalization threshold were tested 4–5 weeks after the spinal cord injury when the rats exhibited hypersensitivity to innocuous mechanical and cold stimuli at flank or upper back areas as described previously(Xu et al., 1992). The rats were shaved and gently held in a standing position. A set of von Frey hairs was used to produce graded pressure to the skin from 0.02g to 100g, 5–10 times in each pressure with the frequency at 1/s. The stimulation which induced consistent vocalization (>75% response rate) was considered as vocalization threshold.

The cold score was examined with ethyl chloride spray (Rönnings Europa AB, Sweden). It was applied onto the shaved flank or upper back area to produce cold stimuli. The response was graded according to the following scale: 0 = no response; 1 = startle-like response, no vocalization, 2 = vocalization, 3 = consistent vocalization combined with avoidance.

2.5 Drugs

For preparation of injection solution, Sinomenine (obtained from the National Institute for Food and Drug Control, Beijing, China, purity > 99%) was dissolved in DMSO (Sigma-Aldrich), then mixed with Cremophor EL oil (Sigma-Aldrich) and saline by vortex mixer (Bibby Scientific, UK) using the volume rate of 1:4:5. Any further dilution was mixed with saline. Sinomenine was applied by single oral administration (p.o.) or injected subcutaneously (s.c.) into the skin over the neck in mice and injected intraperitoneal (i.p.) in rats.

For preparation of injection, gabapentin (Research Biochemicals Inc., USA) was dissolved in saline. The drug was injected intraperitoneal (i.p.) in a volume of 1 ml/kg.

2.6 Statistics

All the experiments were double-blind experiments. Data were presented as median ± MAD. The data were analyzed following paired t-test, Wilcoxon Signed rank test or Two-way analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD). All statistics were made by SPSS, $P<0.05$ was considered to be statistically significant.

3 Results

We have conducted extensive experiments examining the interaction between sinomenine and gabapentin in rodent models of neuropathic pain and our results showed

that there is marked synergistic interaction between these two drugs in producing analgesia.

3.1. Synergistic analgesia effect of sinomenine and gabapentin in a mouse model of peripheral nerve injury.

As previously reported, mice developed chronic hypersensitivity to mechanical stimuli (von Frey hair) after sciatic nerve injury (SNI) in mice (Hao et al., 2000a). These pharmacological experiments were applied two weeks after sciatic nerve injury, when animal exhibited elevated mechanical sensitivity of the hind paws. The analgesic index is paw-withdraw threshold to mechanical stimulation (grams).

A single dose of p.o. administrated sinomenine at 20mg/kg or a single dose of i.p. administrated gabapentin at 30mg/kg have no significant effect on response threshold observed up to 4 hours (tested in every 30-60min) in SNI mice (Fig. 1A). In low dose combination, when gabapentin was first applied at dosage of 7.5mg/kg, with sinomenine administrated at dosage of 10mg/kg 30-60 min later, no analgesic effect was observed on response threshold up to 4 hours (Fig. 1B). However, when sinomenine was applied first and then gabapentin was administrated 30-60 min later, the combination produced significant analgesic effect up to 3 hours (Fig. 1C). Three dosages of combination were tested in the experiment: “sinomenine 10mg/kg + gabapentin 7.5mg/kg”, “sinomenine 20mg/kg + gabapentin 7.5mg/kg” and “sinomenine 20mg/kg + gabapentin 15mg/kg” (Fig. 1C). No side effect was observed in all combination groups at these dosages. Further, the analgesic effect was increased

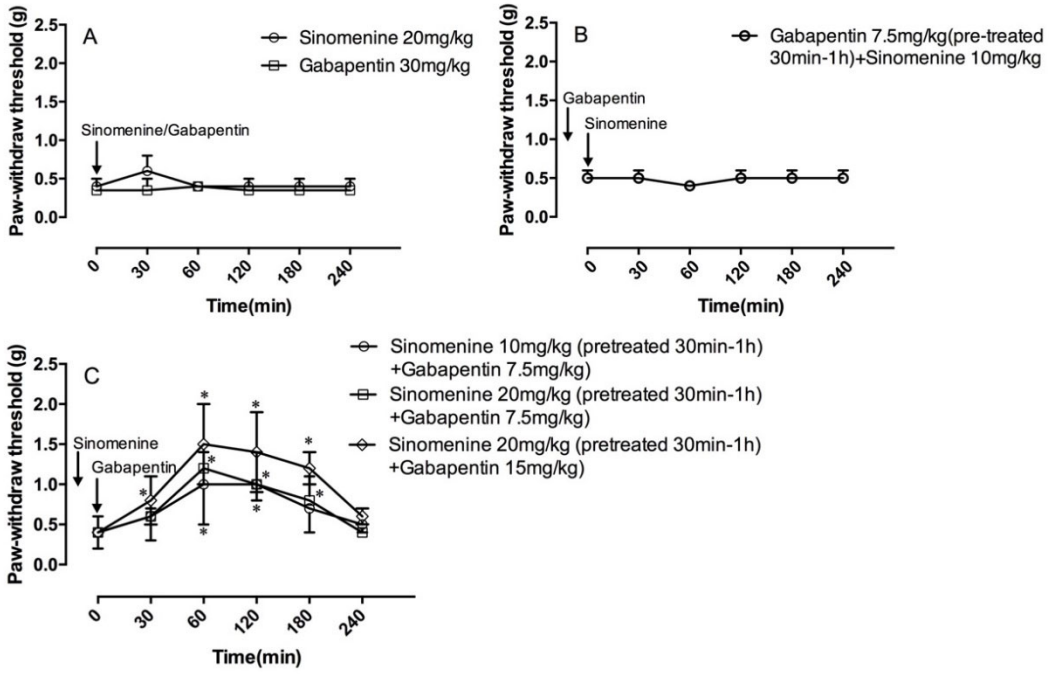

Fig. 1 Effect of single applied 20mg/kg p.o. sinomenine or 30mg/kg i.p. gabapentin (A), combination applied 7.5mg/kg gabapentin 30-60min before 10mg/kg sinomenine(B), combination applied 10, 20mg/kg sinomenine 30-60min before 7.5, 15mg/kg gabapentin(C) on hind paw withdrawal threshold to mechanical stimulation with von Frey hairs in male C57/BL6 mice with sciatic nerve ischemic injury. N = 6 animals in each group, data are presented as median ± MAD. Post-drug time points were compared with pre-drug withdrawal threshold at time 0 using Paired t-test, *P<0.05.

following by increase in dosages of combination.

3.2 Synergistic analgesia effect of sinomenine and gabapentin in rats with spinal cord injury.

As previously report, rats developed chronic hypersensitivity to mechanical (von Frey hair) and cold (ethyl chloride) stimuli after spinal cord ischemia injury (SCI) (Hao et al., 1991, 1992). These pharmacological experiments were applied 4-5 weeks after spinal cord ischemia surgery, when animal exhibited hypersensitivity to innocuous mechanical and cold stimulation. The analgesic index is vocalization threshold to mechanical stimulation (grams) or cold scores to cold stimulation.

