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Ghrelin attenuates imiquimod-induced psoriasiform skin inflammation in mice

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Abstract

Objectives: The endogenous peptide ghrelin, which is produced in the stomach, plays a pivotal role in secretion of growth hormone, regulation of energy metabolism, and cardiovascular protection; however, its anti-inflammatory action has recently gained immense attention, as this peptide is presumed to be effective in patients with heart failure and chronic lung diseases. In dermatology, psoriasis acts as a typical chronic intractable disease, which may be severe, and although biologic agents have been recommended and used for its treatment in recent years, their application is not clear. We administered ghrelin to IMQ-induced psoriasis-like mouse model and examined the anti-inflammatory effect of ghrelin.

Methods: In the present study, we induced psoriasiform skin inflammation via the continuous application of imiquimod cream on the backs of BALB/c mice and examined the gross and histopathological effect of the subcutaneous administration of ghrelin on psoriasiform skin inflammation.

Results: It was confirmed that the administration of ghrelin improved various scores, including erythema, scales, and epidermal thickening scores, in mice with psoriasiform skin inflammation.

Conclusions: The results suggest that ghrelin may be effective in treating psoriasis, including cases involving intractable skin lesions that are resistant to conventional therapies.

KEYWORDS

ghrelin, imiquimod, NF-κB, psoriasis, TNF-α

1 | INTRODUCTION

Psoriasis is a chronic inflammatory skin disease that affects 3% of the population worldwide. Approximately 130 million patients are estimated to suffer from this disease.[1] A certain number of psoriasis cases are intractable and severe, and the patient's QOL is considerably reduced. Psoriasis treatment includes symptomatic therapy involving the long-term application of external preparations, mainly

corticosteroids, as well as oral retinoids and cyclosporine, and phototherapy. Recently, biologic agents that specifically inhibit the action of inflammatory cytokines (TNF-α, IL-23, IL-17), which are intimately involved in the pathology of psoriasis, have been recommended for this condition, and this treatment has been effective for a number of patients with intractable psoriasis. In contrast, psoriasis is assumed to be caused by various immunological factors[2–5]; however, the underlying cause remains unknown. Thus, the aforementioned anticytokine therapies are not completely effective, and the fact remains that patients are often unable to continue treatment with these drugs because of the adverse drug reactions and the cost of the drugs.

Ghrelin is a growth hormone secretagogue (GHS) that was discovered in 1999 from the stomachs of humans and rats. It acts as an endogenous ligand for the growth hormone secretagogue-receptor (GHS-R).[6] It has various physiological actions, including secretion of growth hormone (GH), appetite promotion, enhancing gastrointestinal motility, increasing gastric acid secretion, and improving the cardiac function.[7] The anti-inflammatory action of ghrelin in the gastrointestinal tract,[8] nerves,[9] and various organs, including respiratory organs,[10,11] circulatory organs,[12] and kidneys,[13] has been reported in inflammatory disease models. Clinical studies have been implemented for patients with heart disease[14] and chronic respiratory diseases,[15,16] and it is presumed that ghrelin will become a novel therapeutic option for chronic inflammatory diseases.

GHS-Rs are widely expressed in the body, including the gastrointestinal tract, nerve tissue, and lymphatic tissue, and in immune cells such as T cells, B cells, dendritic cells, monocytes, neutrophils, and macrophages.[17–19] The detailed mechanism of ghrelin's anti-inflammatory activity remains unclear; however, it is assumed that it mainly acts via GHS-R on the immune cells; that is, it is presumed that the action exerted by ghrelin via GHS-R on immune cells inhibits the TNF-α signaling pathway inside the cytoplasm and nucleus after the binding of ghrelin to its receptors on each tissue cell[20,21] and that it inhibits the secretion of cytokines from Th17 cells[22] and the induction of Th17 cell inflammation by incorporating Treg cells.[23]

Based on the findings of reports regarding the anti-inflammatory action of ghrelin, we assume that ghrelin may be effective in treating patients with psoriasis where TNF-α and Th17 are involved during the onset of the disease and its progression. To verify this hypothesis, we induced psoriasiform skin inflammation via the continuous application of imiquimod cream on the backs of BALB/c mice and examined the gross and histopathological effect of the subcutaneous administration of ghrelin on psoriasiform skin inflammation.

