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Effects of ginsenoside Rb_1 at low doses on histamine, substance P, and monocyte chemoattractant protein 1 in the burn wound areas during the process of acute burn wound repair

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Abstract

Aim of the study: We reported recently that the facilitating effects of ginsenosid Rb_1 on burn wound-healing might be due to the promotion of angiogenesis. Increased histamine, substance P (SP), and monocyte chemoattractant protein (MCP)-1 levels caused inflammation, and pain following severe burn wound injury.
Materials and methods: We examined the effects of ginsenoside Rb_1 on the histamine, SP, and MCP-1 levels in burn wound tissue during burn wound repair.
Results and Conclusions: Ginsenoside Rb_1 (1 ng/wound) and basic fibroblast growth factor (bFGF) (2.5 μg/wound) significantly increased the levels of MCP-1 on day 1 compared to the MCP-1 level in vehicle-treated mice. Histamine production of the burn wound area on day 7 was increased by topical application of ginsenoside Rb_1 (100 fg–1 ng/wound) and bFGF. The number of mast cells migrating to the burn wound area was also increased by ginsenoside Rb_1. Conversely, the increased SP production was reduced by ginsenoside Rb_1. This finding suggests that the pain induction by burn injury may be reduced by ginsenoside Rb_1. The facilitating actions of ginsenoside Rb_1 on burn wound healing may be due to the increase in histamine production via the increase in mast cell migration to the burn wound area induced by the rapid elevation of MCP-1.

Keywords: Monocyte chemoattractant protein-1; Histamine; Mast cell; Burn wound healing; Ginsenoside Rb_1

1. Introduction

Ginseng roots (*Panax ginseng* C.A. Meyer) are used clinically in China, Korea, and Japan for various diseases, including atherosclerosis, liver dysfunction, cerebrovascular diseases, and post-menopausal disorder (Yamamoto, 1988). Ginseng root extracts have also been used clinically as topical treatments of atopic supportive dermatis, wounds, and skin inflammation. Zhang et al. (2005) reported previously that intravenous infusion of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$, MW 1109.96), a major ingredient of ginseng roots, is known to ameliorate cortical infarct volume through STAT 5-dependent up-regulation of an anti-apoptic factor, Bcl-xL, and possibly through the promotion of vascular regeneration in stroke-prone spontaneous hypertensive rats. To ascertain the ginsenoside Rb_1-mediated vascular regeneration in a more easily accessible experimental system, we recently demonstrated that topical application of ginsenoside Rb_1 to burn lesions in mice increased the local production and/or secretion of vascular endothelial growth factor (VEGF) and facilitated wound healing by promoting vascular regeneration (Kimura et al., 2006). It is well known that burns initially induce coagulative necrosis and cause scar formation after repair. Macrophages migrate to the injured area to kill invading organisms and produce cytokines and/or chemokines that recruit other inflammation mediators (O'Riordain et al., 1992; Katranovski et al., 1999). Among skin wounds, burn wounds can be extremely painful. Severe burns induce liberation of different mediators such as neuropetides, like substance P (SP), and histamine releases in the skin. Papp et al. (2005) and Papp and Valtonen (2006) reported that the release of substance P in burn-

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injured tissues was a possible cause of the late increase in tissue histamine in burns. In general, pain is divided into two types; one is the so-called pricking pain transmitted by Aδ-fibers frequently containing SP as a neurotransmitter (Nahr and Plaghki, 2003; Mouraux et al., 2004; Matsumoto et al., 2006) and the other is burning pain transmitted by c-fibers, which also frequently contain the same peptide neurotransmitter (Zachariou et al., 1997; Yaksh et al., 1999; Wallengres et al., 1999; Scott et al., 2000, 2005; Papp and Valtonen, 2006). Although histamine, SP, and monocyte chemoattractant protein-1 (MCP-1) caused inflammation, edema, and pain in burn wounds, there are a number of reports that histamine, SP, and MCP-1 also enhance wound healing (Trautmann et al., 2000; Low et al., 2001; Gibran et al., 2002; Spenny et al., 2002; Delgado et al., 2005; Weller et al., 2006; Numata et al., 2006; Rook and McCarson, 2007). In a previous paper, we reported that the facilitating action of ginsenoside Rb_1 may be due to the promotion of anigiogenesis during skin repair via the stimulation of VEGF production, through the increase of hypoxia inducible factor (HIF)-1α expression in keratinocytes, and due to the elevation of interleukin (IL)-1β resulting from macrophage accumulation in the burn wound (Kimura et al., 2006). In a preliminary clinical study, we found that the topical application of ginsenoside Rb_1 (10^{-7}% ointment) to laser-induced lesions shortened the duration of the pain in comparison with vehicle-treated control (data not shown; in preparation). Therefore, in this study, we attempted to clarify the effects of ginsenoside Rb_1 on the relationship between histamine, SP, and MCP-1 in burn wound tissue during the process of burn wound repair.