A single dose of i.p. administrated sinomenine at 20mg/kg or gabapentin at 30mg/kg did not produce any significant analgesia effect against mechanical or cold stimulation (Fig. 2A and 2B). However, when applied in drug combination, small doses of sinomenine combined with gabapentin (sinomenine pretreated 30-60min before gabapentin) produced a strong analgesic effect observable even longer than 4 hours after drug administration. Three dosages of combination were tested in the experiment: “sinomenine 5mg/kg + gabapentin 2mg/kg”, “sinomenine 10mg/kg + gabapentin 4mg/kg” and “sinomenine 20mg/kg + gabapentin 7.5mg/kg (Fig. 2C and 2D). No side effect was observed in all combination groups at these dosages. The analgesic effect was increased following by increase in dosages of

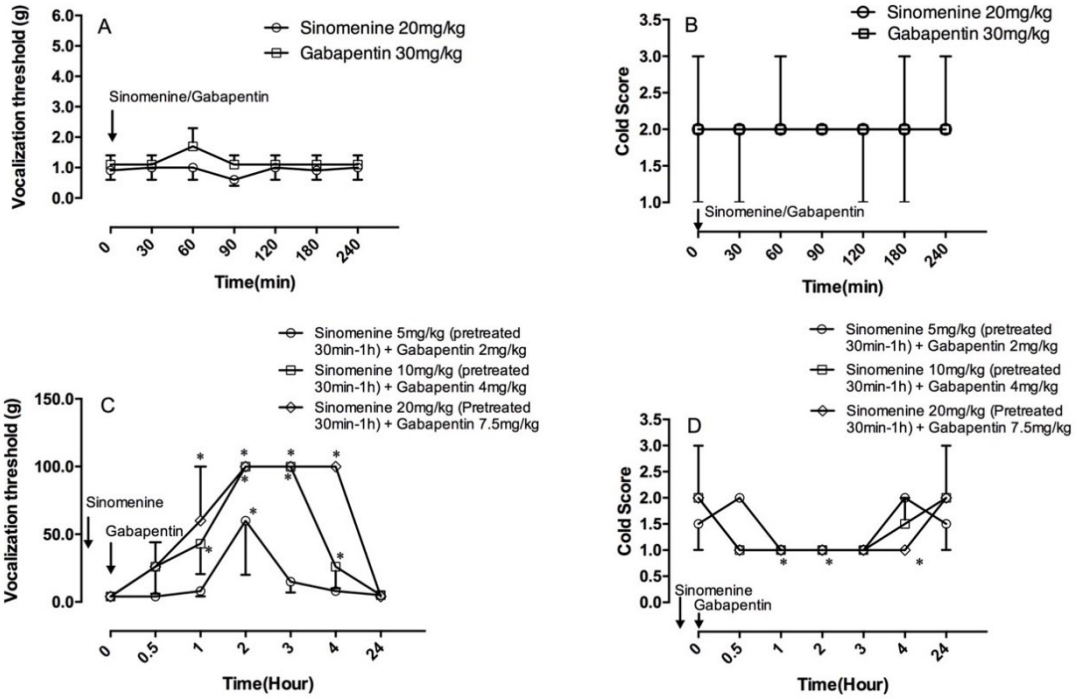

Fig. 2 Effect of single applied 20mg/kg s.c. sinomenine or 30mg/kg i.p. gabapentin (A and B), combination applied 5, 10 and 20mg/kg sinomenine 30-60min before 2, 4 and 7.5mg/kg gabapentin (C and D) on vocalization threshold to mechanical stimulation with von Frey hairs or cold score to stimulation with ethyl chloride in flank area of male SD rats with spinal cord ischemic injury. N = 7 to 8 animals in each group, data are presented as median ± MAD. Post-drug time points were compared with pre-drug vocalization threshold at time 0 using Paired t-test, *P<0.05. Two-way ANOVA with Fisher's Least Significant Difference (LSD) indicated a significant difference between the groups (F=10.8, P<0.001 for C)

combination.

In addition, simultaneous administration of 10mg/kg sinomenine and 4mg/kg gabapentin could also produce significant synergistic analgesia effect to against both mechanical and cold stimulation (Fig. 3A and 3B). No observable side effect in rats were produced.

3.3 Synergistic analgesia effect of repeated administration of sinomenine and gabapentin in rats with spinal cord injury.

To study the analgesic effect of chronic administration of small doses combination of sinomenine and gabapentin in rat model of spinal cord injury, we select 10mg/kg i.p sinomenine combined with 4mg/kg i.p. gabapentin in this pharmacological experiment. The analgesic index is vocalization threshold to mechanical stimulation (grams). In two rounds of experiments, the combined administrations are repeated twice daily and sustained for 7days (Fig. 4A) and 14days (Fig. 4B). Sinomenine was applied 30-60min before gabapentin and performed with dosing interval of 6 hours per day.

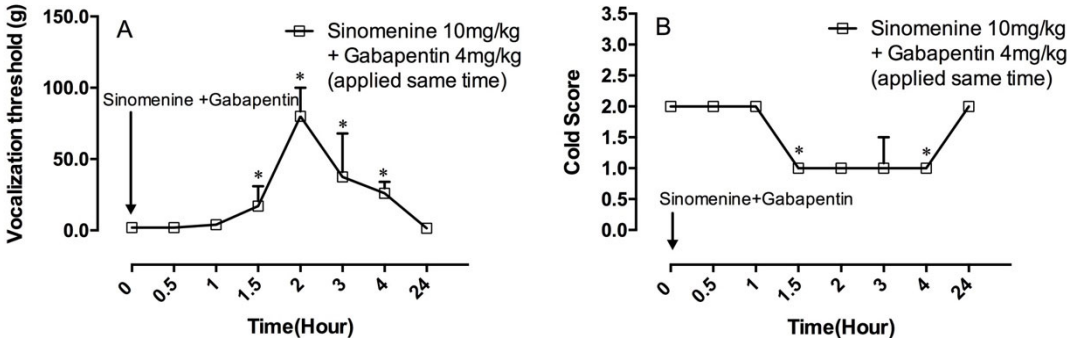

Fig. 3 Effect of simultaneous administrated 10mg/kg i.p. sinomenine and 4mg/kg i.p. gabapentin on vocalization threshold to mechanical stimulation with von Frey hairs (A) or cold score to stimulation with ethyl chloride (B) in flank area of male SD rats with spinal cord ischemic injury. N = 7 to 8 animals in each group, data are presented as median ± MAD. Post-drug time points were compared with pre-drug vocalization threshold at time 0 using Paired t-test, *P<0.05.

