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学位授与の要件	医歯薬学総合研究科病態制御科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Malnutrition delayed wound healing after tooth extraction by HMGB1-related prolonged inflammation （栄養失調下での抜歯後創傷治癒遅延に HMGB1 が及ぼす影響）
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学位論文内容の要旨

Objectives: Malnutrition is a frequent and serious condition of multifactorial origin and is associated with adverse consequences. Elderly people in developed countries and children aged below 5 years in low-income and middle-income countries experience this condition. It is generally believed that malnutrition damages the innate or acquired immune function by imposing a high metabolic load on a depleted capacity for homoeostasis and increases the risk of developing non-communicable diseases and long-term chronic inflammation. Malnutrition impairs wound healing and increases the risk of secondary infections. In wound healing processes, inflammation usually occurs due to the localization and elimination of damaged factors, and promotes the healing of damaged tissues. However, it prevents cell proliferation and injury healing when it is not eliminated in time and leads to continuous chronic inflammation.

High mobility group box-1 (HMGB1) is a DNA-binding protein mainly presented in monocytes, especially macrophages, and functions both inside and outside the cells. It is released when there is danger-induced cellular stress, which regulates inflammation and regeneration of extracellular signaling molecules, as one of damage-associated molecular pattern molecules (DAMPs). Extracellular HMGB1 is transformed into an effective activator of pro-inflammatory cytokine such as interleukin-1 (IL-1) β production through receptors for advanced glycation end products (RAGE) stimulation, then inducing inflammatory response and this is an essential response to initiate wound healing. Bone healing is promoted by inflammation induced by HMGB1, and periodontal inflammation and bone resorption were reduced in mice after the administration of anti-HMGB1 neutralizing antibody based on previous studies conducted in our laboratory. However, it remains unclear whether delayed healing of the tooth extraction wound under malnutrition conditions is related to prolonged inflammation. Therefore, we hypothesize that the delayed healing of tooth extraction wounds caused by malnutrition is related to inflammation induced by HMGB1, ultimately delaying wound healing.

Despite the proven consequences of malnutrition, traditional treatment methods have little effect on wound healing. Therefore, the mechanism of long-term chronic inflammation should be emphasized when developing new treatment methods for malnutrition.

Methods: We employed ten-week-old male C57BL/6J mice and randomized to malnourished group fed with 3% casein diet, and control group fed a 25% casein diet. The left first maxillary molar was extracted as the test site, while the right maxillary molar was left intact as the control site. The mandible and gingival tissues were collected 3 and 7 days after the tooth extraction. Malnutrition condition was confirmed by observing weight and total protein, albumin, glucose, and total cholesterol in serum.

The wound tissue was histologically observed and analyzed to confirm the healing condition. RT-qPCR was used to analyze the mRNA levels of several factors in the inflammation and regeneration lineage, such as IL-1β, CCL2, CD44, Nanog, Runx2. Flow cytometry was used to analyze other inflammation and regeneration-related cells such as mesenchymal stem cells (MSC) and M1/M2 Mø. The bioluminescence in vivo of myeloperoxidase (MPO) as a marker of neutrophil infiltration was used to analyze the level of inflammation in tooth extraction wounds by in vivo molecular imaging analysis system (IVIS). The amount of total and extracellular HMGB1 were analyzed by ELISA and adenosine 5-triphosphate (ATP) was analyzed by fluorometric ATP assay.

