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Original Reports

Ginsenoside Rb1 Attenuates Acute Inflammatory Nociception by Inhibition of Neuronal ERK Phosphorylation by Regulation of the Nrf2 and NF-κB Pathways

Minhee Jang,[*,†] Min Jung Lee,[*,‡] Jong Hee Choi,[*,‡] Eun-Jeong Kim,[*,‡] Seung-Yeol Nah,[§] Hak-Jae Kim,[¶] Sanghyun Lee,[||] Sang Won Lee,[**] Young Ock Kim,[**] and Ik-Hyun Cho[*,‡,††]

**Departments of Convergence Medical Science, †Cancer Preventive Material Development, and ‡Brain Korea 21 Plus Program, College of Oriental Medicine, Kyung Hee University, Seoul, Republic of Korea.*
§Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea.
¶Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea.
||Department of Integrative Plant Science, Chung-Ang University, Anseong, Republic of Korea.
***Department of Medicinal Crop Research Institute, National Institute of Horticultural & Herbal Science, Rural Development Administration, Eumseong, Republic of Korea.*
††Institute of Oriental Medicine, College of Oriental Medicine, Kyung Hee University, Seoul, Republic of Korea.

Abstract: Ginsenoside-Rb1 (Rb1) has anti-inflammatory effects. However, the potential antinociceptive value of Rb1 for the treatment of acute inflammatory nociception is still unknown. In this study, we examined whether Rb1 has any antinociceptive effects on acute inflammatory nociception in Sprague Dawley rats given intrathecal (i.t.) introduction of Rb1 (2, 10, and 50 μg) 20 minutes before injection of formalin (5%, 50 μL) into the plantar surface of the hind paws. I.t. introduction of Rb1 significantly decreased nociceptive behavior during phase II (16–60 minutes), but not phase I (0–10 minutes), after formalin stimulation, corresponding to the reduced activation of c-Fos in the L4 to L5 spinal dorsal horn after formalin stimulation. Rb1 also reduced the phosphorylation of extracellular signal-regulated kinase in the neurons, but not the microglia and astrocytes. Microscopic examination of the microglia and astrocytes revealed no morphological changes due to formalin stimulation and i.t. introduction of Rb1. Interestingly, Rb1 activated the nuclear factor erythroid 2-related factor 2 pathway and inhibited nuclear factor kappa B pathways.

***Perspective:** Our findings indicate that i.t. introduction of Rb1 might effectively inhibit formalin-induced acute inflammatory nociception by inhibition of neuronal extracellular signal-regulated kinase phosphorylation, which is thought to regulate the nuclear factor erythroid 2-related factor 2 nuclear factor kappa B pathways in the spinal dorsal horn, which suggests therapeutic potential for suppression of acute inflammatory pain.*

***Key words:** Ginsenoside-Rb1, intrathecal introduction, inflammatory nociception, phospho-ERK, nuclear factor erythroid 2-related factor 2, nuclear factor-kappa B.*

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Address reprint requests to Ik-Hyun Cho, DVM, PhD, Department of Convergence Medical Science, Brain Korea 21 Plus Program, Institute of Korean Medicine, College of Korean Medicine, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 130-701, Republic of Korea. E-mail: ihcho@khu.ac.kr

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The tetracyclic triterpenoid ginsenoside-Rb1 (Rb1) belongs to the protopanaxadiol group of steroidal saponins, the major bioactive compounds extracted from ginseng.[1,4,29] Rb1 attenuates inflammation in in vivo and in vitro models, including 2,4,6-trinitrobenzene sulfuric acid-induced colitis,[25] lipopolysaccharide-induced lung injury,[52] carbon tetrachloride-induced liver fibrosis,[16] and aortic smooth muscle exposed to tumor necrosis factor (TNF)-α.[34] Rb1 attenuates damage to cerebral cortex neurons through downregulation of nitric oxide, superoxide, and TNF-α expression in hypoxia-activated microglia.[28] Rb1 also attenuates intestinal ischemia reperfusion-induced renal injury and protects neural progenitor cells by activating the Kelch-like erythroid protein with the cap'n collar homology-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2 (Nrf2)–antioxidant response element (ARE) pathway.[39,45] In addition, activation of Nrf2–ARE-antioxidant signaling attenuates the nuclear factor kappa B (NF-κB)-inflammatory response.[27,35] Recently, increasing numbers of studies have highlighted the beneficial effects of Rb1 via regulation of Nrf2-ARE and NF-κB pathways in the nervous system. Rb1 protects 6-hydroxydopamine-induced oxidative stress in human dopaminergic cells by increasing heme oxygenase (HO)-1 expression through the Nrf2-dependent pathway,[19] reducing ischemic cell death,[53] and inhibition of lipopolysaccharide-induced microglial activation by suppression of the NF-κB pathway.[17] Although such reports increase the possibility that Rb1 might be an effective treatment for other neurological diseases, it is as of yet unknown whether it can be used to attenuate inflammatory nociception.