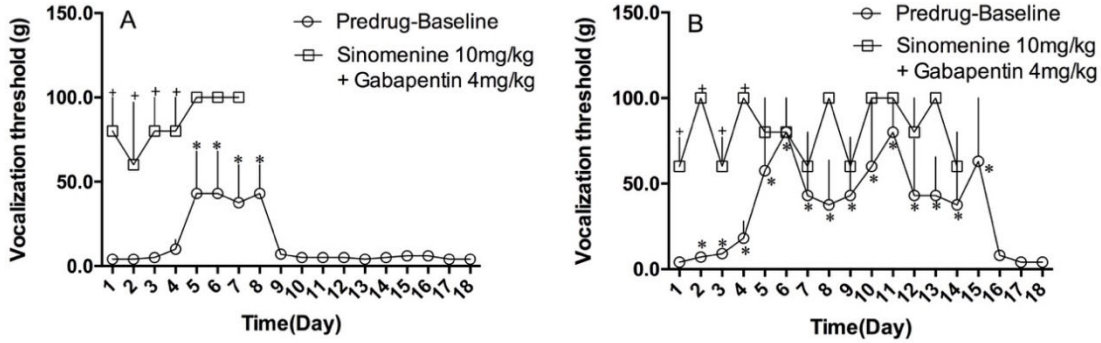

Fig. 4 Effect of repeated combination of 10mg/kg i.p. sinomenine applied 30-60min before 4mg/kg i.p. gabapentin twice/day for 7 days(A) and 14 days(B) on vocalization threshold to mechanical stimulation with von Frey hairs in flank area of male SD rats with spinal cord ischemic injury. N=6 rats in each group, data were presented as median ± MAD. Baseline and post-drug threshold from D2 to D18 were compared with D1 using Wilcoxon Signed Rank test, *P<0.05. Post-drug threshold was compared with baseline value using Wilcoxon Signed Rank test, +p<0.05.

The repeated combination of 10mg/kg sinomenine and 4mg/kg gabapentin last for 7 or 14days were produced significant analgesic effect against mechanical stimulation. More importantly, in the course of chronic administration, the pain threshold before the administration was also significantly increased, which indicates that the drug combination produced a sustained analgesia. In about 2 days after termination of last drug administration, the pain threshold of rats returned to pre-treatment level. The drug composition in rats did not produce observable side effects during chronic administration.

4. Discussion

The clinical management of chronic pain has appeared to be one of the most challenging health issues, facing lack of new strategies. In the present study, we have shown that combined therapy of sinomenine and gabapentin produced synergistic analgesia effect compared to monotherapy against neuropathic pain in central and peripheral nerve injured rats and mice. Moreover, the repeated administration of sinomenine and gabapentin combination produced significant analgesic effect against mechanical stimulation, with a sustained analgesic effect by increasing the baseline threshold even before drug administration, and no observable side effects. These findings strongly advocate the feasibility of applying the combination of sinomenine and gabapentin as a novel neuropathic pain therapy, although the mechanism of such synergic effect produced by sinomenine and gabapentin on chronic pain is still unknown.

We have previously demonstrated sinomenine analgesic property against neuropathic pain (Gao et al., 2013; Gao et al., 2014). Despite of the well-defined analgesic efficacy, sinomenine's mechanism of action has not yet been established. One possible assumption relies on the neuroprotective effect of sinomenine mediated by decrease of production of superoxide ions through inhibiting the microglial NADPH oxidase (Qian et al. 2007) thereby reduce the reactive oxygen species (ROS). Moreover, sinomenine can excert anti-inflammatory property by inhibiting cyclooxygenase (COX-2), thereby reducing the synthesis of the pronociceptive substance prostaglandin E2 (PGE2) (Liu et al., 1994). It is also possible that sinomenine could reduce neuroinflammation through deactivation of microglias (Qian et al. 2007), which may subsequently help to stablize the micro environment of the pain relaying neuronal structure and reduce the neuronal overactivation. Apart from the neuroprotective and anti-inflammatory effects, another explanation refers to the direct antinociceptive effects from sinomenine, which can be mediated through $GABA_A$ receptor (Zhu et al., 2014), or by blocking the acid-sensing ion channel and calcium channels (Wu et al., 2011).

GABA is an important inhibitory neurotransmitter in the mammalian central nervous system, playing an important role in maintaining the balance of excitation and inhibition. A deficiency in GABAergic input will lead to pain, anxiety, restlessness, and fatigue. Chronic pain is often associated with down-regulated and dis-functional GABAergic system， while gabapentin can mimic GABAergic input (Kuzniecky et al., 2002), and in turn mitigate the imbalanced

GABAergic neurotransmission in such situation. Whereas, gabapentin's principal proposed mechanism of action is the interaction with the alpha 2-delta subunit of L-type voltage-regulated calcium channels rather than just enhance GABAergic neurotransmission (Striano and Striano, 2008).

In the present study, we have shown that in rats with neuropathic pain, adequate allodynia reduction could only be observed with the dosages larger than 100 mg/kg for gabapentin (Hao et al., 2000b) and 40 mg/kg (Gao et al., 2013) for sinomenine. When two agents were combined, we found a much less doses (1/10 of the original doses for both drugs), at dosage of 10 mg/kg for gabapentin and 4 mg/kg for sinomenine, can achieve the same antinociceptive efficacy. This indicate that gabapentin and sinomenine can dramatically potentiate each other's analgesic efficacy in combination formula. One possible mechanism of such potentiated combined efficacy is that both sinomenine and gabapentin can modulate / partially block calcium channels (Wu et al., 2011; Striano and Striano, 2008), and enhance GABAergic neural inhibition (Zhu et al., 2014; Kuzniecky et al., 2002), thus worked synergically against chronic pain.

There are established evidences showing both morphine and dextromethorphan can potentiate gabapentin's effect on suppressing chronic pain (Gilron et al., 2005). In the current study, we found sinomenine having similar property to enhance gabapentin's analgesic efficacy. Taking consideration also that the structure of sinomenine is closely related to morphine and dextromethorphan (Yamasaki, 1976), it is rational to postulate that such synergic effect with gabapentin depends on shared structure between morphine, gabapentin and sinomenine, which evidently need to be further validated.

Time is a critical issue when it comes to combined drug administration. Sinomenine and gabapentin can only provide sufficient pain relief when sinomenine was pretreated 30-60 min before gabapentin application or the two drugs applied at the same time. This suggest that gabapentin's effect can be boosted only with the presence of sinomenine, but the opposite scenario does not generate synergy. Why in this drug combination, the pretreatment of sinomenine but not gabapentin is key to synergy is still mysterious to us, however we assume that this may be related to the mechanism of action of both drugs and their duration of effectiveness when applied systemically.

5. Conclusion

In conclusion, our study has shown that the combined therapy of sinomenine and gabapentin has promising synergic effect in alleviating neuropathic pain in central and peripheral nerve injury in rats and mice models, with less dosage and reduced side effects. The repeated administration of combined therapy also produced significant analgesic effect without introducing tolerance. This study, together with other studies, indicate that compared to single agent, the combined therapy entails benefits including improved analgesic effectiveness and reduced adverse effects with smaller doses of individual drugs. However, such drug composition and the application criteria need to be further validated in clinical studies before it can be widely used.

