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Original Article

Hepcidin promotes oral ulcer healing via amphiregulin production in rats

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KEYWORDS
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Abstract *Background/purpose:* Hepcidin is an antimicrobial peptide that regulates iron metabolism. Recently, hepcidin was reported to promote wound healing. However, the mechanism underlying hepcidin signaling in the oral mucosa remains unclear. In the present study, we examined the mechanism by which hepcidin accelerates healing in a rat model of oral ulcerative mucositis.
Materials and methods: In male Wistar rats, 50 % acetic acid was applied to the labial fornix region of the inferior incisors to create a model of oral ulcerative mucositis. The ulcerative mucositis severity was evaluated using an oral mucositis score. Hepcidin expression, phosphorylation of the hepcidin receptor ferroportin and the levels of the epidermal growth factor amphiregulin were assessed using immunohistochemistry and western blotting. Hepcidin was applied to the oral mucosal region of rats with exacerbated oral ulcerative mucositis, which developed following acetic acid treatment after extraction of the submandibular and sublingual glands.
Results: Two and five days after acetic acid treatment, hepcidin levels increased in the ulcerated region and saliva. Ferroportin in the ulcerated region was phosphorylated 2 days after acetic acid treatment. The expression and amount of amphiregulin in the ulcerated region increased on days 2 and 5. In exacerbated oral ulcerative mucositis rats, the oral ulcerative mucositis healing period was prolonged, and hepcidin and amphiregulin levels in the ulcerated region decreased. Daily hepcidin application to the ulcerated region shortened the healing period in exacerbated oral ulcerative mucositis rats.
Conclusion: Oral ulcerative mucositis-induced increase in hepcidin promotes healing through the amphiregulin production of via ferroportin activation.

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Introduction

Oral ulcerative mucositis (OUM) is a painful disease of the intraoral region and is sometimes caused by injury to the oral mucosa, radiation, or immune deficiency due to anticancer drugs. OUM-induced pain sometimes prevents patients from eating and speaking, therefore acceleration of healing can be effective for alleviating pain. Oral mucosal injury results in the destruction of the oral epithelium, swelling, and infiltration of inflammatory cells including leukocytes and macrophages into the dermis.[1–3] Subsequently, fibroblasts and vascular epithelial cells migrate across the ulcerated region, and various growth factors, including epidermal growth factor (EGF) and cytokines, such as tumor necrosis factor-alpha, transforming growth factor beta, and interleukin (IL)-1, are produced for re-epithelialization.[2]

Hepcidin is an iron-regulating hormone that is mainly produced by the liver in response to high iron levels, infections, or inflammation.[4,5] Hepcidin binds the iron exporter ferroportin (FPN) expressed in macrophages, endothelial cells, and neurons and induces FPN internalization and degradation, leading to intracellular iron retention.[6] Hepcidin-FPN signaling has been reported to be involved in wound healing.[4,5,7] Dendritic cell-derived hepcidin inhibits bacterial growth by FPN degradation in the intestine due to reduction of iron supply, facilitating mucosal healing.[4] Moreover, hepcidin-induced FPN internalization promotes production of the EGF amphiregulin, which is a key factor in tissue regeneration.[7] Recently, we reported that *hamp* and hepcidin levels increased in the oral mucosa and trigeminal ganglion of OUM model rats.[8] Therefore, hepcidin may contribute to OUM healing.

Cancer therapies such as radiotherapy and chemotherapy, Sjogren syndrome, and xerostomic medication have sometimes caused a decrease in salivary flow and induce alterations in saliva composition.[9–11] This hyposalivation can delay oral wound healing. In a previous study, the extraction of salivary glands exacerbated OUM severity and prolonged healing.[12] In the present study, we examined whether acetic acid (AA)-induced OUM increased hepcidin and amphiregulin levels in the oral mucosa. Second, we also evaluated the effect of hepcidin on OUM healing, the change in OUM severity following hepcidin application to the ulcerated region in the exacerbated OUM group with prolonged healing periods.

Materials and methods

Animals

Male Wistar rats (n = 176) were purchased from Japan SLC (Hamamatsu, Japan) and housed in clear cages (two rats per cage) in at 21–23 °C and 40–60 % humidity under a 12-h light/dark cycle with food and water provided *ad libitum*. All experiments complied with the ARRIVE guidelines and were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All the experiments were approved by the Animal Experimentation Committee of Nihon University (AP21DEN001 and AP24DEN010). Rats were randomly chosen for each experiment. All observations were performed in a manner blinded to the experimental conditions. Details of animal grouping are shown in Table 1.

Oral ulcerative mucositis model establishment

Filter paper (9 mm^2, Whatman, Maidstone, UK) soaked in 50 % AA was applied to the labial fornix region of the inferior incisors in rats for 30 s under deep anesthesia with intraperitoneal administration of butorphanol (2.5 mg/kg; Meiji Seika Pharmaceutical, Tokyo, Japan), medetomidine (0.375 mg/kg; Xenoac, Koriyama, Japan), and midazolam (2.0 mg/kg; Sand, Tokyo, Japan).

