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Research paper

Polysaccharides derived from *Ganoderma lucidum* fungus mycelia ameliorate indomethacin-induced small intestinal injury via induction of GM-CSF from macrophages

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ABSTRACT

Non-steroidal anti-inflammatory drugs often cause ulcers in the human small intestine, but few effective agents exist to treat such injury. *Ganoderma lucidum* Karst, also known as “Reishi” or “Lingzhi”, is a mushroom. We previously reported that a water-soluble extract from *G. lucidum* fungus mycelia (MAK) has anti-inflammatory effects in murine colitis induced by trinitrobenzene sulfonic acid, and induction of granulocyte macrophage colony-stimulating factor (GM-CSF) by MAK may provide anti-inflammatory effects. However, its effects on indomethacin-induced small intestinal injuries are unknown. The present study investigated the preventative effects of MAK via immunological function and the polysaccharides from MAK on indomethacin-induced ileitis in mice. Peritoneal macrophages (PMs) were stimulated in vitro with MAK and adoptively transferred to C57BL/6 mice intraperitoneally, which were then given indomethacin. Intestinal inflammation was evaluated after 24 h. We performed in vivo antibody blockade to investigate the preventive role of GM-CSF, which derived from PMs stimulated with MAK. We then used PMs stimulated with MAK pre-treated by pectinase in an adoptive transfer assay to determine the preventive role of polysaccharides. Indomethacin-induced small intestinal injury was inhibited by adoptive transfer of PMs stimulated in vitro with MAK. In this transfer model, pre-treatment with anti-GM-CSF antibody but not with control antibody reversed the improvement of small intestinal inflammation by indomethacin. Pectinase pretreatment impaired the anti-inflammatory effect of MAK. PMs stimulated by MAK appear to contribute to the anti-inflammatory response through GM-CSF in small intestinal injury induced by indomethacin. The polysaccharides may be the components that elicit the anti-inflammatory effect.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), including indomethacin and aspirin, are commonly used worldwide for the treatment of musculoskeletal pain and inflammation. However, NSAIDs can cause serious adverse reactions in the form of gastrointestinal lesions [1,2]. Recent advancements in capsule and double-balloon endoscopy have contributed to the increased diagnosis of NSAID-induced small intestinal lesions such as ulcers, bleeding, perforation, and strictures [3–6]. It has become clear that NSAID-induced small intestinal lesions are not as rare as previously thought [7]. For example, Graham et al. reported that 71% of chronic users of NSAIDs have lesions of the small intestine [8]. However, in contrast with upper gastrointestinal injury, few effective agents can prevent and treat small intestinal injury. Therefore, the exploration of preventive and therapeutic agents for NSAID-induced small intestinal injury remains an urgent priority.

Recently obtained data have shown that GM-CSF plays an important role in maintaining intestinal homeostasis. The effect of GM-CSF has been studied in murine models of dextran sodium sulfate-induced colitis, which can be ameliorated by administration of GM-CSF [9,10]. Recent studies have suggested that autoantibodies to GM-CSF are associated with progressive ileal disease in Crohn’s disease patients [11]. Furthermore, a recent phase II, randomized, double-blind, placebo-controlled trial of sargramostim (yeast-derived recombinant human GM-CSF) found that it was effective in the treatment of patients with moderate to severely active Crohn’s disease [12].

Abbreviations: Ab, antibody; mAb, monoclonal antibody; MAK, water-soluble extract from a cultured medium of Reishi mycelia; MLNs, mesenteric lymph nodes; NSAIDs, non-steroidal anti-inflammatory drugs; PM, peritoneal macrophage; TNBS, trinitrobenzene sulfonic acid