6. Acknowledgements

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References

Beniczky, S., Tajti, J., Timea Varga, E., & Vecsei, L. (2005). Evidence-based pharmacological treatment of neuropathic pain syndromes. *J Neural Transm (Vienna), 112*(6), 735-749. doi:10.1007/s00702-005-0300-x

Benyamin, R., Trescot, A. M., Datta, S., Buenaventura, R., Adlaka, R., Sehgal, N., . . . Vallejo, R. (2008). Opioid complications and side effects. *Pain Physician, 11*(2 Suppl), S105-120.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain, 10*(4), 287-333. doi:10.1016/j.ejpain.2005.06.009

Gao, T., Hao, J., Wiesenfeld-Hallin, Z., Wang, D. Q., & Xu, X. J. (2013). Analgesic effect of sinomenine in rodents after inflammation and nerve injury. *Eur J Pharmacol, 721*(1-3), 5-11. doi:10.1016/j.ejphar.2013.09.062

Gao, T., Shi, T., Wang, D.-Q., Wiesenfeld-Hallin, Z., & Xu, X.-J. (2014). Repeated sinomenine administration alleviates chronic neuropathic pain-like behaviours in rodents without producing tolerance. *Scandinavian Journal of Pain, 5*(4), 249-255. doi:https://doi.org/10.1016/j.sjpain.2014.05.006

Geber, C., Baumgartner, U., Schwab, R., Muller, H., Stoeter, P., Dieterich, M., . . . Treede, R. D. (2009). Revised definition of neuropathic pain and its grading system: an open case series illustrating its use in clinical practice. *Am J Med, 122*(10 Suppl), S3-12. doi:10.1016/j.amjmed.2009.04.005

Gilron, I., Bailey, J. M., Tu, D. S., Holden, R. R., Weaver, D. F., & Houlden, R. L. (2005). Morphine, gabapentin, or their combination for neuropathic pain. *New England Journal of Medicine, 352*(13), 1324-1334. doi:DOI 10.1056/NEJMoa042580

Hao, J. X., Blakeman, K. H., Yu, W., Hultenby, K., Xu, X. J., & Wiesenfeld-Hallin, Z. (2000a). Development of a mouse model of neuropathic pain following photochemically induced ischemia in the sciatic nerve. *Exp Neurol, 163*(1), 231-238. doi:10.1006/exnr.2000.7373

Hao, J. X., Xu, X. J., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1991). Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain, 45*(2), 175-185.

Hao, J. X., Xu, X. J., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1992). Photochemically induced transient spinal ischemia induces behavioral hypersensitivity to mechanical and cold stimuli, but not to noxious-heat stimuli, in the rat. *Exp Neurol, 118*(2), 187-194.

Hao, J. X., Xu, X. J., Urban, L., & Wiesenfeld-Hallin, Z. (2000b). Repeated administration of systemic gabapentin alleviates allodynia-like behaviors in spinally injured rats. *Neurosci Lett, 280*(3), 211-214.

Jensen, T. S., & Finnerup, N. B. (2007). Management of neuropathic pain. *Curr Opin Support Palliat Care, 1*(2), 126-131. doi:10.1097/SPC.0b013e3282eeb45f

O'Connor, A. B., & Dworkin, R. H. (2009). Treatment of neuropathic pain: an overview of recent guidelines. *Am J Med, 122*(10 Suppl), S22-32. doi:10.1016/j.amjmed.2009.04.007

Treede, R. D., Jensen, T. S., Campbell, J. N., Cruccu, G., Dostrovsky, J. O., Griffin, J. W., . . . Serra, J. (2008). Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology, 70*(18), 1630-1635. doi:10.1212/01.wnl.0000282763.29778.59

van Hecke, O., Austin, S. K., Khan, R. A., Smith, B. H., & Torrance, N. (2014). Neuropathic pain

in the general population: a systematic review of epidemiological studies. *Pain, 155*(4), 654-662. doi:10.1016/j.pain.2013.11.013

Von Korff, M. R. (2013). Long-term use of opioids for complex chronic pain. *Best Pract Res Clin Rheumatol, 27*(5), 663-672. doi:10.1016/j.berh.2013.09.011

Vorobeychik, Y., Gordin, V., Mao, J., & Chen, L. (2011). Combination therapy for neuropathic pain: a review of current evidence. *CNS Drugs, 25*(12), 1023-1034. doi:10.2165/11596280-000000000-00000

Wiffen, P. J., Derry, S., Bell, R. F., Rice, A. S. C., Tolle, T. R., Phillips, T., & Moore, R. A. (2017). Gabapentin for chronic neuropathic pain in adults. *Cochrane Database of Systematic Reviews*(6). doi:ARTN CD007938

10.1002/14651858.CD007938.pub4

Wu, W. N., Wu, P. F., Chen, X. L., Zhang, Z., Gu, J., Yang, Y. J., . . . Chen, J. G. (2011). Sinomenine protects against ischaemic brain injury: involvement of co-inhibition of acid-sensing ion channel 1a and L-type calcium channels. *Br J Pharmacol, 164*(5), 1445-1459. doi:10.1111/j.1476-5381.2011.01487.x

Xu, X. J., Hao, J. X., Aldskogius, H., Seiger, A., & Wiesenfeld-Hallin, Z. (1992). Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. *Pain, 48*(2), 279-290.

Yamasaki, H. (1976). Pharmacology of sinomenine, an anti-rheumatic alkaloid from Sinomenium acutum. *Acta Med Okayama, 30*(1), 1-20.

Zhu, Q., Sun, Y., Zhu, J., Fang, T., Zhang, W., & Li, J. X. (2014). Antinociceptive effects of sinomenine in a rat model of neuropathic pain. *Sci Rep, 4*, 7270. doi:10.1038/srep07270

IV

Original experimental

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Gabapentin and NMDA receptor antagonists interacts synergistically to alleviate allodynia in two rat models of neuropathic pain

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Abstract

Background and aims: The clinical management of neuropathic pain remains a challenge. We examined the interaction between gabapentin and NMDA receptor antagonists dextromethrophan and MK-801 in alleviating neuropathic pain-like behaviors in rats after spinal cord or sciatic nerve injury.
Methods: Female and male rats were produced with Ischemic spinal cord injury and sciatic nerve injury. Gabapentin, dextromethorphan, MK-801 or drug combinations were injected with increasing doses. Mechanical response thresholds were tested with von Frey hairs to graded mechanical touch/pressure, and ethyl chloride spray was applied to assess the cold sensitivity before and after injuries.
Results: In spinally injured rats, gabapentin and dextromethorphan did not affect allodynia-like behaviors at doses of 30 and 20 mg/kg, respectively. In contrast, combination of 15 or 30 mg/kg gabapentin with dextromethorphan at 10 mg/kg produced total alleviation of allodynia to mechanical or cold stimulation. Further reducing the dose of gapapentin to 7.5 mg/kg and dextromethorphan to 5 mg/kg still produced significant effect. MK-801, another NMDA receptor antagonist, also enhanced the effect of gabapentin in spinally injured rats. Similar synergistic anti-allodynic effect between dextromethorphan and gabapentin was also observed in a rat model of partial sciatic nerve injury. No increased side effect was seen following the combination between gabapentin and dextromethorphan.
Conclusions: In conclusion, the present study suggested that combining NMDA receptor antagonists with gabapentin could provide synergistic effect to alleviate neuropathic pain and reduced side effects.
Implications: Combining NMDA receptor antagonists with gabapentin may provide a new approach in alleviating neuropathic pain with increased efficacy and reduced side effects.

Keywords: anti-convulsant; dextromethorphan; MK-801; nerve injury; spinal cord injury.

