Anti-mechanical Hyperalgesic Effect of Lonicera Japonica in Neuropathic Pain Rats

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Lonicera japonica has been widely used for chronic inflammatory diseases in many Asian countries. Its analgesic effect has not been explored yet. This study aimed to test the analgesic potential of methanol extracts from Lonicera japonica (MELJ) in rat neuropathic mctel. Neuropathic pain was pacts ed by partial sciatic nerve injury. Two weeks after surgery, neuropathic rats received oral administration of MELJ at doses of either 0.0 g/kg, 0.2 g/kg or 0.4 g/kg. At dose of 0.0 g, rats were administered with saline only and used as conracl. The behavioral tests for f 0.0 g, raand ccld hs were adma were weformed up to 2 hours after treatment. The MELJ at the dose 0.4 g/kg dmg gfg, ntly alleviated f 0.0 g, rahyperalgesia, but not cold hyperalgesia. These results showed that the MELJ had, although transient, analgesic effect on mechanical hyperalgesia in the rat neuropathic model.

Key words : lonicera japonica, neuropathic pain, mechanical hyperalgesia

Introduction

Neuropathic pain is chronic pain caused by injury of peripheral nerve or soft tissue. It is characterized by spontaneous burning pain, hyperalgesia and allodynia[11]. Although analgesics including opiates have been commonly used to alleviate chronic neuropathic pain, their side effects and resistance in neuropathic conditions frequently limit clinical applications[4]. In condition that peripheral nerves or soft tissue are damaged, opioid-containing spinal terminals disappear which results in opioid-resistance[1,6]. Moreover, it is well known that besides of gastrointestinal or neurological complications, their long-term use causes abuse and endocrine and immune alterations[2,8]. Clearly, more effective treatments with fewer side effects and tolerance are required. Oriental herbal medicine has been used empirically for chronic pain patients. Herbal extracts, for example, ginseng have been reported to have antinociceptive effects[17,21].

Lonicera japonica, (Jinyinhua) is one of popular anti-inflammatory drugs (like antibiotics) in Oriental medicine.

Previous studies have demonstrated strong anti-inflammatory effects of Lonicera japonica in rodent edema and arthritic model[12,14,19]. Although Lonicera japonica is known to contain promising components with bioactivities[10], besides of anti-inflammatory components, it is largely unexplored concerning the biological effects of their components, especially analgesic effects. We explored whether Lonicera japonica had analgesic effects on neuropathic pain.

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Materials and Methods

1. Animals

Young adult male Sprague-Dawley rats (n=21, 200-250 g) were housed in group cages (4-5 per cage) with water and food available ad libitum. The room was light/dark controlled and kept at 21-24℃.

2. Neuropathy surgery

The neuropathy was produced by partial sciatic nerve injury on the left hindlimb as described previously[13]. In brief, under halothane anesthesia, a segment of the sciatic nerve was exposed between the mid-thigh level and the popliteal fossa by skin incision and blunt dissection through the biceps femoris

muscle. The three major divisions of the sciatic nerve (the tibial, sural and common peroneal nerves) were clearly separated by individual perineurium. The tibial and sural nerves were tightly ligated using 6.0 silk threads and cut with fine scissors, while the common peroneal nerve was left intact. The wound was closed. Rats were allowed to have 2 weeks to recover from the surgery prior to behavioral testing.

3. Paw hypersensitivity tests

Two weeks after the neuropathy surgery, rats were placed on a metal mesh floor under a custom-made transparent plastic dome (8×8×18 cm). They were allowed to adapt to the environment for 30 minutes. To measure mechanical hyperalgesia, innocuous mechanical stimuli were applied with a von Frey filament (bending force, 0 mN) to the sensitive area of the hind paw (medial aspects of ankle joint). The von Frey filament was applied 10 times (once every 3-4sec) to each hind paw. And,0to quantity cold hyperalgesia of the paw, brisk foot withdrawal in response to acetone (99% meigma) application was measured. The acetone was applied 10 times (once every 5 min) to each paw. Paw withdrawal freqmencies were expressed as a percentce :

response rate = number of foot withdrawals / 10× 100

4. Methanol extraction and drug treatment

Lonicera japonica (500 g) was purchased from Jungdo Inc (Seoul, Korea). It was extracted three times in a reflux condencer with 80% methanol. The solution was combined, filtered through Whatman No. 1 filter paper, and concentrated using a rotary vacuum evaporator followed by lyophilization (-66℃, 10 mmHg). The yields of Lonicera japonica were 21.98% (w/w). It was dissolved in saline (0.9%) and administrated orally at the doses of 0.0 (n=7), 0.2 g/kg (n=7) and 0.4 g/kg (n=7) in a volume of 10 ml/kg body weight. The control group received saline only in the same volume. The behavioral tests were performed before and 10, (30), 60 and 120 min after oral administration.

