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Analgesic effect of sinomenine in rodents after inflammation and nerve injury

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ABSTRACT

Sinomenine is an alkaloid originally isolated from the root of the plant *Sinomenium acutum*. It is used in traditional medicine in China to treat rheumatic arthritis. In the present study, we evaluated the potential antinociceptive effects of sinomenine in rodents with nociceptive, inflammatory and neuropathic pain. In normal rats and mice, systemic sinomenine produced moderate antinociceptive effect in the hot plate and tail flick tests. Sinomenine also exerted analgesic effects on mechanical and heat hypersensitivity in mice after carrageenan induced inflammation. Finally, sinomenine effectively alleviated mechanical and cold allodynia in rats and mice after injury to peripheral nerve or spinal cord. The analgesic effect of sinomenine is not associated with side effects and is not reversed by the opioid receptor antagonist naloxone. Our results showed that sinomenine has a wide spectrum analgesic effect in rodent models of nociceptive, inflammatory and neuropathic pain.

1. Introduction

Around 20% of adults experience chronic pain (Breivik et al., 2006) which is associated with considerable individual suffering and with significant costs to society (Breivik et al., 2006; McDermott et al., 2006; Jensen and Finnerup, 2007). Neuropathic pain resulting from injury to the central and peripheral nervous system is one of the main causes of chronic pain (Treede et al., 2008; Geber et al., 2009). Management of chronic pain, especially of neuropathic origin, remains a pressing challenge (O'Connor and Dworkin, 2009). Despite extensive research and the development of animal models, few analgesics are available for treating neuropathic pain and do not provide adequate pain relief in many patients (O'Connor and Dworkin, 2009).

Botanicals, compounds extracted from plants, have contributed significantly to our arsenal of pharmacological treatment of diseases. Furthermore, traditional Chinese herbal medicine harbors a potentially rich source of drug candidates for which Western drug companies are turning to with ever increasing urgency (Bloomberg News, 2012). The discovery of artemisinin for treating malaria is probably the best example of a successful development of a novel drug from Chinese herbal medicine (Miller and Su, 2011), a feat that will likely be repeated many times in the future.

Chinese herbal medicine has a long tradition in treating pain, but most experimental studies have been carried out using crude extracts or preparations with multiple components (Yu et al., 2008). Sinomenine (Fig. 1) is an alkaloid and a morphinan derivative isolated from the root of the climbing plant *Sinomenius acutum* (in China known as Fang-ji or Qing-teng) (Yamasaki, 1976). The whole *Sinomenium acutum plant*, including root and stem, has been used as traditional remedy in China for treating conditions such as rheumatoid arthritis, arrhythmia and neuralgia (Yamasaki, 1976, Xu et al., 2008). The main active component of *Sinomenium acutum*, sinomenine has been shown to have a variety of pharmacological effects, including anti-inflammatory, antiarrhythmic and immunosuppressive properties (Yamasaki, 1976). However, the potential analgesic effect of sinomenine has not been well established. In the present study, we have systematically assessed potential antinociceptive and analgesic effects of sinomenine in rodents using models of nociceptive, inflammatory and neuropathic pain.

2. Material and methods

2.1. Animals

All experiments were approved by regional animal research ethics committee and were carried out on Sprague-Dawley rats of both sexes (Harlan, Horst, The Netherlands) or male C57BL/6 mice, (Charles River, Sollentuna, Sweden). Rats were housed 4 per cage

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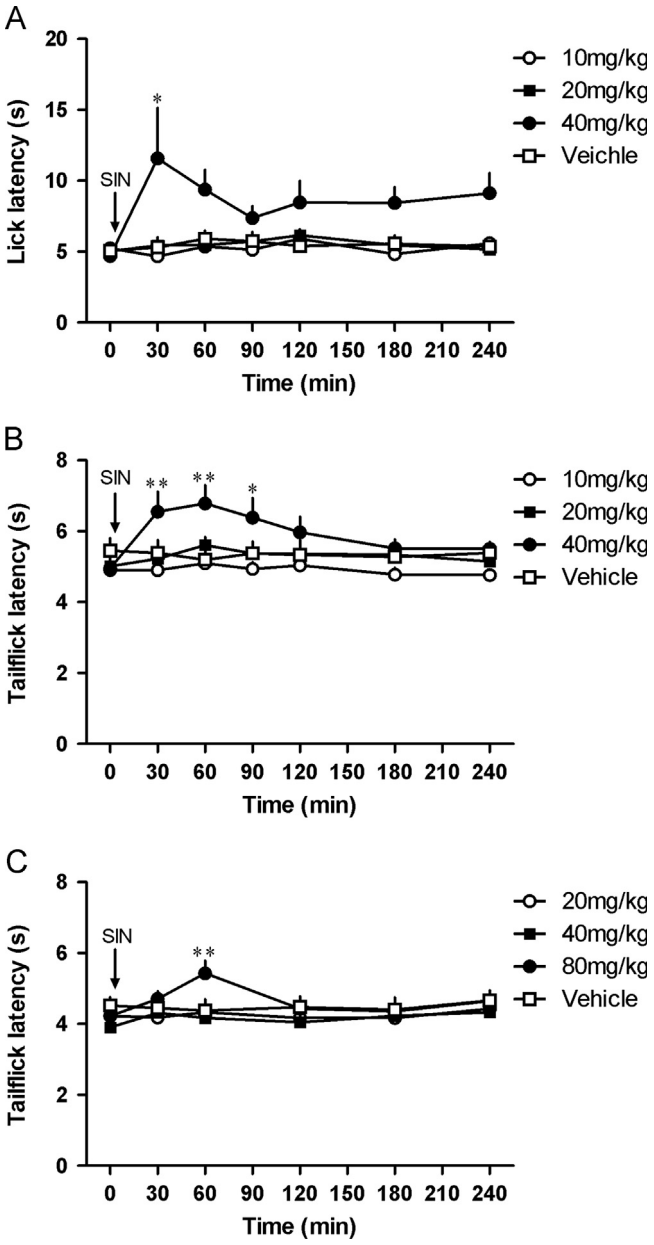