1 Introduction

Anticonvulsants, such as carbamazepine or phenytoin, have been traditionally used for the management of neuropathic pain. Their efficacy has, however, not been unequivocally established for many types of neuropathic pain and they are often associated with side effects [1, 2]. More recently, the antiepileptics gabapentin has been increasingly used as an analgesic in neuropathic pain [1–3]. Although it was found to exert analgesic effect superior to placo in a large number of randomized, placebo controlled, double-blind clinical trials in conditions such as postherpetic neuralgia, painful diabetic neuropathy and central neuropathic pain, gabapentin only provide some degree of pain relief in a minority of neuropathic pain patients [4–7].

Another class of compounds believed to be useful in neuropathic pain is antagonists of NMDA receptors for glutamate [1, 8, 9]. Such promise was derived from the well established involvement of NMDA receptors in plasticity after injury to the nervous system as well as its pivotal role in central sensitization and hyperalgesia [8]. However, NMDA antagonists in general produced many side effects and clinical trials with several clinically available compounds with NMDA receptor blocking property in neuropathic pain have produced at best conflicting results [1, 8–10].

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It is also well established in rodent models that the antinociceptive effect of morphine is potentiated by NMDA receptor antagonists that is mediated by an interaction between the activation of the μ-opioid and NMDA receptors at cellular level [11, 12]. NMDA antagonists also reversed morphine tolerance [11]. The interaction between NMDA receptor antagonists and other analgesics is however less known. In the present study, we evaluated the analgesic interaction between NMDA receptor antagonists, MK-801 and dextromethorphan, and gabapentin using two rat models of neuropathic pain after spinal cord or sciatic nerve injury.

2 Materials and methods

Male and female Sprague-Dawley rats (Mollegård, Denmark) weighing 200–250 g at the start of the experiments were used. All experimental procedures were approved by the local research Ethics Committee.

2.1 Photochemically-induced ischemic spinal cord injury

Ischemic spinal cord injury was produced in female SD rat weighing 200 g according to methods described previously [13]. In brief, rats were anesthetized with chloral hydrate (300 mg/kg, i.p.) and a midline incision was made on the skin overlying vertebral segments T 12-L 1. The animals were positioned beneath an argon laser beam and irradiated for 10 min with the beam directed towards vertebral segment T 12 or T 13 (spinal segments L 3–5). Immediately prior to and 5 min after the start of the irradiation, erythrosin B (Red N°3, Aldrich-Chemie, Steinheim, Germany) dissolved in 0.9% saline was injected intravenously through the tail vein at a dose of 32.5 mg/kg. A tunable argon ion laser (Innova model 70, Coherent Laser Product Division, Palo Alto, CA, USA) operating at 514 nm was used. The average beam output power was 160 mW. The beam covers the entire width of the vertebra and the length is approximately 1–2 mm. After irradiation, the wound was closed in layers and the rats were allowed to recover. Bladder was empted manually for 1 week.

2.2 Assessment of mechanical and cold sensitivity after spinal cord injury

The behavioral assessments were conducted blindly as the groups of drugs administered. Vocalization thresholds to graded mechanical touch/pressure were tested with calibrated von Frey hairs (ranging from 0.04 to 0.2155 mN, Stoelting, Chicago, IL, USA). During testing the rats were gently restrained in a standing position and the von Frey hair was pushed onto the skin until the filament became bent. The frequency of stimulation was about 1/s and at each intensity, the stimuli were applied 5–10 times. The intensity of stimulation which induced consistent vocalization (>75% response rate) was considered as pain threshold.

The response of rats to brushing stimulation was tested with the blunt point of a pencil gently stroking the skin on the trunk in a rostro-caudal direction. The frequency of the stimulation was about 1 Hz and responses were graded with a score of 0 = no observable response; 1 = transient vocalization and moderate effort to avoid probe; 2 = consistent vocalization and aversive reactions and 3 = sustained and prolonged vocalization and aggressive behaviors. Normal rats exhibited no reactions to such brush stimuli (score 0).

Responses to cold was tested with ethyl chloride spray applied to the shaved allodynic skin area. The response was graded with a score of 0 = no observable response; 1 = localized response (skin twitch and contraction), no

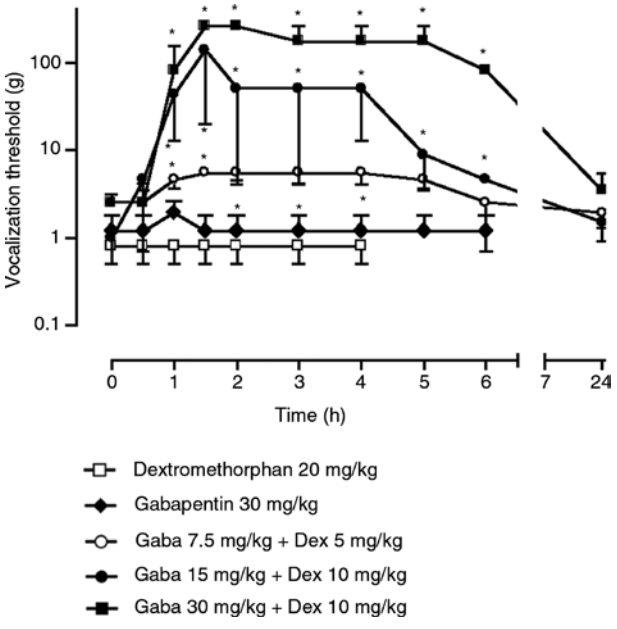

Fig. 1: Effects of gabapentin at 30 mg/kg, dextromethorphan at 20 mg/kg or combination of the two compounds on vocalization threshold to von-Frey hair stimulation in spinally injured rats. The data is expressed as median ± MAD (median absolute deviation) and 6–8 rats were included in each group. * = $p < 0.05$ compared to time 0 with Wilcoxon signed-rank test. Friedman ANOVA with repeated measures indicated a significant general difference for the three drug combination groups ($p < 0.01$).

vocalization; 2 = transient vocalization, moderate struggle and 3 = sustained vocalization and aggression. Normal rats usually had response score of 0 or 1.

2.3 Photochemically-induced sciatic nerve injury

Male SD rats were anesthetized by chloral hydrate (300 mg/kg, i.p.) and the left sciatic nerve was exposed. The nerve trunk was gently dissected free from the surrounding tissue over a distance of about 1 cm proximal to trifurcation. The exposed nerve was irradiated with an argon ion laser for 2 min. The irradiation was performed with a knife-edged beam across the nerve. Aluminum foil was placed under the nerve to isolate the surrounding tissue and to reflect light. Just before the irradiation, erythrosin B (Aldrich, USA 32.5 mg/kg dissolved in 0.9% saline) was injected i.v. via the tail vein. After the surgery the wounds were closed in layers and the rats were returned to the cages for subsequent behavioral tests.

2.4 Assessment of mechanical sensitivity after sciatic nerve injury

The behavioral assessments were conducted blindly as the groups of drugs administered. To test of sensitivity to mechanical stimulation, the rats were placed in plastic cages with a metal mesh floor. The plantar surface of the hind paws was stimulated with a set of calibrated von Frey hairs (ranging from 0.04 to 0.2155 mN, Stoelting, Chicago, IL, USA) with increasing force until the animal withdrew the limb. Each monofilament was applied 5 times. The withdrawal threshold was taken as the force at which the animal withdrew the paw from at least three out of five consecutive stimuli.