5. Statistical analysis

Behavioral data are presented as mean ± SEM (standard error of the mean). The data were analyzed by two-way repeated-measurement analysis of variance (ANOVA), followed by post hoc testing. P values ≤ 0.05 were considered statistically significant.

Results

Most neuropathic animals exhibited paw withdrawal frequencies over 90% to von Frey stimuli 2 weeks after neuropathic surgery, indicating the development of mechanical hyperalgesia. Methanol extracts from Lonicera japonica (MELJ) was administered orally at doses of 0.0 g/kg (saline only), 0.2 g/kg and 0.4 g/kg, and then mechanical sensitivity was measured before, 10, 30, 60 and 120 min after administration. Oral administration of MELJ at dose 0.2 g/kg did not change mechanical hyperalgesia. However, the dose 0.4 g/kg significantly decreased the mechanical hyperalgesia on 60 minutes after treatment (71.7 ± 17.2%), compared to control (100 ± 0.0) ($p<0.05$), and its values returned to baseline level (93.3 ± 3.3%) to 120 min after treatment(Fig. 1).

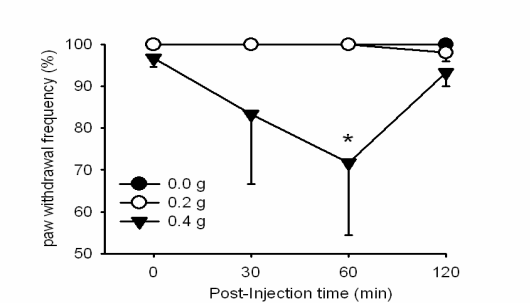

Fig. 1. Mechanical hyperalgesia Test

Neuropathic animals were hypersensitive to acetone preparation 2 weeks after surgery (96.0 ± 4.0%), indicating the development of cold hyperalgesia. Oral administration of MELJ at doses of 0.2 g/kg or 0.4 g/kg did not reverse the cold hyperalgesia by acetone preparation, compared to values of pretreatment or control(0.0 g) (Fig. 2).

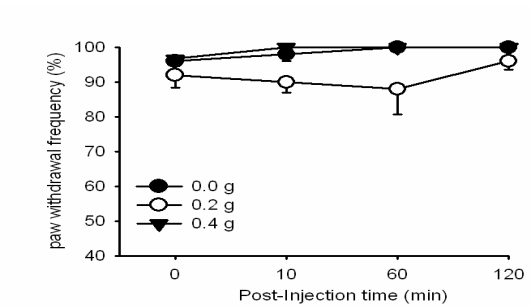

Fig. 2. Cold hyperalgesia Test

Lonicera japonica has been well known to have anti-inflammatory effects[12,14,19]. However, its analgesic potential has not been investigated so far. This simple study showed that Lonicera japonica was, although transient, effective on mechanical hyperalgesia in neuropathic rats. Lonicera japonica contains several components with bioactivity such as saponins, phenolics, terpenoids, loganin, flavones, 3-Caffeoyl-quinic acid, 3,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, methyl ester

and 3,5-dicaffeoylquinic acid butyl ester[18]. Although it is largely unexplored concerning biological effects of those components, it has been shown that saponin from Lonicera japonica has strong anti-inflammatory activity[12] and that phenolics from Lonicera japonica have the inhibitory effect on platelet activation and the cytoprotective effect on hydrogen peroxide-induced cell injury[5]. Saponins from ginseng and Neorautanenia mitis are known to have analgesic effects[3,15,16,20]. Phenolics from medicinal plants such as Calophyllum brasiliense leaves and Barringtonia racemosa have also been reported to be anti-nociceptive[7,9]. We assume that suppression of mechanical hyperalgesia by Lonicera japonica might be due to components such as saponins and phenolics.

In conclusion, to our knowledge, this is first study to show that Lonicera japonica has anti-mechanical hyperalgesic effects. However, to apply to neuropathic patients in Oriental clinic, further investigations concerning the analgesic effects of individual components of Lonicera japonica and its underlying mechanisms are required.

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