2 | MATERIALS AND METHODS

2.1 | Mice

The mice used in this study (BALB/cAnNCrlCrlj, female, 8 weeks old, body weight 15-20 g) were purchased from Charles River Laboratories, Japan. The mice were housed at room temperature (20-26°C), under 30%-70% humidity, in a 12-hour light and dark cycle (light period: 7 AM to 8 PM, dark period: 8 PM to 7 AM), in a specific pathogen-free (SPF) environment, and were allowed ad libitum access to feed and water. After 5 days acclimatization, they were divided into a total of four groups (n = 10-11 per group) and were used for the experiments. The four groups were as follows: hydrophilic ointment alone applied to the observation site (left abdomen; control: n = 10), subcutaneous injection of phosphate-buffered saline (PBS) after the application of IMQ (PBS: n = 11), and two groups with the subcutaneous injection of ghrelin dissolved in PBS after the application of IMQ (ghrelin 400 μg/kg, ghrelin 1600 μg/kg: n = 10 per group).

The experimental use of animals in this study was approved by the institutional animal care and use committee of Osaka Medical College, Japan.

2.2 | IMQ-induced psoriasis-like mouse model and drug treatment

The drug-induced psoriasis-like mouse model was created by an already established model (IMQ-induced psoriasis-like mouse mode[24]) using the aforementioned mice and the repeated daily application of imiquimod (Beselna®; Mochida Pharmaceutical), thereby creating localized psoriasiform skin inflammation.

Initially, 3 days before commencing the experiment, the fur on the left abdomen (30 × 20 mm) of the mice was shaved with electric clippers (THRIVE) and was then completely removed with depilatory cream (Epilat®; Kracie Holdinds Ltd). IMQ (50 mg/application) was applied once a day for 4 days to the skin of the shaved left abdomen for all groups—with the exception of the control group—from the day of experiment commencement (Day 1). Psoriasis-like skin inflammation was induced to create the drug-induced psoriasis-like mouse model (IMQ; Days 1-4). In the control group, hydrophilic ointment (Nikko Pharmaceutical Co., Ltd) was applied in a similar manner to IMQ (Days 1-4). The two groups treated with ghrelin (ghrelin 400 μg/kg, ghrelin 1600 μg/kg) then received a subcutaneous injection of ghrelin (human/mouse/rat ghrelin [1-5] amide, [Dap3]-Octano; Phoenix Pharmaceuticals, Inc) dissolved in PBS (200 μL/dose) at the location at which psoriasiform skin inflammation had been induced (evaluation site), immediately after the application of IMQ, once a day for 4 days from the commencement of the experiment. PBS (200 μL/dose) without dissolved ghrelin was administered to the PBS group. The evaluation site, a 15 × 15 mm area at which of PBS or ghrelin dissolved in PBS was subcutaneously administered (evaluation site), was marked on the left abdomen.

2.3 | Measurement and evaluation of macroscopic dermatitis score

The symptoms on the evaluation site were observed from Days 1 to 5, and the severity of the rash was evaluated as a macroscopic score. The macroscopic dermatitis score referenced the PASI score, which scores erythema, scales, and induration on a 5-point scale from 0 to 4 (0, none; 1, slight; 2, moderate; 3, marked; and 4, extensively marked)

(Figure 2A-C: each 0-4 points), and the total score was used as the dermatitis score (Figure 2D: total 0-12 points) to observe and compare the changes over time in each group (Days 1-5). The measurement of each score was implemented by observing the skin symptoms daily before the application of IMQ and at the completion of the experiment.

2.4 | Histological examination

On the final experiment day (Day 5), the skin symptoms were observed, and after evaluating various gross scores, the mice were euthanized and the skin tissue was collected from the evaluation site with an 8 mm dermapunch (Biopsy Punch®; Kai Industries). Skin samples were obtained from all individuals. After fixing the skin tissue in 10% neutral buffered formalin solution, paraffin and tissue slices (~5 μm) were created and hematoxylin and eosin (HE) staining was performed by Kyodo Byori Inc. HE-stained slices (magnification ×100) of 10 randomly selected samples were evaluated measuring of an epidermal thickness and using a pathological score[25] to assess the histopathological severity of psoriasiform skin inflammation findings in each group.