2. Materials and methods

2.1. Preparation of ginsenoside Rb_1

Ginseng saponins were isolated by the method described by Shibata and co-workers (Nagai et al., 1971; Sanada et al., 1974a,b; Shibata et al., 1985; Shibata, 2001). Briefly, ginsenoside Rb_1 was isolated and purified from the crude saponin fractions of roots of Panax ginseng CA Meyer (Korean Red Ginseng) by repeated column chromatography on silica gel with $CHC1_3$–MeOH–H_2O (65:35:10, v/v) and ocatdecylsilyl silica with MeOH–H_2O (1:1–7:3, v/v). The purity of ginsenoside Rb_1 (Fig. 1) used in this study was more than 99.99%, as determined by high-performance liquid chromatography. Ginsenoside Rb_1 was used in white vaseline ointment as indicated in the text.

2.2. Animals

Male Balb/c mice (5-week old) were obtained from Clea Japan (Osaka, Japan), housed for 1 week in a room with controlled temperature at 25 ± 1 °C and relative 60% humidity, and given free access to standard laboratory diet and water. Mice were treated according to the Ethical Guidelines of the Animal Center, Graduate School of Medicine, Ehime University, and the experimental protocol was approved by the Animal Studies Committee of Ehime University.

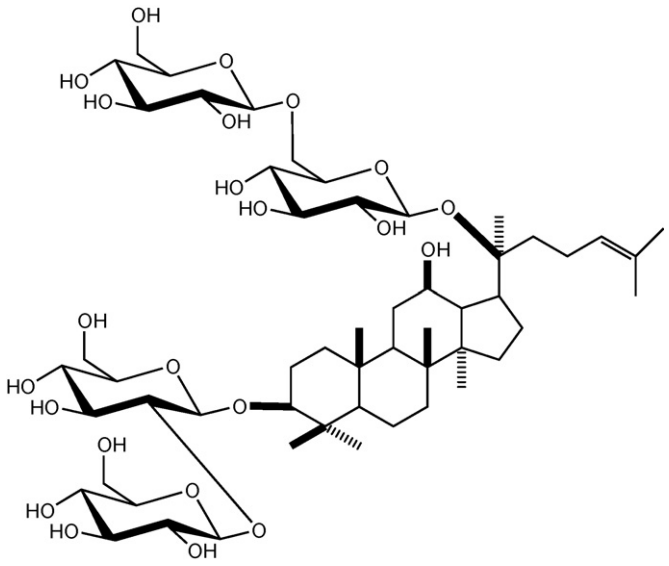

Fig. 1. The structure of ginsenoside Rb_1

2.3. Measurement of histamine, substance P (SP), and monocyte chemoattractant protein-1 (MCP-1) in the burn wounds

The burn wounds were created on the backs of male Balb/c mice under anesthesia with pentobarbital (50 mg/kg body weight, *i.p.*). Hair was removed from the backs of mice using hair remover under anesthesia. The backs of the mice were subsequently wiped with 37 °C-distilled water and 70% ethanol. A customized soldering iron was used to cause the burn to the skin on the backs of the mice. The custom-made tip of the soldering iron was 7 mm in diameter with a heating ring of 1 mm thickness around the outside of the tip, which reached a temperature of 250 °C. After the burn wound was made by applying the soldering iron to the skin for 10 s, a sterile biopsy punch (8 mm diameter, Kai Industries Co. Tokyo, Japan) was used to excise the burn skin, leaving the underlying fasciae intact. All surgical treatments were performed under anesthesia with pentobarbital. A polyethylene filter pellet (about 8 mm diameter, 3 mm thickness) containing the indicated amounts of ginsenoside Rb_1 was applied to the burn wound surface, and covered with film dressing (PERME-AIDS, Nitto Medical Co., Tokyo, Japan). On days 1, 3, 5, and 7 the filter pellets were removed and replaced with fresh filter pellets. For control mice, filter pellets containing 0.9% NaCl alone were applied according to the same schedule. Immediately after removal, phosphate-buffered saline (PBS, pH 7.0, 200 μL) was added to each filter pellet and mixed for 10 min. The filter pellet was then removed and the extract centrifuged at 1000 × *g* for 10 min at 4 °C. The supernatant fractions from the centrifugation were used to determine the contents of histamine, SP, and MCP-1 using Histamine ELISA kit (SPI BIO, France), mouse substance P ELISA kit (Assay Design, Inc., MI, USA), and mouse MCP-1 ELISA kit (R&D Systems Inc., MN, USA), respectively.