2.5 Drugs and statistics

Gabapentin, dextromethorphan and MK-801 were obtained from Research Biochem Inc. (Natick, MA, USA) and dissolved in physiological saline. All drugs were

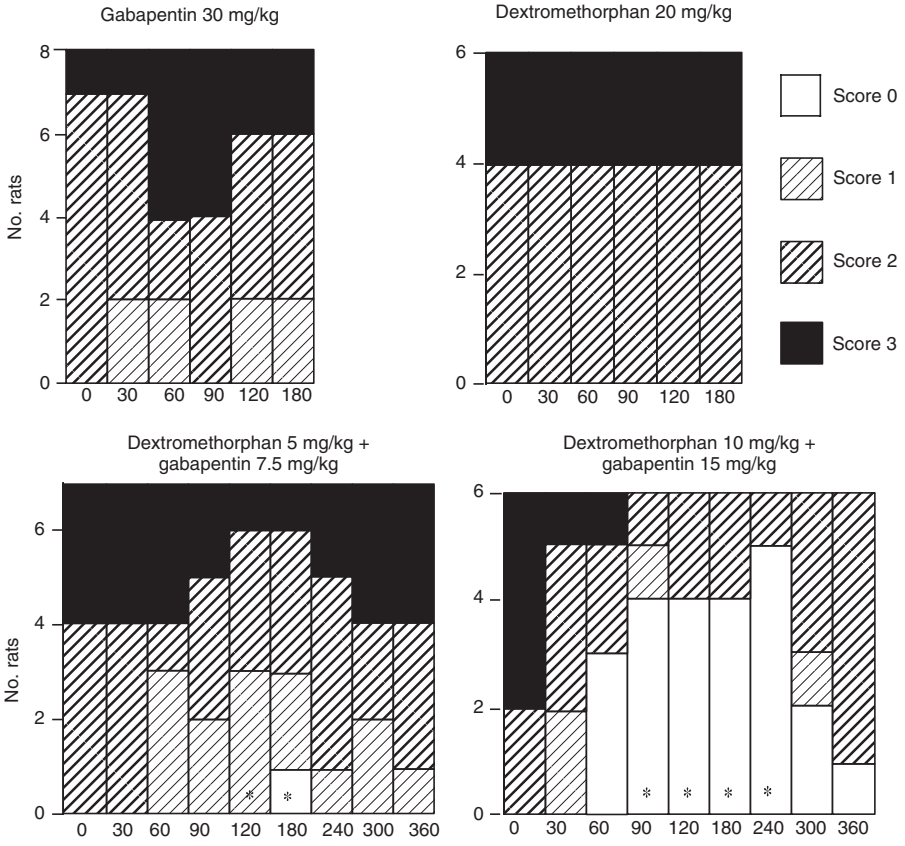

Fig. 2: Effect of gabapentin at 30 mg/kg, dextromethorphan at 20 mg/kg or combination of the two compounds on responses of spinally injured rats to brushing stimulation. The responses were graded and number of rats exhibiting different level of responses were shown. * = $p < 0.05$ compared to time 0 with Wilcoxon signed-ranks test.

injected i.p. in a volume of 0.1 mL/kg. The behavioral assessments were conducted blindly. Data are expressed as median ± median absolute deviation (MAD) and analyzed with Friedman one way analysis of variance for repeated measurements and Wilcoxon signed-ranks test.

3 Results

3.1 Spinally injured rats

As previously described, some spinally injured rats developed allodynia-like behavior manifested as reduction in vocalization threshold to mechanical touch stimulation applied by the von-Frey hairs or by brush and as increased response to cold stimulation applied by ethyl chloride spray. And saline treatment has no effect on either mechanical or cold sensitivity on our neuropathic pain models [13–15].

I.p. dextromethorphan or gabapentin did not alleviate allodynia-like behaviors at the doses up to 20 or 30 mg/kg, respectively (Figs. 1–3). Further increasing the dose of dextromethorphan or gabapentin produced numerous side effects, including sedation and motor impairment for gabapentin and hyperactivity for dextromethorphan. Combining gabapentin (7.5, 15 or 30 mg/kg) with low doses of dextromethorphan (5 or 10 mg/kg) significantly