2.5 | Statistical analyses

The Steel Dwass test was used to evaluate the statistical significance between each group. *P* values of <.05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | The ameliorating effect of ghrelin in IMQ-induced psoriasis-like lesions

The changes over time that increased for each gross score in the IMQ application groups (PBS, ghrelin 400 μg/kg, ghrelin 1600 μg/

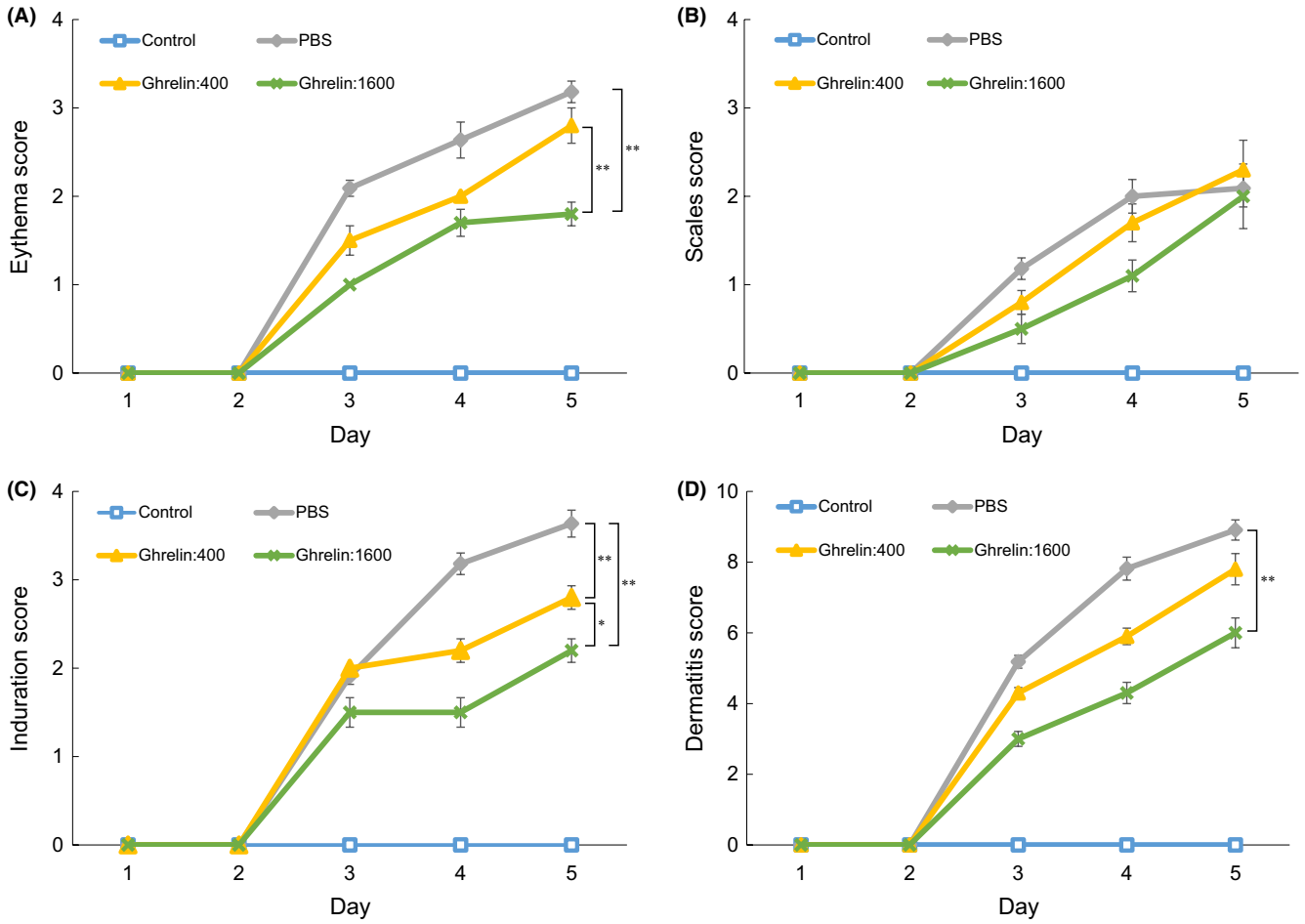

FIGURE 1 Change of the cutaneous symptom scores and clinical score in the IMQ-induced psoriasis-like mouse model during ghrelin treatment. In PBS, ghrelin 400 μg/kg, and ghrelin 1600 μg/kg groups, the psoriasiform skin inflammation symptoms of erythema, scales, and induration were induced and progressed as the days passed. In comparison with the PBS groups, the erythema (A), scales (B), induration (C), and dermatitis (D) scores, which are the comprehensive scores of these three symptoms, decreased in a dose-dependent manner with ghrelin treatment, and the erythema (A), induration (C), and dermatitis scores (D) were significantly lower in the ghrelin 1600 μg/kg group (*P* < .05, *P* < .01, *P* < .01, respectively). Each score is displayed as the mean ± standard deviation (Mean ± SD). **P* < .05; ***P* < .01; IMQ, imiquimod cream; PBS, phosphate-buffered saline

kg groups) are presented in Figure 1A-D. No changes were observed over time in the gross scores of the control group (Figure 1A-D). The ghrelin 1600 μg/kg group showed the lowest erythema score (Figure 1A) among the IMQ application groups from Days 3 to 5, and a significant difference was observed between this group and PBS and ghrelin 400 μg/kg groups. The ghrelin 1600 μg/kg group tended to have lower scale scores (Figure 1B) in comparison with the other IMQ application groups from Days 3 to 5; however, no clear significant difference was observed between the ghrelin groups and PBS groups. The ghrelin 1600 μg/kg groups tended to have lower induration scores (Figure 1C) in comparison with the other IMQ application groups on Day 3; however, the scores did