To exacerbate OUM in rats, the salivary glands were removed 2 weeks before OUM induction.[12] Briefly, under deep anesthesia as described above, the bilateral submandibular and sublingual glands were exposed, ducts and blood vessels were ligated, and the glands were extracted (2EXT). Subsequently, the labial fornix region of the inferior incisors was treated with AA for 30 s (2EXT-OUM). In some rats, the salivary glands were exposed without ligation of the salivary ducts or blood vessels, after which OUM inducted (Sham-OUM).

Table 1 Animal grouping.

Fig.	Experiments	Naive	OUM	Sham -OUM	2EXT -OUM	Total (n)
Figs. 1-3	HE, IHC	6	12			18
Fig. 1	Scoring					11
	CFUs	5	21			26
Fig. 2	ELISA	7	12			19
	WB (IP)	5	5			10
Fig. 3	WB	8	16			24
Fig. 4	Scoring			11	11	22
	IHC			6	6	12
Fig. 5	Scoring				17	17
	CFUs			5	12	17
						176

OUM, oral ulcerative mucositis; Sham-OUM, sham operation and OUM induction; 2EXT-OUM, extraction of the submandibular and sublingual glands and OUM induction; HE, hematoxylin and eosin; IHC, immunohistochemistry; CFUs, colony forming units; ELISA, enzyme-linked immunosorbent assay; WB, western blotting; IP, immunoprecipitation.

Oral ulcerative mucositis severity assessment

To assess OUM severity, a visual score for oral mucositis was used: Score 0, normal; Score 0.5, possible presence of redness; Score 1, slight but definite redness; Score 2, severe redness; Score 3, focal pseudomembrane without a break in the epithelium; Score 4, broad pseudomembrane with a break in the epithelium within the AA-treated mucosal area; Score 5, virtual loss of epithelial and keratinized layers over the AA-treated mucosal area; and Score 6, severe swelling of the lower lip with an OUM score of 5.[3,13]

In some 2EXT-OUM rats, a cotton ball soaked with rat hepcidin (1 μg/10 μL/rat, 4467-v, Peptide institute, Osaka, Japan) or saline were applied daily to the ulcerated region for 5 min from day 0 to day 10 after AA treatment under anesthesia with 2 % isoflurane inhalation. Daily application was performed after the assessment of the OUM severity.

Histology and immunohistochemistry

Details of the antibodies used in the present study are reported in Table 2. Under deep anesthesia, the rats were perfused with saline and 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and the lower lip including the oral mucosa and mental skin was removed. After post-fixation, the tissues were subsequently embedded in paraffin and sliced into 14 μm-thick sagittal sections and mounted onto MAS coated glass (Matsunami, Tokyo, Japan). The paraffin sections were incubated with primary antibodies overnight at 4 °C, followed by incubation with secondary antibodies for 2 h at room temperature (RT). Immunofluorescence was observed under a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan). The mean number of the hepcidin- or amphiregulin-immunoreactive cells in 3 sections of the oral mucosa per rat were calculated. Some sections were stained with hematoxylin and eosin.

Immunoprecipitation and western blotting

Under deep anesthesia mentioned above, the rats were perfused with saline and the lower lip including the oral mucosa and mental skin was removed. The tissues were homogenized in RIPA buffer (L1A5274, Nacalai Tesque, Kyoto, Japan) with a protease inhibitor (1:100, 1807736A, Takara, Otsu, Japan). Following centrifugation, the supernatants were collected, and concentration of total protein were measured using BCA protein assay kit (Takara). Proteins (20 μg) were mixed with Laemmli buffer supplemented with 2-mercaptoethanol, and denatured for 5 min at 95 °C. For immunoprecipitation, the tissue was homogenized in lysis buffer (10 mm Tris—HCl, pH 7.4; 150 mM NaCl; 1 % Triton X-100 and 0.5 % NP-40) with a protease inhibitor (1:100, Takara) and a phosphatase inhibitor (1:100, Cell signaling, Danvers, MA, USA). The FPN antibody was reacted with Dynabeads Protein G (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The immunoprecipitated samples were mixed with Laemmli buffer and denatured for 5 min at 95 °C, followed by mixed with 2-mercaptoethanol.

Samples were electrophoresed using a 10 % Mini-PROTEAN TGX Precast Gel (Bio-Rad, Hercules, CA, USA) and transferred onto polyvinylidene difluoride membrane (Trans-Blot Turbo Transfer Pack, Bio-Rad). Membranes were blocked with TBST containing 5 % Blocking-One (Nacalai Tesque) for 1 h at RT and then incubated overnight at 4 °C with primary antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibodies 2 h at RT. The bands were visualized using an image analyzer (Amersham Image Quant 800, Cytiva, Tokyo, Japan) after reaction in Western Lightning ELC Pro (PerkinElmer, Waltham, MA, USA) or Immobilon (Millipore, Burlington, MA, USA). The band intensity was quantified using ImageJ and normalized to FPN or GAPDH expression.