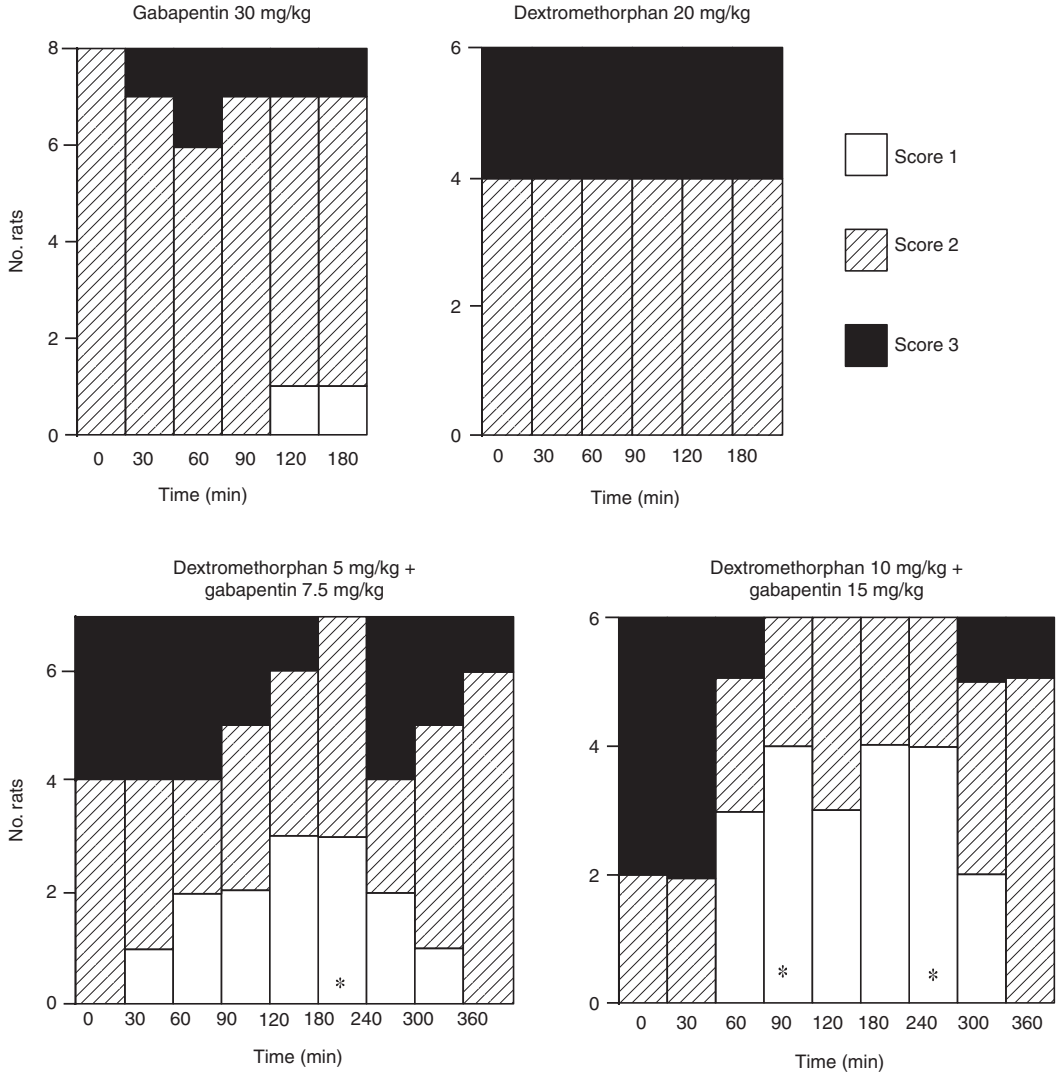

Fig. 3: Effect of gabapentin at 30 mg/kg, dextromethorphan at 20 mg/kg or combination of the two compounds on responses of spinally injured rats to cold stimulation. The responses were graded and number of rats exhibiting different level of responses were shown.
* = $p < 0.05$ compared to time 0 with Wilcoxon signed-ranks test.

increased vocalization threshold to von-Frey hair stimulation (Fig. 1), reduced and normalized increased response to brush (Fig. 2) or cold (Fig. 3) stimulation. The antiallodynic effect of dextromethorphan and gabapentin is long-lasting, but reversible. At doses used, gabapentin, dextromethorphan or combination did not produce observable side effects, such as sedation, motor impairments or hyperactivity.

I.p. MK-801 at 0.05 or 0.1 mg/kg also did not affect mechanical allodynia-like behavior in spinally injured rats whereas combination of small doses (0.01 or 0.05 mg/kg) of MK-801 with 15 mg/kg gabapentin again significantly increased vocalization threshold to von-Frey stimulation (Fig. 4). The cold allodynia was also similarly reduced (not shown).

3.2 Sciatic nerve injury

Rats subjected to ischemically-induced sciatic nerve injury developed mechanical hypersensitivity seen as bilaterally decreased paw withdrawal threshold to von-Frey hair stimulation which peaked at 1–2 weeks when the experiments were conducted. The mechanical hypersensitivity is more severe on the ipsilatera side to the irradiation than the contralateral side and the data from the ipsilateral were presented. I.p. dextormethorphan or gabapentin did not affect mechanical allodynia in sciatic nerve injured rats in the present experiments as doses up to 40 or 100 mg/kg despite the presence of side effects (Fig. 5). In contrast, combining gabapentin 30 mg/kg with 20 mg/kg dextromethorphan significantly increased paw withdrawal threshold to von-Frey stimulation in nerve injured rats (Fig. 5).

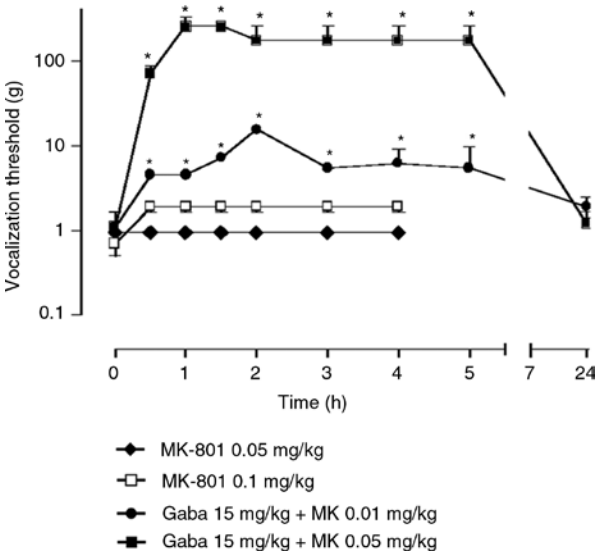

Fig. 4: Effect of MK-801 at 0.05 or 0.1 mg/kg or combination with MK with gabapentin 15 mg/kg on vocalization threshold of spinally injured rats to von Frey stimulation. The data is shown as median ± MAD (median absolute deviation) and * = $p < 0.05$ compared to time 0 with Wilcoxon signed-ranks test. Friedman ANOVA with repeated measures indicated a significant general difference for the two drug combination groups ($p < 0.01$).

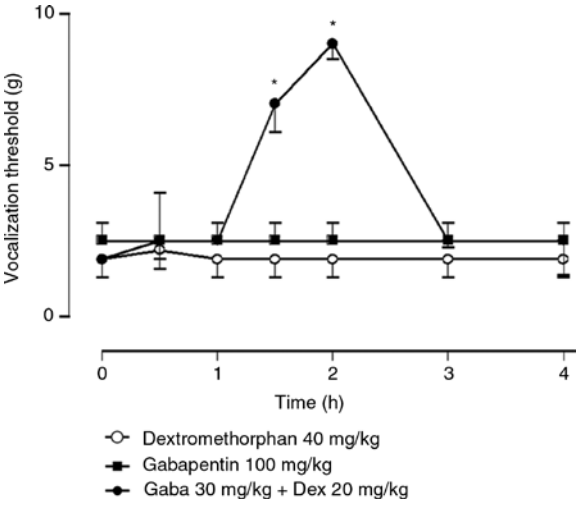

Fig. 5: Effects of gabapentin at 100 mg/kg, dextromethorphan at 40 mg/kg or combination of dextromethorphan 20 mg/kg and gabapentin 30 mg/kg on paw withdrawal threshold after partial sciatic nerve injury. The data is expressed as median ± MAD (median absolute deviation) and 6–8 rats were included in each group. * = $p < 0.05$ compared to time 0 with Wilcoxon signed-rank test. Friedman ANOVA with repeated measures indicated a significant general difference for the drug combination groups ($p < 0.01$).

4 Discussion

The present results showed that combining NMDA receptor antagonists and gabapentin produced synergistic antiallodynic effect in two rat models of neuropathic pain after spinal cord or sciatic nerve injury. The effect of the combination is clearly synergistic rather than additive since either drug along did not produce any effects at doses equal or even larger than that of the combination [16, 17]. At effective doses, the combination did not produce increased side effects in comparison to either drug alone. Thus, the present results support a potential clinical application of this combination strategy, particularly with NMDA antagonists that are clinically available, in treating patients with neuropathic pain of central and/or peripheral origins.

The mechanisms by which synergism between dextromethorphan and gabapentin occurs are unclear. The analgesic effect of gabapentin may be related to its binding to the α2δ subunit of the voltage-dependent calcium channels (VDCCs) [18, 19]. Thus, such synergism may be derived from a simultaneous reduction in calcium entry through blockade of VDCCs and NMDA receptor/channels. In this context, it is noteworthy that gabapentin per se often produced limited effect on various types of Ca^{2+} currents [20–23]. In addition, such interaction may also occur directly at the NMDA receptor complex. Previous work has shown that the anti-hyperalgesic effect of gabapentin was blocked by D-serine, an agonist at the glycine site of the NMDA receptor [24, 25]. The direct effect of gabapentin on NMDA receptor is, however, an enhancement of NMDA-evoked current in isolated neurons that may be difficult to reconcile with its analgesic effect [26, 27]. Gabapentin is also able to reduce glutamate release in some systems that may also contribute to its interaction with an additional blockade of the NMDA receptors [28, 29]. Finally, although dextormethorphan can also act on VDCCs [30], the fact that MK-801 also enhances the effect of gabapentin made it unlikely that such interaction take place solely at the VDCCs.

