Intracerebroventricular Gisenosides are Antinociceptive in Proinflammatory Cytokine-Induced Pain Behaviors of Mice

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Several ginsenoside (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg3) are neuroprotective and antinociceptive agents. In this study, we assessed the effects of these ginsenosides following intracerebroventricular (i.c.v.) administration on the nociceptive behaviors induced by intrathecal injection of pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ)). The ginsenosides, Rb1, Rb2, Rc, Rd, Re, Rf and Rg1, significantly attenuated the nociceptive behavior induced by TNF-α, IL-1β, and IFN-γ injection, but ginsenoside Rg3 did not. These results suggest that several ginsenosides may regulate the nociceptive processing induced by pro-inflammatory cytokines.

Key words: Ginsenoside, Anti-nociception, Pain behaviors, Pro-inflammatory cytokines, TNF-α, IL-1β, IFN-γ

INTRODUCTION

Traditionally, Panax ginseng is used for a panacea or promoting longevity in far-east Asian traditional medicine. Most pharmacological action of ginseng is produced by ginsenosides, which belong to the steroidal saponin family (Tsang et al., 1985; Attele et al., 1999).

Ginsenosides administered systemically (i.p.), intracerebroventricularly (i.c.v.) or intrathecally (i.t.) regulate nociception in pain models (Shin et al., 1999; Nah et al., 2000; Yoon et al., 1998; Suh et al., 1997, 1999; Choi et al., 2003b; Rhim et al., 2002; Mogil et al., 1998). Ginsenosides differ in the structure of their sugar moieties and their effects on the nervous system. For example, i.t. administered ginsenosides attenuated analgesia induced by morphine, a mu opioid ligand, and U-50,488H, a kappa opioid ligand, in mice (Kim et al., 1992; Suh et al., 1997, 2000), while ginsenoside Rf potentiated U-50,488H-mediated analgesia (Nemmani and Ramarao 2003). Ginsenosides can also regulate inflammatory pain processing. Ginsenosides Rb1, Rb2 and Re, produce analgesic effects on pain behaviors elicited by formalin injected subcutaneously (s.c.) or writhing responses induced by 1% acetic acid (i.p.) injection (Shin et al., 1999). In addition, we also demonstrated that ginsenosides inhibit pain behavior induced by substance P (i.t.) injection (Choi et al., 2003b), a typical neurotransmitter in inflammatory pain (Hunt and Mantyh 2001). Ginsenosides can regulate inflammatory pain processing (Shin et al., 1999; Yoon et al., 1998; Mogil et al., 1998; Nah et al., 2000; Choi et al., 2003b), but little information is available on the effect of ginsenosides in pain behaviors related to pro-inflammatory cytokines (i.e. TNF-α, IL-1β, and IFN-γ).

Injection of mouse pro-inflammatory cytokines evoke nociceptive behaviors (Choi et al., 2003c) and contribute to the pathophysiology of pathological pain states in hyperalgesia or allodynia (Watkins et al., 1994; Tadano et al., 1999; Reeve et al., 2000). However, the supraspinal action of ginsenosides in regulating nociceptive behavior induced by pro-inflammatory cytokines by i.t. injection is not well-characterized. Thus, we examined the modulatory role of ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg3) injected supraspinally on the nociceptive behavior induced by pro-inflammatory cytokines (TNF-α, IL-1β, and IFN-γ) injected i.t.
MATERIALS AND METHODS

These experiments were approved by the Hallym University Animal Care and Use Committee. All procedures were conducted in accordance with the ‘Guide for Care and Use of Laboratory Animals’ published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Experimental animals

Male ICR mice (MJ Ltd., Seoul, Korea) weighing 23-25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5°C with an alternating 12 h light-dark cycle for at least 5 days before the experiments were started and food and water were available ad libitum. The animals were allowed to adapt to the experimental condition in the laboratory for at least 2 h before pain testing. To reduce variation, all experiments were performed during the light phase of the cycle (10:00-17:00).

Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection of drugs

The i.t. injections were made according to the procedure of Hylden and Wilcox (Hylden and Wilcox 1981) using a 25 µL Hamilton syringe with a 30 gauge needle. The i.c.v. administration followed the method described by Haley and McCormick (Haley and McCormick 1957). The mouse was grasped firmly without anesthesia by the loose skin behind the head. The skin was pulled taut. A 30-gage needle attached to a 25 mL syringe was inserted perpendicularly through the skull into the brain and solution was injected. The injection site was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The i.c.v. and i.t. injection volumes were 5 µL and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space or in the spinal cord. The dye injected i.c.v. was distributed through the ventricular spaces and reached the ventral surface of the brain and the upper cervical portion of the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but within a short distance (about 0.5 cm), and no dye was found visually in the brain. The success rate for the prior injections with this technique was over 95%.

Drug treatment and pro-inflammatory cytokine-induced nociceptive behavioral test

One group of mice was pretreated i.c.v. once with 50 µg of ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg3 10 min before pro-inflammatory cytokines (TNF-α (100 pg/5 µL), IL-1β (100 pg/5 µL) or IFN-γ (100 pg/5 µL)) were i.t. injected. Immediately after cytokine injection, each mouse was placed in an observation chamber (20 cm high, 20 cm diameter) and responses such as licking, biting, and scratching directed toward the lumbar and caudal region of the spinal cord were recorded for 30 min. The cumulative response time(s) of scratching and biting episodes were measured with a stop-watch timer.

Drugs

Ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg3) were obtained from Korea Ginseng and Tobacco Research Institute (Taejon, Korea). The purity of Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg3 used in the present study was 98.63%, 98.13%, 95.97%, 97.87%, 99.23%, 99.33%, 98.10%, and 99.00%, respectively. TNF-α, IL-1β, and IFN-γ were purchased from R&D Systems Inc. (Minneapolis, MN., U.S.A.). Morphine hydrochloride was purchased from Sam-Sung Pharm. Co. (Seoul, Korea). All drugs for injection, except Rd, were dissolved in sterile saline (0.9% NaCl solution). Ginsenoside Rd was prepared in saline containing 20% dimethyl sulfoxide (DMSO) as vehicle. All drugs were prepared just before use. Drug doses were chosen based on our previous study (Choi et al., 2003b).

Fig. 1. Structure of ginsenosides. Ginsenosides have a common steroid ring with three different side chains. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara(p), arabinopyranoside; Ara(f), arabinofuranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links two carbohydrates.