Table 2 Antibody table. Details of sources and concentrations of antibodies used for immunohistochemistry and western blotting in this study.

Antibody	Species	Dilution	Company (Catalog #)
Anti-hepcidin	Rabbit	1:400 (IHC)	Abcam (AB30760)
Anti-amphiregulin	Mouse	1:500 (IHC, WB)	Santa Cruz (sc-74540)
Anti-FPN	Rabbit	1:1000 (IHC) 1:500 (WB)	Novus biologicals (NBP1-21502)
Anti-Iba1	Goat	1:1000 (IHC)	Abcam (ab5076)
Anti-phosphorylated tyrosine	Mouse	1:100 (WB)	Cell signaling (9411S)
Anti-GAPDH	Mouse	1:1000 (WB)	Santa Cruz (sc-47724)
Anti-rabbit IgG (AF 488)	Donkey	1:1000 (IHC)	Abcam (A-11008)
Anti-goat IgG (AF 568)	Donkey	1:1000 (IHC)	Abcam (A-11057)
Anti-mouse IgG (AF 568)	Goat	1:1000 (IHC)	Abcam (A-11004)
Anti-rabbit IgG, HRP	Donkey	1:2000 (WB)	Cytiva (NA934V)
Anti-mouse IgG, HRP	Sheep	1:2000 (WB)	Cytiva (NA931V)

IHC, immunohistochemistry; WB, western blotting; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; AF, Alexa Fluor; HRP, horseradish peroxidase.

Quantification of hepcidin

Under the deep anesthesia, the trachea of the rats was cannulated for secure breathing, and pilocarpine (4 mmol/kg; Kanto Chemical, Tokyo, Japan) was intraperitoneally administered. Thirty minutes after administration, the saliva was collected for 20 min using a pipette. The saliva samples were centrifuged and the total protein concentration of the supernatants was measured using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The hepcidin concentration in the supernatant was measured using a Rat Hepcidin ELISA kit (MBS017183; MyBioSource, San Diego, CA, USA).

Quantification of bacterial counts

The oral mucosal region was extracted from naive, OUM (Day 2–5), Sham-OUM (Day 5) and 2EXT-OUM (Day 5) rats under anesthesia as described previously. The mucosal tissue was incised with a surgical scalpel and placed in a pre-weighed 1.5-mL plastic tube containing 500 μL of sterilized PBS. The tube was then subjected to ultrasonication for 30 s to leach out oral bacteria. An anaerobic incubation process was conducted in an airtight container with AnaeroPack-Anaero, an O_2-absorbing and CO_2-generating agent (Mitsubishi Gas Chemical, Saga, Japan), followed by overnight incubation at 37 °C. Colony-forming units (CFUs) of duplicate bacterial culture plates were manually counted. The number of CFUs was presented as the number per wet tissue weight.

Statistical analysis

Data are presented as the mean ± standard error of the mean or as median, and interquartile range (25–75 %). The dotted plots indicate individual sample sizes, where n represents the number of rats tested. Data normality was assessed using the Shapiro–Wilk test. The Mann–Whitney U test was used to compare the two groups as a nonparametric procedure for OUM score analysis. An unpaired *t*-test was used to compare the two groups as a parametric procedure for immunohistochemical analysis. Dunnett's post hoc tests and Kruskal–Wallis tests were performed following one-way analysis of variance (ANOVA) to analyze the immunohistochemistry and western blotting or CFU analysis, respectively. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the GraphPad Prism 8 software package (GraphPad Software, San Diego, CA, USA).

Results

Characteristics of oral ulcerative mucositis

Ulceration was observed in the AA-treated mucosal area on day 2, which gradually healed by day 5 and

