High-mobility group box protein 1 (HMGB1) was initially identified as a nuclear protein implicated in maintaining the nucleosome structure and the regulation of gene transcription. HMGB1 acts as a cytokine released actively by immune cells and passively by necrotic cells or damaged cells under inflammatory or injurious conditions. It is widely accepted that HMGB1 is a danger signal, acting as a link between cellular damage and inflammation, activating the immune system. After released, HMGB1 is capable of activating an inflammatory response, then transferring the injury signal to nearby immune cells. Cells undergoing apoptosis also release HMGB1, but they are immunologically inactive. HMGB1 interacts with several receptors that can be activated by exogenous (TLR-2, TLR-4) and endogenous (RAGE) ligands. Myocardial infarction (MI) is an irreversible necrosis of heart muscle caused by the occlusion of a coronary artery secondary to prolonged lack of oxygen supply, leading to tissue necrosis and defect formation. Increasing evidence exists for the importance of extracellular HMGB1 in the pathophysiology of MI. HMGB1 was upregulated and released by ischemic tissue necrosis in vivo. The prolonged presence of active inflammation can be harmful for the injured heart and eventually results in heart failure. Periodontitis is a chronic inflammatory disease caused by gram-negative anaerobic bacteria that results in bone resorption, destruction of the connective tissue, and loss of teeth. *Porphyromonas gingivalis* (*P.g.*) is a major pathogen in human periodontitis. Recently, there might be a connection between periodontitis and cardiovascular disease (CVD), including MI. Ten to fifteen % of the periodontal patients had been linked to CVD. Patients with periodontitis were at risk for bacteremia from brushing or dental treatment. The periodontal bacteria may invade the peripheral vessels, and some bacteria may infiltrate in the heart. *P.g.* bacteremia could induce MI in mice and it might possibly induce severe MI by repeat *P.g.* infection. Periodontopathic pathogens deteriorated ventricular remodeling after MI and other cardiovascular diseases. We hypothesized that infection with *P.g.* could cause an adverse outcome after MI via HMGB1. Thus, the purpose of this study was to investigate the effect of *P.g.* on HMGB1 expression after MI in mice.

Male C57BL/6J wild type mice were obtained from Japan Clea, Co. Coil-shaped chambers (subcutaneous chamber model) were surgically implanted in the dorsal region of each mouse, used as a biological compartment to inoculate bacteria by injection. *P.g.* (0.1 mL of 10^6 CFUs/mL) or phosphate-buffered saline (PBS) (0.1 mL) was injected into the chambers to induce inflammation once a week for two weeks. Then the mice were subjected to MI induction. Left lateral thoracotomy was performed, and the left anterior descending (LAD) coronary artery was ligated using a nylon suture. The mice were allowed to recover on a warmed surface. The mice were evaluated for up to five days
and fourteen days after MI and then sacrificed to obtain samples. Plasma samples were obtained when the mice were sacrificed on days 5 and 14, and the level of plasma HMGB1 was determined by an enzyme-linked immunosorbent assay (ELISA) with an immunoassay kit. The control levels of HMGB1 on day 0 prior to the MI induction (P.g. inoculation only), and mice with sham operation were also measured. Twelve sections were prepared through the heart on day 5 for immunohistochemistry. The sections were incubated with rabbit polyclonal anti-HMGB1 as the primary reagent (1:1000) at 4 °C for overnight and incubated with biotinylated donkey anti-rabbit antibodies (1:200) as the secondary reagent in room temperature for 1 hour. The sections were developed with an avidin–biotin alkaline phosphatase and observed under a microscope. In all cases, parallel incubations with nonimmune IgGs of the relevant species served as negative control. The immunohistochemical results were determined by counting the positively stained cells in each section under a light microscope. The positive cells were counted in each whole heart sample and compared them among the following myocardial areas: 1) infarcted area (anterior wall), 2) peri-infarcted area (lateral and septal wall), and 3) remote viable area (inferior wall). All data are expressed as the mean ± SEM. All statistical analyses were performed using an unpaired Student's t-test. A value of $P < 0.05$ was considered to be significant.

The results show the plasma level of HMGB1 protein significantly increased in the P.g.-inoculated MI mice group compared to the PBS-injected MI mice on day 5 (27.75 ± 0.89 versus 12.67 ± 0.20, $P < 0.05$), but not on day 14 (24.31 ± 2.23 versus 21.05 ± 1.21). The control serum samples on day 0 prior to the MI induction and mice with sham operation showed low HMGB1 levels. In order to confirm the effect of P.g. infection on HMGB1 expression in MI hearts, we performed immunohistochemistry on day 5. In the PBS-injected MI group, HMGB1 was mainly expressed in cardiomyocytes, immune cells, and vascular endothelial cells. However, HMGB1 was seen broadly in degenerated cardiomyocytes, extracellular fields, immune cells, and vascular endothelial cells in the P.g.-inoculated MI group. A significant increase in the number of HMGB1 positive cells was observed in the P.g.-inoculated MI group compared to the PBS-injected MI group. (6023.33 ± 1406.74 versus 3139.33 ± 940.44, $P < 0.05$). We compared the HMGB1 positive cell numbers among the following myocardial areas: 1) infarcted area (anterior wall), 2) peri-infarcted area (lateral and septal wall), and 3) remote viable area (inferior wall). The HMGB1 positive cells in all areas in the P.g.-inoculated MI group showed significant increase compared to those in the PBS-injected MI group. This study suggests that infection with P.g. after MI enhanced myocardial HMGB1 expression, there is a possible relationship between periodontitis and post-infarction myocardial inflammation through HMGB-1. High serum levels of HMGB1 may cause cardiac inflammation and dysfunction after MI. P.g. elevated the early release of HMGB1 from post-MI damaged cells, necrotic cell and inflammatory cells that were likely to trigger and sustain the initial inflammation. Moreover, the myocardial ischemia and chronic inflammation induced by a periodontal pathogen influenced all areas of the hearts. The direct relationship between P.g. infection and HMGB1 expression is unknown. Because P.g. is recognized via TLRs, its infection can aggravate HMGB1-induced MI. HMGB1 enhances the production of other pro-inflammatory cytokines, which might further promote inflammatory cell adhesion and infiltration into the myocardium and enhance tissue injury. Excessive inflammation post-MI has been shown to impair infarct healing. There are several patents proposed for controlling the production, secretion and neutralization of HMGB1 such as anti-HMGB1 antibodies, anti-TLR-2 antibodies and HMGB-A box as a competitive antagonist of HMGB1. Early HMGB1 increase might influence MI hearts, thus, the therapeutic target phase of HMGB1 on MI with P.g. infection might be early time points than late phases after MI. Future studies are needed to clarify its effects and safety before it is used in clinical settings.
Photos

- Experiment at laboratory of department of periodontology in Tokyo Medical and Dental University
- Presentation of research work at 12th asian pacific society of periodontology meeting in 2017, Seoul.