Fig. 1. Effect of 10 mg/mg, 20 mg/kg or 40 mg/kg i.p. sinomenine or vehicle on hot plate (A) and tail flick (B) response in normal male SD rats and the effect of i.p. sinonemine at 20 mg/kg, 40 mg/kg, 80 mg/kg or vehicle on tail flick latency (C) in normal male C57BL/6 mice. $N=6$–8 rats and $N=6$–12 mice in each group. The data are presented as mean ± S.E.M. ANOVA with repeated measures indicated a significant overall difference among rats in both tests ($F=6.441$, $P<0.01$ for A and $F=3.548$, $P<0.05$ for B) but not in the tail flick test in mice (C). Newman-Keul test also showed overall difference between 40 mg/kg sinomenine and vehicle group for hot plate test and tail flick test in rats but not in mice. The comparisons between pre-drug baseline response and various post drug times was with the Dunnett's test after ANOVA, $^{*}P<0.05$, $^{**}P<0.01$.

and mice 6 per cage 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. Hot plate and tail flick tests in rats and mice

The antinociceptive effect of sinomenine in normal rats was assessed using a hot plate (IITC, Woodland Hills, CA) which was maintained at 54 ± 1 °C. The latency to lick a hind paw was measured with an accuracy of 0.1 s and the cut-off value was set at 30 s to prevent tissue damage. Before testing, the rats were habituated in the testing room for at least 30 min. The rats were trained on the hot plate for 4 days with 2 trials/day to obtain a stable baseline response prior to the experiment.

In the tail flick test the rats were gently restrained and a radiant heat source (Ugo Basile, Italy) was focused 1–2 cm from the tip of the tail. Response latency was automatically recorded. The intensity of the stimulation was adjusted so that the baseline latency was from 4–6 s. The cut-off value was 10 s.

The tail flick test was similarly conducted in mice by focusing the radiant heat source 1–1.5 cm from the tip of the tail. Response latency was automatically recorded. The intensity of the stimulation was adjusted so that the baseline latency was from 4–6 s. The cut-off value was 10 s.

2.3. Carrageenan induced inflammation in mice

Mice were anaesthetized with 75 mg/kg ketamine + 1 mg/kg medetomidine in a volume of 1 ml/kg and λ-carrageenan (Sigma-Aldrich, 20 μl, 2%) was injected subcutaneously (s.c.) into the plantar surface of one hind paw. Mechanical and heat hypersensitivities of the inflamed hind paw was tested 24 h after the injection.

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 Frey hairs (Stoelting, Chicago, IL, USA) with increasing force. Each filament was applied 5 times and response threshold was reached when the animal withdrew the paw at least 3 times. The cut-off value was 4 g.

To test heat hyperalgesia, mice were gently restrained and a radiant heat source (Ugo Basile, Italy) was focused on the plantar surface of hind paw. The intensity of the stimulation was adjusted so that the baseline latency was 4–6 s and the cut-off value was set at 10 s.

2.4. Photochemically induced sciatic nerve injury in mice and rats

Detailed methods for producing sciatic nerve ischemic injury have been described previously for rats (Kupers et al., 1998) and mice (Hao et al., 2002). Briefly, animals were anaesthetized by 75 mg/kg ketamine + 1 mg/kg medetomidine and the left sciatic nerve was exposed. After i.v. injection of 32.5 mg/kg of the photosensitizing dye erythrosine B (Red N°3, Aldrich-Chemie, Steinheim, Germany), the sciatic nerve was irradiated under an argon ion laser (514 nm, 160 mW, Innova model 70, Coherent Laser Product Division, Palo Alto, CA) for 45 s or 2 min for mice or rats, respectively. The effect of sinomenine was tested 2 weeks after sciatic nerve injury, when the animals exhibited mechanical and cold hypersensitivity of the hind paws.

The withdrawal response of the ipsilateral hindpaw to mechanical stimulation after nerve injury was tested using a set of calibrated von Frey 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 for both mice and rats as follows: 0 = no response, 1 = startle response without evident paw withdrawal, 2 = withdrawal of the stimulated hind paw, 3 = sustained withdrawal of the simulated hind paw with flinching or licking.

2.5. Photochemically-induced spinal cord injury in rats

The methods of producing photochemically induced spinal cord ischemic injury in rats have been described in detail previously (Hao et al., 1991, 1992). Briefly, the rats were anesthetized and a midline incision was made in the skin overlying vertebral segments T12-L1. Following i.v. injection of erythrosine B (32.5 mg/kg),