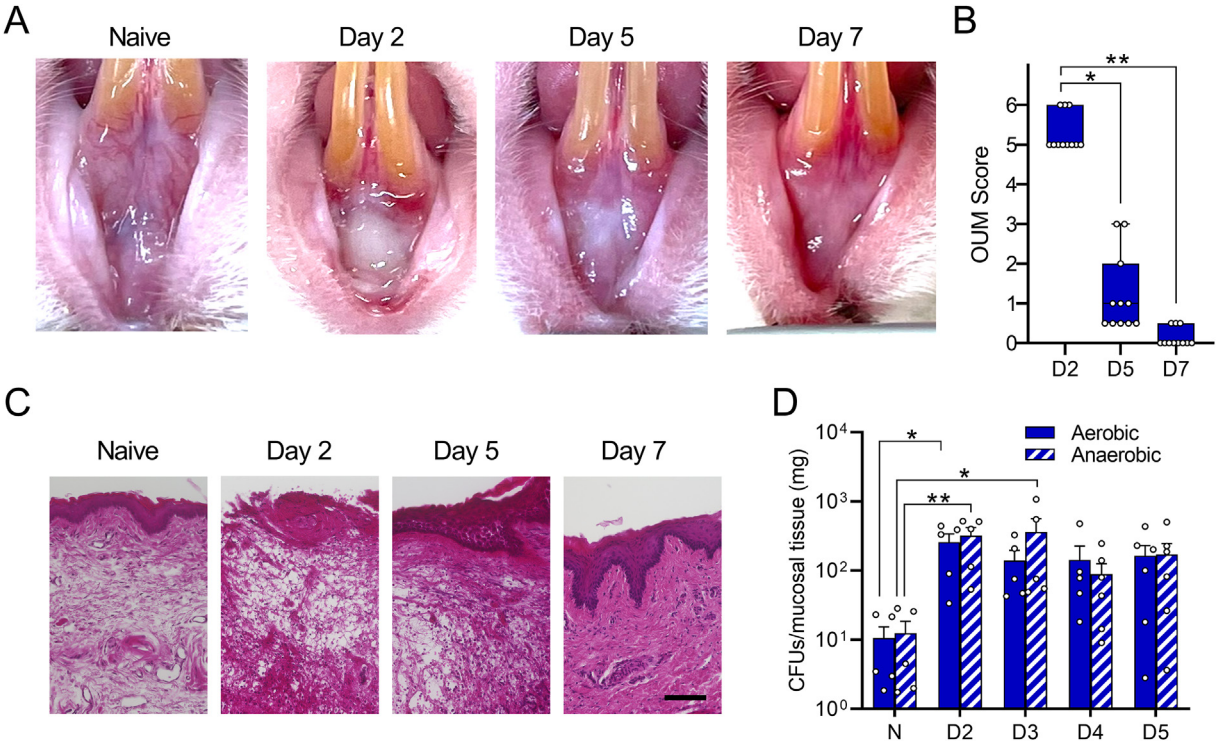

Figure 1 Characteristics of OUM in rats. (A) Representative images of mucosa before acetic acid (AA) treatment (Naive) and on days 2, 5 and 7 after AA treatment. (B) Changes in OUM scores (n = 11 in each group). *$P < 0.05$, **$P < 0.01$. Data was indicated by median and interquartile range (25–75 %). (C) Mucosal region histology in Naive and OUM rats. Scale bar, 100 μm. (D) CFUs in oral mucosal tissue. *$P < 0.05$, **$P < 0.01$. Error bars indicate the SEM. OUM, oral ulcerative mucositis; CFUs, colony forming units; SEM, standard error of the mean; N, Naive; D2, 2 days after AA treatment; D5, 5 days after AA treatment; D7, 7 days after AA treatment.

disappeared on day 7 (Fig. 1A). The OUM score significantly decreased on days 5 and 7 (Score 1—2) compared to that on day 2 (Score 5—6) (Fig. 1B). The epithelial and keratinized layers were lost in the AA-treated area and some rats showed lower lip swelling on day 2 (Fig. 1C). The number of CFUs under aerobic and anaerobic conditions in the ulcerative region increased on day 2 compared to that in naive rats (Fig. 1D).

Hepcidin production in oral ulcerative mucositis rats

Hepcidin was observed in the OUM region on days 2 and 5 after AA treatment (Fig. 2A). The number of hepcidin-immunoreactive cells significantly increased on days 2 and 5 compared to that of naive rats (Fig. 2B). Since hepcidin has been detected in the saliva of humans and rats,[8,14] changes in hepcidin levels in the saliva were examined in OUM rats. The hepcidin levels in pilocarpine-induced saliva were significantly higher in OUM rats than in naive rats (Fig. 2C). FPN, the target protein for hepcidin binding, was expressed in macrophages in the oral mucosa (Fig. 2D). The result was consistent with previous reports that FPN is expressed in macrophages.[6,15] Next, to determine whether FPN was phosphorylated in the ulcerated region in OUM rats, proteins from the OUM region were immunoprecipitated with an anti-FPN antibody and phosphorylated FPN was examined using an anti-p-tyrosine (p-tyr) antibody. The relative amount of p-tyr/FPN in the oral mucosa was significantly increased in OUM rats compared with naive rats (Fig. 2E).

Amphiregulin expression in the ulcerated region

Amphiregulin was observed in the oral mucosa at 2 and 5 days after AA treatment (Fig. 3A). The number of amphiregulin-immunoreactive cells significantly increased on days 2 and 5 compared to that of naive cells (Fig. 3B). The amount of amphiregulin in the ulcerated region increased on day 2 (Fig. 3C).