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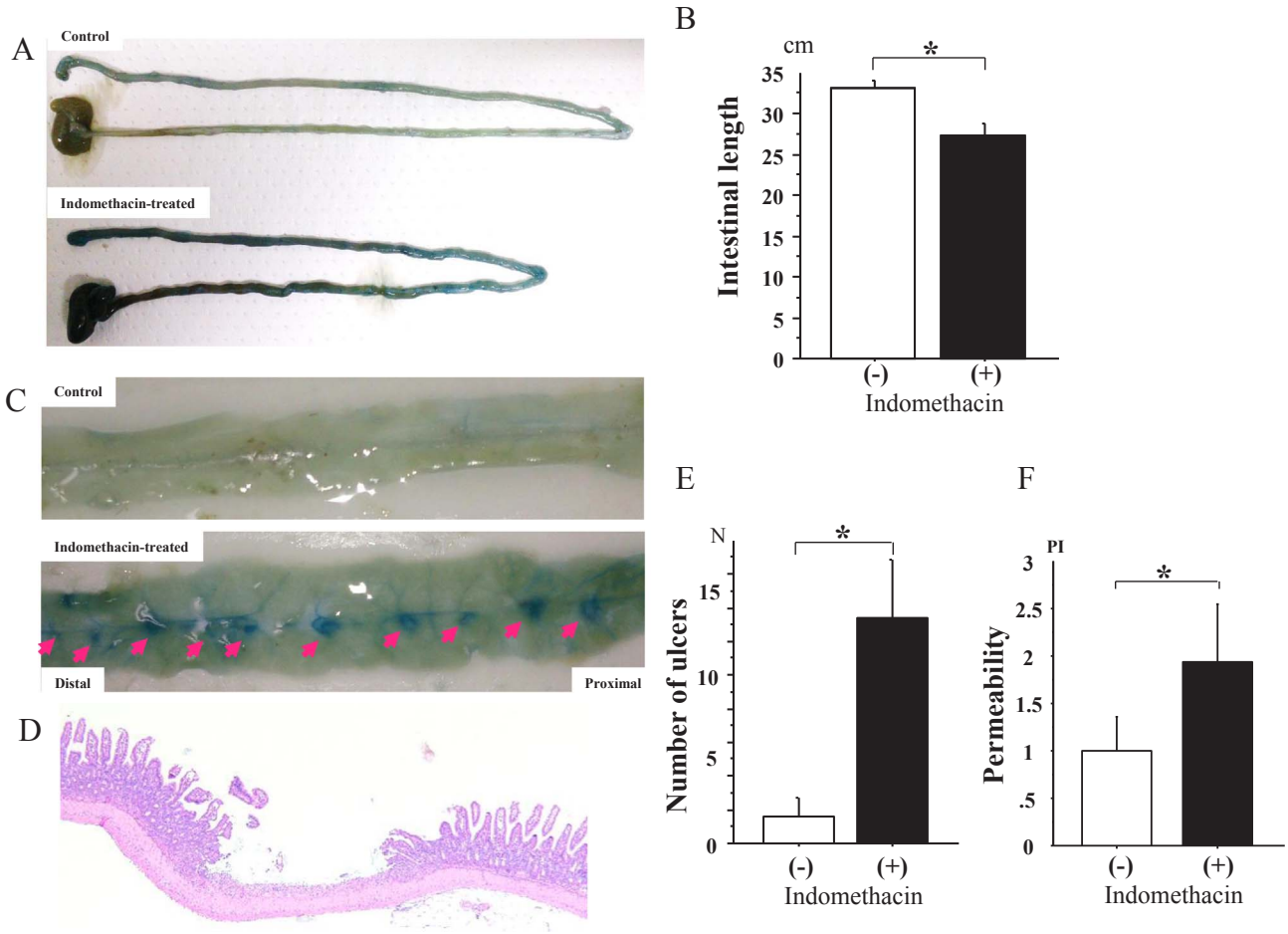

Fig. 1. Small intestinal damage after indomethacin administration. (A) The whole small intestine after indomethacin administration. (B) The length of the small intestine was shorter. Date are expressed as means ± SD, n = 5. $^{*}P < 0.05$ versus untreated controls. (C) Injured mucosa stained dark blue with ulcer formations by injection of 1% Evans blue (arrows). (D) Histological findings in the small intestine. Destruction and necrosis of intestinal epithelium extending to the base of the crypts was observed. (E) The number of macroscopic ulcers and (F) the permeability of blood vessels in the small intestine. Data are presented as mean ± SD (n = 4–5 for each group). $^{*}P < 0.05$ versus untreated controls. Data are presented as mean ± SEM of three independent experiments.

Ganoderma lucidum Karst, belonging to the Basidiomycetes class of fungi, is well known as "Reishi", a traditional food in China and Japan [13]. It contains various bioactive substances, including polysaccharides, proteins, nucleotides, fatty acids, terpenoids, sterols, and cerebrosides [14]. Reishi has multiple immunologic functions, such as activation of natural killer cells in BALB/c mice [15], induction of CD40/CD86 on human peripheral blood monocytes [16], and cytokine-induced killer cells in C57BL/6 mice [17]. Recently, it was reported that Reishi contains a fraction named "F3", which stimulates mouse spleen cell proliferation and cytokine production, especially that of GM-CSF [18]. Although a water-soluble extract from a cultured medium of Reishi mycelia (MAK) and F3 are purified by different methods, there appears to be a strong likelihood that components of Reishi may contribute to GM-CSF-mediated immune responses.

We previously reported that murine trinitrobenzene sulfonic acid (TNBS)-induced colitis was prevented via GM-CSF production by feeding with MAK [19]. So far, however, there have been no reports showing the effect of MAK on small intestinal inflammation. In this work, we investigated the role of MAK in indomethacin-induced small intestinal injury. This study shows that peritoneal macrophages (PMs) stimulated by MAK are effective in the prevention of intestinal inflammation. In vitro, MAK stimulated PMs to produce GM-CSF in a dose-dependent manner. Finally, the protective effect of PMs on intestinal inflammation is dependent on GM-CSF. Therefore, GM-CSF may be a candidate for the treatment of small intestinal inflammation.

2. Materials and methods

2.1. Mice

Specific pathogen-free C57BL/6(B6) mice were purchased from CLEA Japan (Tokyo, Japan). All mice were housed under specific pathogen-free conditions in micro-isolator cages in the animal facility at Hiroshima University, and only male mice (9–14 weeks old) were used. The animals were maintained in accordance with the "Guidelines for the Care and Use of Laboratory Animals" established by Hiroshima University. Normal tap water was also provided *ad libitum*. The MAK was provided by Noda Shokkin-Kogyo Co., Ltd. (Chiba, Japan). The preparation of MAK (overall yield ≈ 10%) was as follows: a pure culture of G. lucidum mycelia was inoculated into a solid culture medium that was composed of bagasse and defatted rice bran and cultured until just before the formation of the fruit body (for 3–4 months); subsequently, the entire medium overgrown with G. lucidum mycelia was extracted with hot water, and then the extract was sterilized by filtration and lyophilized for powderization.

2.2. Preparation of peritoneal macrophages

Peritoneal cells were collected by washing the peritoneal cavity with ice-cold PBS. The cells were seeded at 1×10^6 cells/well in 96-well plates to allow them to adhere to the surface and incubated in humidified 5% CO_2 at 37 °C for 1–2 h in RPMI 1640 medium