It is interesting to note that the synergism between dextromethorphan and gabapentin produces larger effect in spinally injured rats than in rats with sciatic nerve injury. This is possibly due to the fact that both drugs are less potent in the periphery vs. central model [17, 31] and may reflect different mechanisms for these two neuropathic pain models. Nonetheless, it is tempting to suggest that this combination may be particularly useful in treating spinal cord injury pain, a difficult clinical problem [32]. Our results also support the clinical observation [9] in which they showed that combination of dextromethorphan and gabapentin alleviated neuropathic pain in patients with spinal cord injury.

5 Conclusion

In conclusion, the present study suggested that combining NMDA receptor antagonists with gabapentin could provide a new approach in alleviating neuropathic pain with increased efficacy and reduced side effects.

Authors' statements

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Conflict of interest: We declare no conflicts of interests.

Informed consent: Not applicable.

Ethical approval: All experiments were approved by the local research ethics committee and were conducted in accordance with research guidelines of the International Association for the Study of Pain. We have used the minimal number of animals that is required to obtain results for statistical analysis.

References

[1] Sindrup SH, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain 1999;83:389–400.
[2] Jensen TS. Anticonvulsants in neuropathic pain: rationale and clinical evidence. Eur J Pain 2002;6(Suppl A):61–8.
[3] Nicholson B. Gabapentin use in neuropathic pain syndromes. Acta Neurol Scand 2000;101:359–71.
[4] Backonja M, Beydoun A, Edwards KR, Schwartz SL, Fonseca V, Hes M, LaMoreaux L, Garofalo E. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. J Am Med Assoc 1998;280:1831–6.
[5] Rice AS, Maton S. Postherpetic Neuralgia Study Group. Gabapentin in postherpetic neuralgia: a randomised, double blind, placebo controlled study. Pain 2001;94:215–24.
[6] Rowbotham M, Harden N, Stacey B, Bernstein P, Magnus-Miller L. Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. J Am Med Assoc 1998;280:1837–42.
[7] Serpell MG. Neuropathic pain study g. Gabapentin in neuropathic pain syndromes: a randomised, double-blind, placebo-controlled trial. Pain 2002;99:557–66.
[8] Parsons CG. NMDA receptors as targets for drug action in neuropathic pain. Eur J Pharmacol 2001;429:71–8.
[9] Sang CN. Glutamate receptor antagonists in central neuropathic pain following spinal cord injury. In: Yezierski RP, Burchiel KJ, editors. Spinal cord injury pain: assessment, mechanisms, managements. 23. Seattle: IASP Press, 2002:365–77.
[10] Collins S, Sigtermans MJ, Dahan A, Zuurmond WW, Perez RS. NMDA receptor antagonists for the treatment of neuropathic pain. Pain Med 2010;11:1726–42.
[11] Inturrisi CE. Preclinical evidence for a role of glutamatergic systems in opioid tolerance and dependence. Semin Neurosci 1997;9:110–9.
[12] Wiesenfeld-Hallin Z. Combined opioid-NMDA antagonist therapies. What advantages do they offer for the control of pain syndromes? Drugs 1998;55:1–4.
[13] Xu XJ, Hao JX, Aldskogius H, Seiger A, Wiesenfeld-Hallin Z. Chronic pain-related syndrome in rats after ischemic spinal cord lesion: a possible animal model for pain in patients with spinal cord injury. Pain 1992;48:279–90.
[14] Gao T, Hao JX, Wiesenfeld-Hallin Z, Xu XJ. Quantitative test of responses to thermal stimulation in spinally injured rats using a Peltier thermode: a new approach to study cold allodynia. J Neurosci Methods 2013;212:317–21.

[15] Gao T, Hao J, Wiesenfeld-Hallin Z, Wang DQ, Xu XJ. Analgesic effect of sinomenine in rodents after inflammation and nerve injury. Eur J Pharmacol 2013;721:5–11.
[16] Xu XJ, Alster P, Wu WP, Hao JX, Wiesenfeld-Hallin Z. Increased level of cholecystokinin in cerebrospinal fluid is associated with chronic pain-like behavior in spinally injured rats. Peptides 2001;22:1305–8.
[17] Hao JX, Xu XJ. Treatment of a chronic allodynia-like response in spinally injured rats: effects of systemically administered excitatory amino acid receptor antagonists. Pain 1996;66:279–85.
[18] Rose MA, Kam PC. Gabapentin: pharmacology and its use in pain management. Anaesthesia 2002;57:451–62.
[19] Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem 1996;271:5768–76.
[20] Rock DM, Kelly KM, Macdonald RL. Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons. Epilepsy Res 1993;16:89–98.
[21] Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. Neuropharmacology 1998;37:83–91.
[22] Fink K, Meder W, Dooley DJ, Gothert M. Inhibition of neuronal Ca(2+) influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. Br J Pharmacol 2000;130:900–6.
[23] Martin DJ, McClelland D, Herd MB, Sutton KG, Hall MD, Lee K, Pinnock RD, Scott RH. Gabapentin-mediated inhibition of voltage-activated Ca^{2+} channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. Neuropharmacology 2002;42:353–66.
[24] Singh L, Field MJ, Ferris P, Hunter JC, Oles RJ, Williams RG, Woodruff GN. The antiepileptic agent gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by D-serine. Psychopharmacology (Berl) 1996;127:1–9.
[25] Jun JH, Yaksh TL. The effect of intrathecal gabapentin and 3-isobutyl gamma-aminobutyric acid on the hyperalgesia observed after thermal injury in the rat. Anesth Analg 1998;86:348–54.
[26] Shimoyama M, Shimoyama N, Hori Y. Gabapentin affects glutamatergic excitatory neurotransmission in the rat dorsal horn. Pain 2000;85:405–14.
[27] Gu Y, Huang LY. Gabapentin actions on N-methyl-D-aspartate receptor channels are protein kinase C-dependent. Pain 2001;93:85–92.
[28] Dooley DJ, Mieske CA, Borosky SA. Inhibition of K(+)-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. Neurosci Lett 2000;280:107–10.
[29] Maneuf YP, Hughes J, McKnight AT. Gabapentin inhibits the substance P-facilitated K(+)-evoked release of [(3)H]glutamate from rat caudial trigeminal nucleus slices. Pain 2001;93:191–6.
[30] Lipton SA. Prospects for clinically tolerated NMDA antagonists: open-channel blockers and alternative redox states of nitric oxide. Trends Neurosci 1993;16:527–32.
[31] Hao JX, Xu XJ, Urban L, Wiesenfeld-Hallin Z. Repeated administration of systemic gabapentin alleviates allodynia-like behaviors in spinally injured rats. Neurosci Lett 2000;280:211–4.
[32] Yezierski RP, Burchiel KJ. Spinal injury pain: assessment, mechanism, management. Progress in pain research and management. Seattle: IASP Press, 2002:9–23.