Acceleration of exacerbated oral ulcerative mucositis healing

In a previous study, hyposalivation prolonged the AA-induced OUM healing period.[12] Therefore, we investigated the effect of hepcidin on ulcer healing in rats with exacerbated OUM (2EXT-OUM). Ulceration was observed on days 2, 5, and 7 in the 2EXT-OUM rats and on day 2 in the sham-OUM rats (Fig. 4A). OUM severity in 2EXT-OUM rats was significantly enhanced from days 2—7 compared to that in Sham-OUM rats (Fig. 4B). The number of hepcidin-immunoreactive cells in the ulcerated region of 2EXT-OUM rats was significantly decreased compared to that in Sham-OUM rats (Fig. 4CD). Consistently, the number of amphiregulin-immunoreactive cells in the ulcerated region in 2EXT-OUM rats was lower than that in Sham-OUM rats (Fig. 4CE). Additionally, compared to vehicle application, hepcidin application significantly reduced the severity of OUM on days 5 and 7 in 2EXT-OUM rats (Fig. 5A and B). On day 5 after AA treatment, the CFUs under aerobic

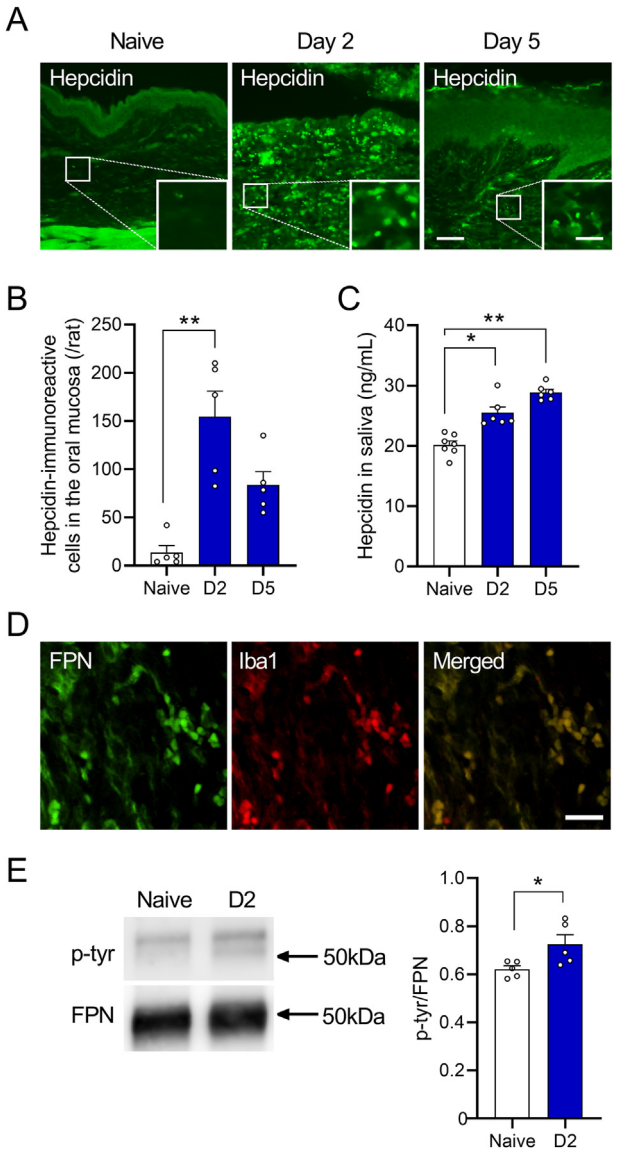

Figure 2 Hepcidin production in OUM model rats. $*P < 0.05$, $**P < 0.01$. Error bars indicate the SEM. (A) Expression of hepcidin in the oral mucosa in Naive, day 2 and day 5 after acetic acid (AA) treatment in OUM rats. Inset in each shows the magnified image. Scale bar, 50 μm. Scale bar in inset, 15 μm. (B) Mean number of hepcidin-immunoreactive cells in the oral mucosa (n = 5 in each group). (C) Hepcidin concentration in saliva (n = 6—7 in each group). (D) Expression of FPN in the oral mucosa on day 2 after AA treatment. FPN (green), Iba1 (red) and FPN and Iba1-immunoreactivity (Merged, yellow). Scale bar, 20 μm. (E) FPN phosphorylation in the oral mucosa of Naive and OUM rats on day 2 after AA treatment. The bands represent FPN and p-tyr of FPN in the oral mucosa following immunoprecipitation with anti-FPN antibody. Relative amount of p-tyr/FPN in the oral mucosa (n = 5 in each group). SEM, standard error of the mean; OUM, oral ulcerative mucositis; FPN, ferroportin; Iba1, ionized calcium-binding adapter molecule 1; p-tyr, phosphorylated-tyrosine; D2, 2 days after AA treatment; D5, 5 days after AA treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