not differ from that in the IMQ only group to a statistically significant extent. Nevertheless, on Days 4 and 5, the ghrelin 1600 μg/kg group showed the lowest thickness score of the IMQ application groups, and a significant difference was observed between this group and PBS and ghrelin 400 μg/kg groups. The ghrelin 1600 μg/kg group showed the lowest dermatitis score (Figure 1D), which is the total of the erythema, scales, and thickness scores, from Days 3 to 5, and a significant difference was observed between this group and PBS groups.

Figure 2 shows representative images on the final day of the experiment (Day 5). PBS, ghrelin 400 μg/kg, and ghrelin 1600 μg/kg groups showed characteristic psoriasis skin rashes, including erythema, desquamation, and induration, in comparison with the control group, which was not treated with IMQ. When the five groups that received the application of IMQ (PBS, ghrelin 400 μg/kg, and ghrelin 1600 μg/kg groups) were compared, the ghrelin 1600 μg/kg groups showed milder psoriasiform skin inflammation (eg, erythema, desquamation, and induration) in comparison with the other groups.

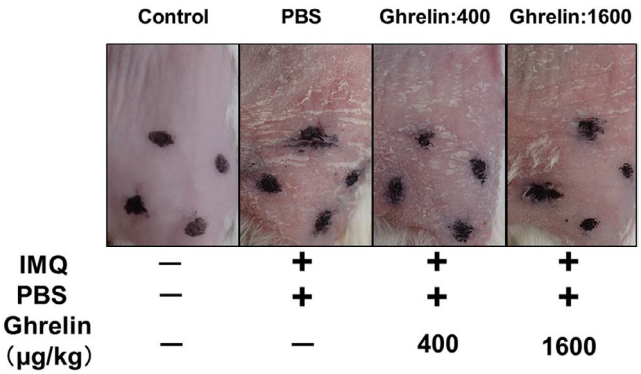

FIGURE 2 Representative images depicting the effect of ghrelin on imiquimod-induced psoriasis-like dermatitis in mice. The figure presents the findings of the gross changes in IMQ-induced dermatitis in each group of mice on Day 5. Erythema formation with induration and scales was seen in the IMQ-treated groups, but these changes tended to be milder with the subcutaneous administration of ghrelin (particularly in the 1600 μg/kg group). IMQ, imiquimod cream; PBS, phosphate-buffered saline

3.2 | Ghrelin ameliorates psoriasis-like changes in IMQ-treated lesions on HE-stained sections

Images of HE-stained tissue specimens from the site at which PBS or ghrelin dissolved in PBS was subcutaneously administered (evaluation site), which was collected from the left abdomen of each group on the final experiment day (Day 5), are shown in Figure 3, and the pathological scores are shown in Figure 4.