Results: The reduction in body weight and in total protein, albumin, and glucose levels in serum indicate a successful establishment of a malnutrition animal model. In hematoxylin-eosin stained images, mononuclear cells, probably immune cells, filled the tooth extraction socket in the malnourished group, whereas new bone was detected in the control group on day 7. CD44 and Nanog mRNA tended to be expressed and MSCs population increased in tooth-extracted socket wounds in the control group compared with in the malnourished group on day 7 detected by qRT-PCR. The MPO activity and the mRNA expression of the pro-inflammatory cytokine IL-1β tended to increase on day 3 in tooth-extracted socket wounds in the control group, but returned to baseline on day 7. However, they obviously increased in the tooth-extracted socket wound in the malnourished group on day 7. The total HMGB1 in both control and malnourished groups increased from day 3 to day 7. In particular, the extracellular HMGB1 increased on day 3 but recovered to baseline in the control group on day 7, whereas it remained increase in the malnourished group on day 7. Furthermore, an immunohistochemical analysis and the concentration of HMGB1 in serum also shown the secretion of HMGB1 increased in the malnourished group on day 7. M1 Mø was obviously decreased, whereas M2 Mø was obviously increased by Flow cytometry analysis and the ATP concentration also increased in malnutrition group on day 7.

Conclusions: These results suggest that the significant increase secretion of HMGB1 associated with up-regulation of ATP and promote the production of IL-1β by activating RAGE receptors,

which may interfere inflammation resolution and wound healing. In summary, malnutrition alters HMGB1-related inflammation associated with delayed wound healing after tooth extraction.

論文審査結果の要旨

Malnutrition causes prolonged inflammation, resulting in delayed wound healing. Children in developing country and elderlies even in developed country have risk of this situation. High mobility group box-1 (HMGB1) is one of the damage-associated molecular patterns (DAMPs) that presents in the nuclei of macrophages and is secreted into the extracellular milieu in response to stimuli. It stimulates the production of interleukin-1β (IL-1β) through the receptors for advanced glycation end products (RAGE), inducing an inflammatory response, which is an essential response to initiate wound healing. The hypothesis of this study was that malnutrition may interfere with this cascade, causing abnormal inflammation and ultimately delaying wound healing.

Tooth-extracted mice under malnutrition condition by low-casein diet were used for comparing to normal diet-fed mice. Malnutrition condition was confirmed by observing those weight, total protein, albumin, glucose, and total cholesterol in serum after 14-day feeding. The wound tissue in the tooth-extracted socket after 7 days, was observed by hematoxylin-eosin staining. Several factors in inflammation-regeneration lineage including IL-1β, mesenchymal stem cells and M1/M2 Mø, myeloperoxidase activity, total and extracellular HMGB1, and ATP, were analyzed by using RT-qPCR, flow cytometry, *in vivo* molecular imaging analysis system, ELISA, immunohistochemistry, and fluorometric ATP assay, respectively.

Successful establishment of a malnutrition animal model was confirmed by the reduction in body weight and factors levels in serum. Under the malnutrition condition, delayed wound healing was observed in hematoxylin-eosin stained images. CD44, Nanog mRNA and MSCs population tended to be decreased. The MPO activity and the mRNA expression of the pro-inflammatory cytokine IL-1β tended to increase on day 7. The total HMGB1 in both control and malnourished groups increased from day 3 to day 7. In particular, the extracellular HMGB1 increased on day 3, but recovered to baseline in the control group on day 7, whereas it remained increasing in the malnourished group on day 7. Furthermore, an immunohistochemical analysis and the concentration of HMGB1 in serum also showed that the secretion of HMGB1 increased in the malnourished group on day 7. M1 Mø was obviously decreased, whereas M2 Mø was obviously increased and the ATP concentration also increased on day 7.

Significant increase in secretion of HMGB1 associated with up-regulation of ATP and promotion of IL-1β production by activating RAGE receptors may interfere with resolution of inflammation and wound healing. Therefore, malnutrition could alter HMGB1-related inflammation associated with delayed wound healing after tooth extraction.

These findings are scientifically significant, providing useful knowledge that will promote the advance in oral science. The contents of this study covered the article, “Malnutrition delayed wound healing after tooth extraction by HMGB1-related prolonged inflammation” (DOI: 10.1016/j.intimp.2021.107772) which has been published in the International Immunopharmacology (Volume 96, July 2021) after the international peer-review. Therefore, the thesis defense committee hereby accept this article as a doctoral dissertation in dentistry.