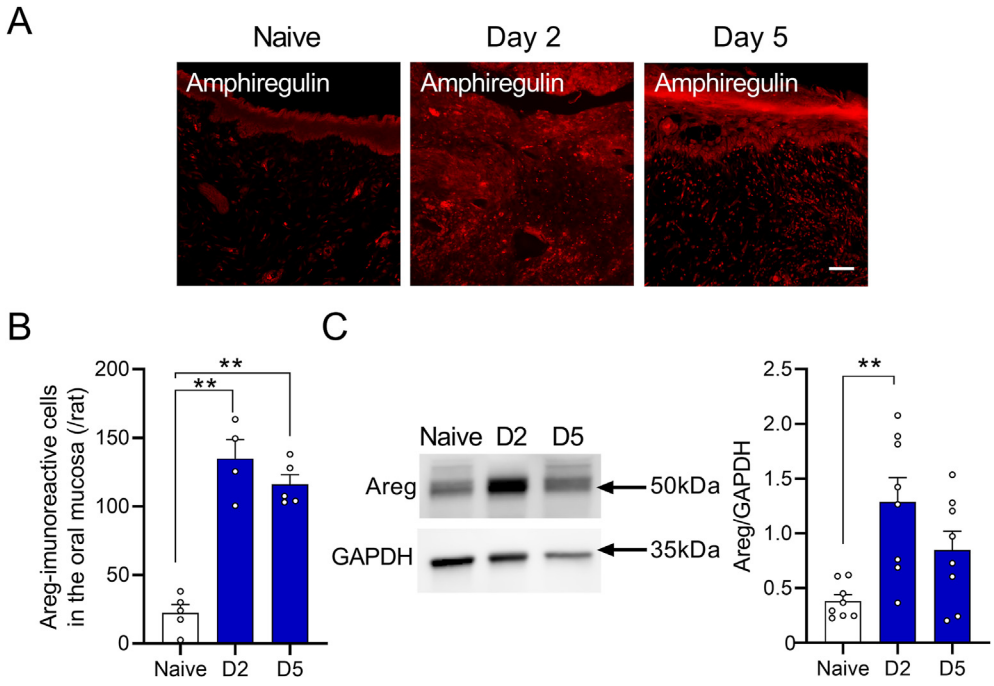

Figure 3 Areg expression in the ulcerated region. $^{**}P < 0.01$. Error bars indicate the SEM. (A) Areg-immunoreactive cell expression in Naive, day 2 and day 5 after acetic acid (AA) treatment in OUM rats. Scale bar, 50 μm. (B) Mean number of Areg-immunoreactive cells in the oral mucosa (n = 4–5 in each group). (C) Relative amount of Areg/GAPDH in the oral mucosa on Naive, D2 and D5 in OUM rats (n = 8 in each group). SEM, standard error of the mean; Areg, amphiregulin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; D2, 2 days after AA treatment; D5, 5 days after AA treatment.

conditions in the ulcerated region of 2EXT-OUM rats were significantly higher compared with that in Sham-OUM rats (Fig. 5C). The CFUs in the ulcerated region of 2EXT-OUM rats were not changed by hepcidin application (Fig. 5C).

Discussion

The present study demonstrated that hepcidin levels increased in the ulcerated region. OUM also accelerated FPN phosphorylation and amphiregulin production in the oral mucosa. Furthermore, daily hepcidin application to the ulcerated region shortened the healing period in rats with exacerbated OUM induced by AA treatment after salivary gland extraction. These results suggest that hepcidin is involved in OUM healing via FPN phosphorylation and amphiregulin production.

Hepcidin is produced mainly in hepatocytes to regulate iron levels, but is also produced in macrophages, and the kidney, brain, and pancreas in human and rodents.[15] In a previous study, hepcidin mRNA *Hamp* was detected in the oral mucosa and the trigeminal ganglion of OUM rats.[8] In the present study, the number of hepcidin-immunoreactive cells increased in the ulcerated region on day 2 and decreased on day 5 after AA treatment. An increase in the levels of the inflammatory cytokine IL-6 upregulates hepcidin expression via the Janus kinase 2/signal transducer and activator of transcription 3 pathway.[16] Since IL-6 protein level was significantly increased in the OUM region compared to healthy mucosa,[3] the OUM-induced hepcidin increase was likely caused by enhancement of IL-6 levels signaling in the oral mucosa. Furthermore, hepcidin levels in pilocarpine-induced saliva increased in OUM rats. Hepcidin in saliva is probably derived from the salivary glands because hepcidin and the mRNA *Hamp* was detected in the salivary glands.[8,17] As OUM increased the number of CFUs in the ulcerated region, hepcidin production in the saliva was possibly enhanced for antimicrobial purposes.

FPN is an iron exporter expressed in macrophages, enterocytes, and hepatocytes.[18] Our results demonstrate that FPN is co-expressed with the macrophage marker Iba1 in the oral mucosa. Phosphorylation of the tyrosine residues of FPN has been reported in response to hepcidin binding at the plasma membrane, which subsequently induces FPN internalization.[19] In the present study, the level of phosphorylated FPN in the ulcerated region was higher than that in the healthy mucosa, suggesting that OUM induced an increase in hepcidin binding to FPN in macrophages infiltrated by OUM, and FPN was internalized and degraded. Although the phosphorylation of tyrosine residues is not always required for FPN internalization,[20] hepcidin-induced FPN phosphorylation is likely to be involved in amphiregulin production.

