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Chlorogenic acid administered intrathecally alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model

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ABSTRACT

Chlorogenic acid (CGA), one of the most abundant dietary polyphenols, is known to have various physiological properties. Although CGA is reported to have an antinociceptive effect on acute and inflammatory pain, little is known about its effect on neuropathic pain or its action site. The aim of the present study was to determine whether intrathecally administered CGA can ameliorate hyperalgesia in a neuropathic pain model. Chronic constriction injury to the sciatic nerve was induced in male Sprague-Dawley rats. CGA (0.5, 1, or 2 mg) was administered intrathecally to examine the effects on mechanical, thermal, and cold hyperalgesia using the electronic von Frey test, plantar test, and cold plate test, respectively. A rotarod test was also performed to assess motor function. To identify the neurotransmitter pathway involved in the spinal action of CGA, the present study examined the effect of intrathecal pretreatment with several antagonists of spinal pain processing receptors on the action of CGA in the electronic von Frey test and cold plate test. Spinally applied CGA dose-dependently alleviated mechanical and cold hyperalgesia. Conversely, CGA had no effect on thermal hyperalgesia. At the highest dose, CGA affected motor performance. The antihyperalgesic action of CGA was partially reversed by bicuculline, an γ-aminobutyric acid$_A$ (GABA$_A$) receptor antagonist, at a dose that did not affect baseline behavioral responses. These findings suggest that CGA ameliorates mechanical and cold hyperalgesia partly by activating GABAergic transmission in the spinal cord, and that CGA may be useful for novel treatments for neuropathic pain.

1. Introduction

Chlorogenic acid (5-caffeoylquinic acid; CGA) is a phenolic product naturally isolated from the leaves and fruits of plants, including the coffee bean and green tea leaves. It is one of the most plentiful dietary polyphenols and is formed by the esterification of caffeic acid with quinic acid (Fig. 1). CGA has been shown to have antioxidant, anticarcinogenic, antidiabetic, and antihypertensive properties (Greenberg et al., 2006; Butt and Sultan, 2011; Zhao et al., 2012). Recently, several animal studies showed that systemically administered CGA, as well as extracts from plants containing CGA, exerted antinociceptive activity in acute and inflammatory pain models (Yonathan et al., 2006; Marrassini et al., 2010; Gorzalczany et al., 2011). Furthermore, one study demonstrated an antihyperalgesic effect of CGA injected intraperitoneally in a rat neuropathic pain model (Bagdas et al., 2013). However, little is known about the site (central or peripheral) and the mechanism of antinociceptive action by CGA. The spinal dorsal horn plays a crucial role in modulating transmission of various pain modalities in acute, persistent, and chronic pain. Peripheral nerve injuries induce neural plasticity that elicits central sensitization of the spinal neurons and enhances nociceptive transmission (Costigan et al., 2009; Price et al., 2009; Woolf, 2011). This is considered an important mechanism contributing to neuropathic pain.

The aim of the present study was to examine if centrally administered CGA (via intrathecal injection) can alleviate mechanical, thermal, and cold hyperalgesia induced in a rat neuropathic pain model, and whether CGA is a candidate for a novel remedy against neuropathic pain. To determine which neurotransmitter pathway is involved in the spinal actions of CGA, the present study examined the effects of several antagonists of spinal pain processing receptors, including γ-aminobutyric acid$_A$ (GABA$_A$), glycine, 5-hydroxytryptamine (5-HT), α_2 adrenergic and μ-opioid receptors, on the action of CGA. Regarding adverse effects for clinical application, the effect of CGA on motor performance was also examined.

2. Material and methods

2.1. Animals and drug preparation

The present study was approved by the Ethics Committee of Animal Care and Experimentation at the University of

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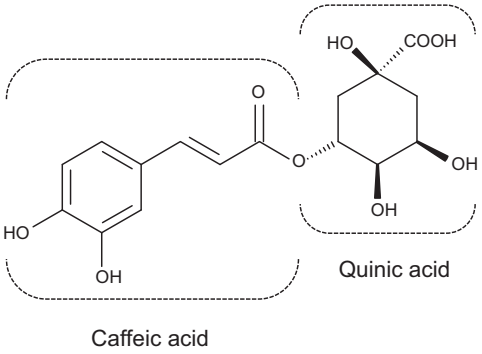

Fig. 1. Chemical structure of chlorogenic acid (CGA). CGA is formed by the esterification of caffeic acid with quinic acid.

Occupational and Environmental Health, Japan. A total of 226 male Sprague-Dawley rats (Kyudo, Fukuoka, Japan) weighing 170–260 g were used. Rats were housed with free access to food and water, and were maintained on a 12-h light–dark cycle at a constant room temperature of 22 °C ± 2 °C with a humidity of 50% ± 5%. All experiments were performed at the same time (between 10:00 and 16:00) during the light period. Rats were assigned randomly to treatment groups, with the experimenter blind to the drug treatments. All experimental groups consisted of six or eight rats, unless otherwise stated.