Images of HE-stained tissue specimens (Figure 3) from PBS and ghrelin 400 μg/kg groups show characteristic histopathological findings of psoriasis, including epidermal hyperplasia, parakeratosis,

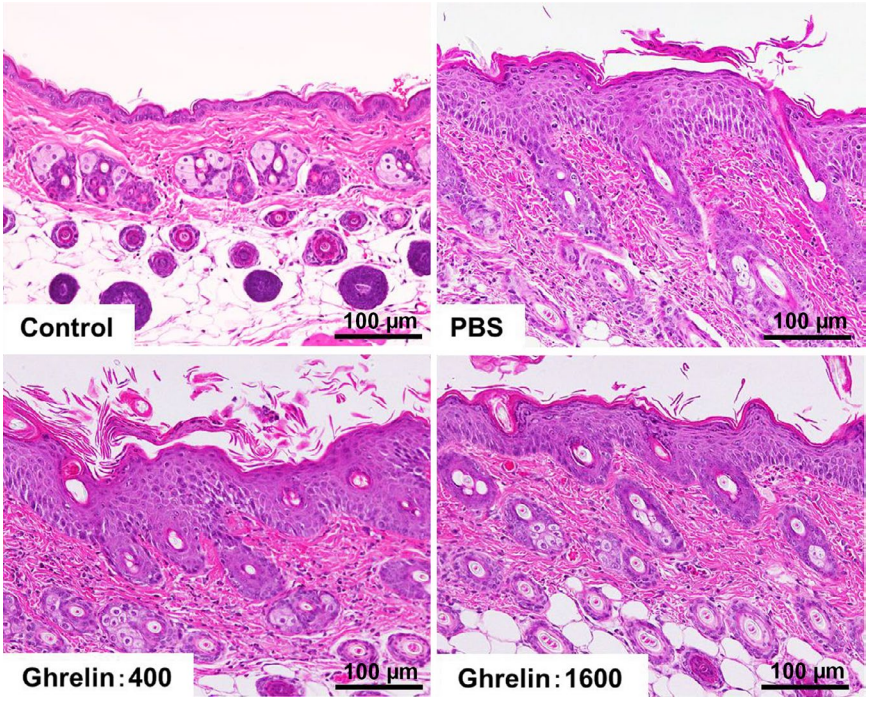

FIGURE 3 Representative histopathological change of the IMQ-induced psoriasis-like mouse model during ghrelin treatment. The figure shows representative histopathological findings (HE staining, scale bar: 100 μm) for each group of mice (×200). In the ghrelin 1600 μg/kg groups, parakeratosis disappeared and the epidermal thickening was milder in comparison with the PBS groups. PBS, phosphate-buffered saline

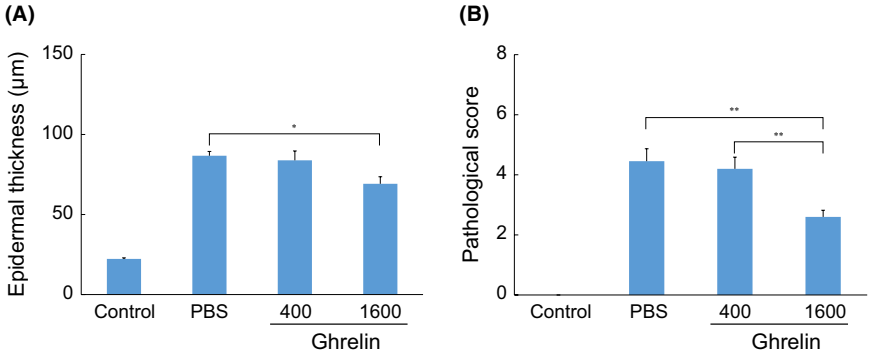

FIGURE 4 Epidermal thickness and pathological score, evaluated by hematoxylin and eosin staining, on Day 5 during ghrelin treatment. HE-stained slices (magnification ×100) of 10 randomly selected samples were evaluated based on an epidermal thickness (A) and a pathological score (B), which was determined based on epidermal thickening, parakeratosis, hypogranulosis, and microabscess formation. The epidermal thickness (A) of the ghrelin 1600 μg/kg group was significantly decreased compared with PBS groups ($P < .05$). And the pathological score (B) of the ghrelin 1600 μg/kg group was significantly lower in comparison with PBS and ghrelin 400 μg/kg groups ($P < .01$). Each score is displayed as the mean ± standard deviation (Mean ± SD). *$P < .05$; **$P < .01$; IMQ, imiquimod cream; PBS, phosphate-buffered saline