OUM healing can be delayed by a reduction in salivary flow and alteration in saliva composition, such as reduction of growth factors and antimicrobial peptides, which may be caused by Sjogren syndrome, xerostomic medications, and radiation exposure.[9–11] In the present study, OUM severity was enhanced and the OUM healing period was prolonged in 2EXT-OUM rats compared with Sham-OUM rats, consistent with a previous report.[12] In addition, hepcidin and amphiregulin levels were significantly lower

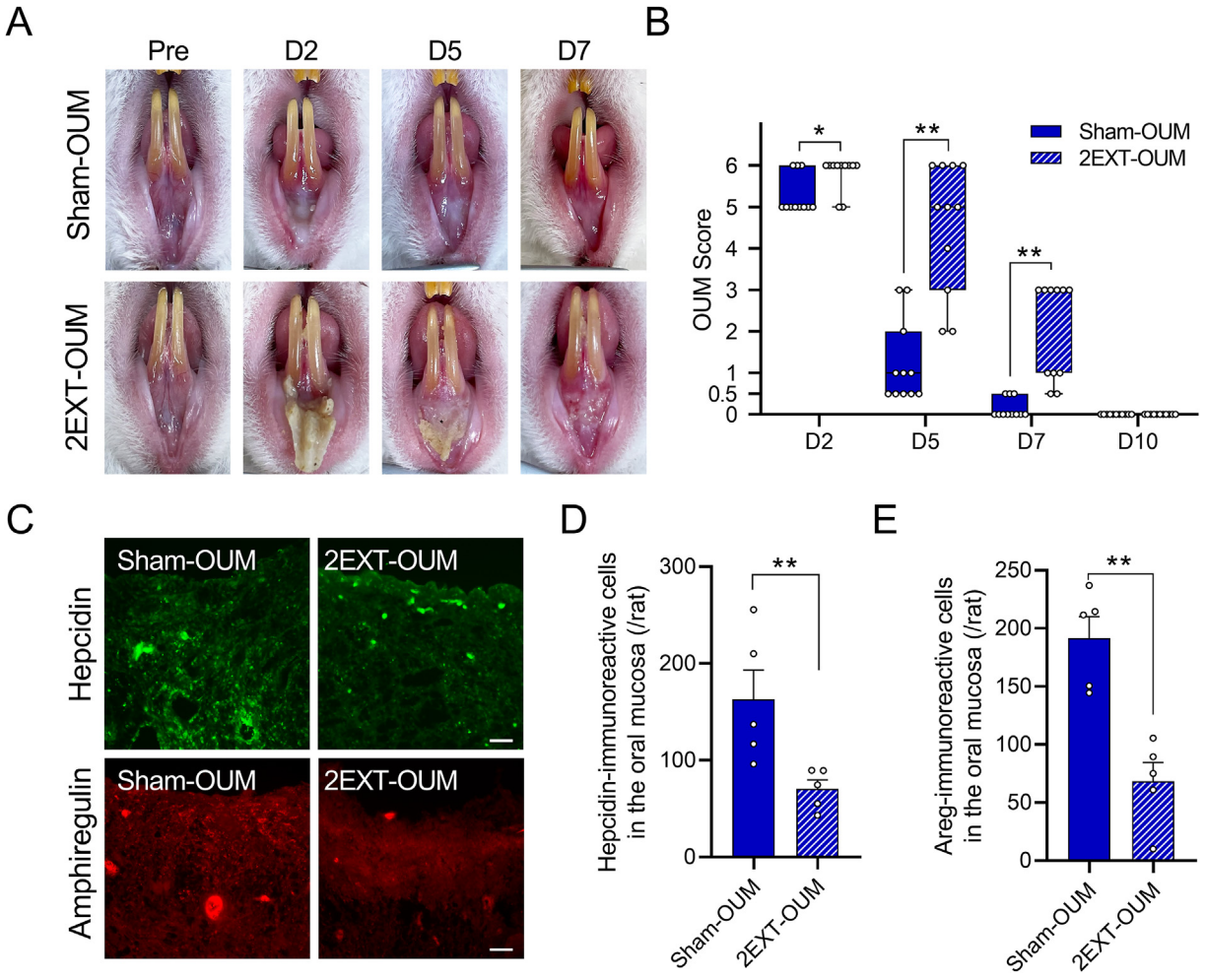

Figure 4 OUM healing in exacerbated OUM rats. (A) Daily oral mucosal changes in Sham-OUM and 2EXT-OUM rats. (B) Changes in OUM scores in Sham-OUM and 2EXT-OUM rats (n = 11 in each group). *$P < 0.05$, **$P < 0.01$. Data was indicated by median and interquartile range (25—75 %). (C) Expression of hepcidin (green) and Areg (red) in the oral mucosa on day 2 after acetic acid (AA) treatment in Sham-OUM and 2EXT-OUM rats. Mean number of (D) hepcidin-immunoreactive cells and (E) Areg-immunoreactive cells in the oral mucosa (n = 5 in each group). *$P < 0.05$, **$P < 0.01$. Error bars indicate the SEM. Pre, before AA treatment; Sham-OUM, sham operation and OUM induction; 2EXT-OUM, extraction of the submandibular and sublingual glands and OUM induction; Areg, amphiregulin; D2, 2 days after AA treatment; D5, 5 days after AA treatment; D7, 7 days after AA treatment; D10, 10 days after AA treatment; SEM, standard error of the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in OUM-2EXT rats than in sham-OUM rats. Amphiregulin, a member of EGF family, has been expressed in epithelial cells and various immune cells including leukocytes in response to inflammation.[21,22] In a mouse model of acute lung injury, hepcidin-FPN signaling in macrophages increased amphiregulin production, leading to the promotion of tissue repair.