CGA, pentobarbital sodium, (+)-bicuculline, strychnine hydrochloride, methysergide maleate salt, ondansetron hydrochloride dihydrate, yohimbine hydrochloride, naloxone hydrochloride, urethane, and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, Missouri, USA). The polyethylene catheters (PE-10) were obtained from Becton, Dickinson and Company (San Jose, California, USA). CGA and bicuculline were dissolved in 20% DMSO (diluted with 0.9% physiological saline). The other drugs were dissolved in the saline.

2.2. Intrathecal catheter implantation

For the intrathecal administration of drugs, lumbar catheters were placed in all rats according to the procedure outlined by Yaksh and Rudy (1976). Under anesthesia using pentobarbital sodium (60 mg/kg, intraperitoneal), a stretched PE-10 polyethylene catheter (8.5 cm, 10 μL void volume) was inserted into the intrathecal space and advanced caudally to the rostral edge of the lumbar enlargement through an incision in the atlanto-occipital membrane. The rats were allowed to recover for 5 days and housed individually before the experimental procedures began. Proper location of the catheter was confirmed by hind limb paralysis lasting for 10–15 min after the injection of 10 μL of 2% lidocaine 2 days before the behavioral assessment. For assays, 10 μL of CGA (0.5, 1, or 2 mg) or 20% DMSO was administered intrathecally, followed by 10 μL of saline to flush the catheter. Each rat received only one injection.

2.3. CCI model

On the same day as the intrathecal catheter implantation, peripheral neuropathy was induced using a slight modification of the procedure described by Bennett and Xie (1988). Briefly, rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneal), after which the left sciatic nerve was exposed at mid-thigh level. Proximal to the sciatic trifurcation, four loose 4-0 silk ligatures were tied around the nerve at 1-mm intervals. To confirm the influence of nerve injury, a sham operation was performed with exposure of the left sciatic nerve without ligation. The CCI rats were tested on the seventh day post-operation, unless otherwise stated. After the experiments, the rats were euthanized using an injection of urethane (3 g/kg, intraperitoneal).

2.4. Behavioral assessment

To examine chronic pain, a constriction injury was applied to the sciatic nerve, which induced neuropathic pain characterized by hyperalgesia and allodynia. The electronic von Frey test, the plantar test, and cold plate test were performed to assess mechanical, thermal, and cold hyperalgesia, respectively. The rotarod test was used to evaluate motor function.

2.5. Electronic von Frey test

In the CCI rats, mechanical hyperalgesia was assessed by measuring the withdrawal threshold of the left hind paw in response to a mechanical stimulus using an electronic von Frey aesthesiometer (model 2391C, IITC Life Science, Woodland Hills, California, USA) (Terada et al., 2011; Hara et al., 2012; Kataoka et al., 2013). Each animal was placed on a metallic grid floor in a plastic observation chamber, which provided access to the plantar surface of the hind paw. Animals were allowed to acclimate to the environment for 10 min. A rigid tip attached to the meter was applied to the left plantar surface from under the floor. The withdrawal threshold was defined as the average force (g) required to cause withdrawal of the stimulated paw in three trials. The effects of CGA and 20% DMSO administered intrathecally on mechanical nociception were assessed repeatedly for 240 min post-injection.

To look at systemic effect of CGA, 2 mg CGA (0.5 mL dissolved in 0.4% DMSO) was administered intraperitoneally and then the effect on mechanical hyperalgesia was examined at 30 min post-injection ($n=8$).

2.6. Plantar test

Thermal hyperalgesia was assessed by measuring hind paw withdrawal latency in response to radiant heat using a plantar test apparatus (model 7360, Ugo Basile, Comerio, Italy) according to the method described by Hargreaves et al. (1988). Each rat was placed into a compartment enclosure on a glass surface. A mobile heat source was then positioned under the plantar surface of the hind paw and activated with a light beam, giving withdrawal latencies of 8–10 s in sham-operated rats. The digital timer automatically recorded the response latency for paw withdrawal to the nearest 0.1 s. A cutoff time of 20 s was imposed to prevent tissue damage in the absence of a response. The mean withdrawal latencies (s) for the left hind paw were determined from the average of two trials separated by a 5-min interval to prevent thermal sensitization. The effects of CGA and 20% DMSO administered intrathecally were assessed repeatedly for 120 min post-injection in CCI rats.

2.7. Cold plate test

Cold hyperalgesia was assessed in CCI rats using a Hot/Cold Plate (model 35100, Ugo Basile) on the 14th day after the operation. Rats were placed on a cold stainless steel plate maintained at 2 °C and the latency (s) to the first lifting or shaking of the CCI hind paw was measured. A cutoff time of 180 s was imposed to prevent tissue damage. The effects of CGA and 20% DMSO administered intrathecally on cold nociception were assessed repeatedly for 240 min post-injection in the CCI rats.