hypogranulosis, and microabscess formation. In comparison with these groups, the parakeratosis had disappeared, and the epidermal hyperplasia was milder in ghrelin 1600 μg/kg groups.

The HE-stained slices (magnification ×100) of 10 randomly selected samples were evaluated using an epidermal thickness (Figure 4A) and a pathological score (Figure 4B) for each individual—with epidermal thickening, parakeratosis, hypogranulosis, and microabscess formation as indices—in order to evaluate the severity of psoriasiform skin inflammation in each group for the skin histopathological findings (HE staining) on the final experiment day (Day 5). The ghrelin 1600 μg/kg group showed the thinnest epidermal thickness (Figure 4A) among the IMQ application groups, and significant differences were observed between the ghrelin 1600 μg/kg group and PBS groups. And the ghrelin 1600 μg/kg group had the lowest pathological score (Figure 4B) of the IMQ application groups, and significant differences were observed between the ghrelin 1600 μg/kg group and PBS and ghrelin 400 μg/kg groups.

4 | DISCUSSION

Psoriasis is a chronic intractable skin disease that develops through interaction between immune cells and epidermal keratinocytes. The activation of immune cells, such as dendritic cells, Th17 cells, neutrophils, and macrophages and the overexpression of inflammatory cytokines, including TNF-α, IL-23, and IL-17, are known to play an important role in the pathogenesis of psoriasis from large-scale studies that have been conducted with the aim of elucidating the pathology of psoriasis and to investigate treatments.[26–28] Mice are almost exclusively used as animal models for psoriasis in these studies, and various models are presently in use. These models also include an IMQ-induced psoriasis-like mouse model that creates localized psoriasiform skin inflammation via the daily application of IMQ. IMQ stimulates the dendritic cells and increases the production of cytokines, such as TNF-α and IFN-α, which are involved in the pathology of the IMQ-induced psoriasis-like mouse model.[29,30] Reports reveal that psoriasiform skin inflammation is not triggered in IL-17 or IL-23p19 receptor knockout mice, even with the application of IMQ,[24] and it has also been reported that the application of IMQ activates the NF-κB pathway.[31–33] Thus, this IMQ-induced psoriasis-like mouse model is considered useful for dermatitis, and as the pathogenesis of dermatitis also resembles human psoriasis, this model is also useful as an animal model of human psoriasis.

Ghrelin is an endogenous hormone that is mainly produced by X/A-like cells in the stomach, which acts as an endogenous ligand for GHS-R.[6,34] Ghrelin has various physiological effects, including appetite promotion, regulation of energy metabolism, and enhancement of the secretion of growth hormone (GH) and insulin-like growth factor-1 (IGF-I).[7] Ghrelin also has an anti-inflammatory effect, which has been reported in various inflammatory disease models.[8–13] Recently, clinical studies have been conducted on the use of ghrelin for the treatment of heart failure[14] and chronic respiratory failure,[15,16] and hexarelin,[35] a chemically stable synthetic agonist of GHS-R, is being studied in mice. Thus, ghrelin is presumed to be a novel drug candidate for various chronic inflammatory diseases.