[7] Application of hepcidin to the ulcerated region of OUM-2EXT rats accelerated healing. Furthermore, the CFUs in the ulcerated region were not changed after hepcidin application. These results indicate that the accelerated ulcer healing was due to the applied hepcidin binding to FPN in the ulcerated region, which contributing to amphiregulin production, and not to the antimicrobial effect of hepcidin. However, this does not explain the decrease in hepcidin levels in the oral mucosa of OUM-2EXT rats. Since hepcidin has been known to be produced in response to the production of inflammatory factors such as IL-6, exacerbated OUM could induce hepcidin production. This will be investigated in future studies.

In conclusion, elevated hepcidin levels in OUM enhanced FPN phosphorylation in macrophages and likely promoted amphiregulin production. Furthermore, hepcidin application to the OUM region in exacerbated OUM rats accelerated ulcer healing, suggesting that amphiregulin is involved in wound healing via the hepcidin-FPN axis. Recently, hepcidin and FPN were identified as potential targets for the treatment of iron-related disorders such as iron deficiency and microcytic anemia.[23] Several drugs have been evaluated in clinical trials.[23,24] However, high concentrations of plasma hepcidin cause side effects, such as iron deficiency and anemia.[25] Therefore, to facilitate wound healing without adverse effects, local regulation of hepcidin levels is crucial.

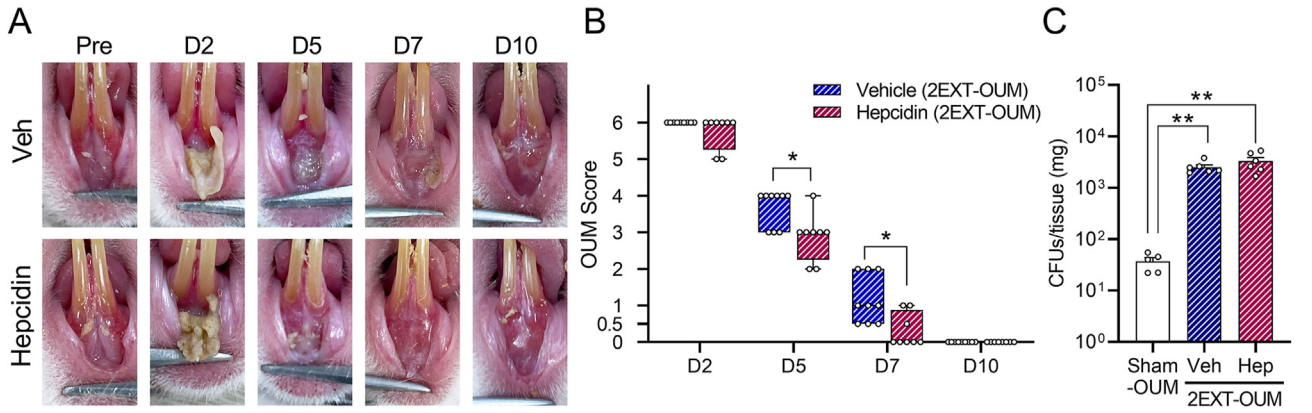

Figure 5 Effect of hepcidin application to the ulcerated region on OUM healing. (A) Typical images of OUM region following Veh or hepcidin application to the oral mucosa in 2EXT-OUM rats. (B) Daily OUM score changes (n = 8—9 in each group). *$P < 0.05$, Data was indicated by median and interquartile range (25—75 %) (C) CFUs in 2EXT-OUM rats on day 5 after acetic acid (AA) treatment. (n = 5—6 in each group). Error bars indicate the SEM. **$P < 0.01$. Pre, before AA treatment; Veh: vehicle, Hep: hepcidin; OUM, oral ulcerative mucositis; Sham-OUM, sham operation and OUM induction; 2EXT-OUM, extraction of the submandibular and sublingual glands and OUM induction; D2, 2 days after AA treatment. D5, 5 days after AA treatment. D7, 7 days after AA treatment; D10, 10 days after AA treatment; CFUs, colony forming units; SEM, standard error of the mean.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

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