Extracellular signal-regulated kinase (ERK) 1/2, mitogen-activated protein kinase subfamily members, were found to be activated in spinal dorsal horn (DH) neurons in response to peripheral noxious stimulation (injury and inflammation)-induced hyperalgesia.[18,22,23,36,54] Interestingly, phospho (p)-ERK is present in spinal DH neurons immediately after nerve injury (10 minutes–6 hours), in microglia cells 2 days after injury, and in astrocytes 3 weeks later.[54] This sequential induction of p-ERK in different cell types at different times is important for the development of neuropathic pain at different phases.[54] Furthermore, intrathecal (i.t.) introduction of a specific or upstream inhibitor (PD98059, etc), which causes suppression of ERK activity reduces the nociceptive response in complete Freund's adjuvant–induced arthritis[42] and formalin-induced inflammatory pain,[33] and reduces visceral pain caused by intracolonic capsaicin[12] and peritoneal acetic acid.[3] These studies point to the critical role of ERK in the development and persistence of inflammatory or neuropathic pain hypersensitivity.[22-24] Recently, Rb1 was reported to protect PC12 pheochromocytoma cells from caspase–3-dependent apoptosis through inhibition of activation of the ERK1/2 pathway.[15] In addition, it was also observed to alleviate hypoxia hypercapnia-induced pulmonary vasoconstriction by inhibition of the ERK1/2 pathway.[44] However, very little is known about the possible link, including molecular signaling mechanisms, between p-ERK and Rb1 evoked by acute inflammatory pain.

These recent reports increase the likelihood that Rb1 might be an effective treatment for nociception. To address this issue, we investigated whether i.t. injection of Rb1 has an antinociceptive effect in a formalin-induced inflammatory nociceptive rat model. We showed that i.t. injection of Rb1 attenuated formalin-induced inflammatory nociception by inhibition of the phosphorylation of neuronal ERK that is thought to regulate the Nrf2 and NF-κB pathways in the spinal cord. Our findings indicate that Rb1 could be applied as a therapeutic strategy in patients with acute nociception, after further investigation.

Methods

Animals and Ethical Statements

Adult male Sprague Dawley rats (weight, 200–220 g) were purchased from Narabiotec Co, Ltd (Seoul, Korea), kept under a 12-hour light and dark cycle (lights on 07:00–19:00) at room temperature (RT; 23 $\pm$ 2°C) and the humidity of 55 $\pm$ 10%, and given food and water ad libitum. The animals were allowed to habituate to the housing facilities for 1 week before commencement of the experiments. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee or Ethics Committee of Animal Experiments of Kyung Hee University, Republic of Korea. Proper randomization of the laboratory animals and handling of the data were performed in a blinded manner in accordance with the recent recommendations from a National Institutes of Health workshop on preclinical models of neurological diseases.[30]

Experiment Group and Formalin-Induced Behavioral Test

The formalin-induced nociceptive response was investigated as described previously.[33] Briefly, male Sprague Dawley rats were randomly subdivided into the following groups (n = 10–12 per group): normal control group (saline, subcutaneous [s.c.] + vehicle, i.t.), formalin-stimulated group (formalin, s.c. + vehicle, i.t.), formalin-stimulated group after i.t. introduction of Rb1 (formalin, s.c. + 2, 10, or 50 μg of Rb1, i.t.), and Rb1 group (Rb1 alone, i.t.-introduced; saline, s.c. + 50 μg of Rb1, i.t.). After intraplantar injection of the right hind paw with formalin (5%, 50 μL), rats were placed in a clear plastic cage (20 $\times$ 26 $\times$ 12 cm) without bedding, and the total time of the nociceptive responses, including licking and/or rubbing of the stimulated area or lifting the paw, was counted in 5-minute intervals for 60 minutes. The behavioral tests were performed blinded under constant conditions (temperature, 23 $\pm$ 3°C; humidity, 55 $\pm$ 5%) between 9:00 am and 12:00 pm in a quiet room. For the main experiment, the i.t. introduction of Rb1 (Sigma-Aldrich, St Louis, MO) was carried out 20 minutes before formalin stimulation. Moreover, for additional experiments (to investigate the antinociceptive time window of Rb1 and the antinociceptive effect of Rb1 after formalin stimulation), the i.t. introduction of Rb1 was carried out at 1, 3, and 5 hours before formalin