The detailed mechanism through which ghrelin exerts its anti-inflammatory effect remains unclear; however, it is presumed that it directly inhibits the immune function via the target cells (immune cells in particular) of GHS-R[21] and regulates the immune cell function by inhibiting overactivity of the sympathetic nervous system via the vagus nerve.[12,36] GHS-Rs are widely expressed in the body, including the gastrointestinal tract, pancreas, nerve tissue, and lymphatic tissue, and their expression in immune cells has been confirmed in T cells, B cells, dendritic cells, monocytes, and neutrophils.[17–19] Ghrelin's GHS-R-mediated anti-inflammatory mechanism is presumed to inhibit the function of NF-κB, a nuclear transcription factor that plays a pivotal role in the immune response.[21,37,38] NF-κB is activated by cytokines such as TNF-α and is known to cause the overexpression and release of various inflammatory mediators.[21,39] Moreover, it is involved in numerous physiological phenomena,

including acute and chronic inflammatory responses, cell proliferation, and apoptosis, and plays a pivotal role in the pathology of psoriasis.[40] In particular, it is presumed that the mechanism of ghrelin's GHS-R-mediated anti-inflammatory action inhibits the signal transduction to NF-κB by TNF-α inside the cytoplasm and nucleus, after ghrelin binds to GHS-R.[20,21] Reports have revealed that the mechanism of ghrelin's anti-inflammatory action involves the inhibition of the secretion of Th17 cell-derived chemokines and cytokines[22] and the inhibition of Th17 cell inflammation by Treg cells.[23] Thus, ghrelin may have an inhibitory effect on the pathogenesis of psoriasis and may control the disease.

In the present study, we focused on the possibility of controlling psoriasis by the administration of ghrelin, which has an anti-inflammatory effect. We hypothesized that the onset of psoriasiform skin inflammation in an IMQ-induced psoriasis-like mouse model could be inhibited by the administration of ghrelin, and we attempted to prove this hypothesis by examining the effect of ghrelin on psoriasis.

In this study, no significant differences were observed in the gross score (Figure 1), epidermal thickness score (Figure 4B), and pathological score (Figure 4B) between PBS and low-dose ghrelin groups (400 μg/kg group); however, the gross score, epidermal thickness, and pathological scores of the ghrelin 1600 μg/kg group were significantly lower than those of PBS groups. This result suggests that the expression of psoriasiform skin inflammation in the IMQ-induced psoriasis-like mouse model may be inhibited by the anti-inflammatory action of ghrelin.

In the previous report, intraperitoneal administration of ghrelin reduced psoriasiform skin inflammation in the induced psoriasis-like mouse model.[20] The aforementioned results have implied that topical administration of ghrelin is also effective in inhibiting psoriasiform skin inflammation in the induced psoriasis-like mouse model, the pathology of which resembles human psoriasis. The mechanism of the anti-inflammatory action by the intraperitoneal administration of ghrelin is supposed to be based on the suppression of NF-κB route of immune cells such as macrophage and the induction of inflammatory cytokine.[20] However, because local administration of ghrelin was effective to psoriasis-like inflammatory skin in mice in this study, other anti-inflammatory mechanisms different from intraperitoneal administration may exist. Recently, CD8-positive T cells in the epidermis and Th17 cells in the dermis produce IL-17 in the lesions of psoriasis, act on epidermal keratinocytes to induce various cytokines, chemokines, and antimicrobial peptides, and are implicated in the formation of psoriasis-like eruptions such as epidermal hyperplasia.[41–43] In past reports, ghrelin has been shown to exhibit anti-inflammatory functions against T cells in vitro.[22,23,44,45] The expression of GHS-R has also been confirmed in T cells and dendritic cells.[17–19] Topical administration of ghrelin may attenuate psoriasis-like skin inflammation by locally suppressing inflammation induced by T cells and dendritic cells such as Th17 cells present in the affected skin and secretion of cytokines such as IL-17. This suggests that ghrelin may be a future treatment option for patients with psoriasis. Low molecular weight compounds, such as methotrexate and cyclosporine, which have been used to date as systemic treatments for psoriasis, are effective for treating psoriasis rash, but their mechanisms of action are diverse. Hence, serious consideration of the hepatotoxicity, nephrotoxicity, and drug interactions associated with these drugs is required. Biologic agents, which have recently been established as a new treatment option for severe psoriasis, exert their therapeutic effect by specifically binding to the target molecules; however, it is essential to be aware of infection and malignant tumors while using these drugs. In contrast, ghrelin is originally an endogenous hormone; hence, it is expected to have few adverse drug reactions. Thus, the future development of ghrelin preparations as a novel drug for psoriasis with an anti-inflammatory action and few adverse reactions is warranted. The clinical validation of the rash-improving effect of ghrelin in patients with psoriasis should be immediately addressed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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