論文内容の要旨

論文題目　Vibration attenuates a deterioration of deep tissue injury in rats
（ラット深部組織損傷褥瘡における振動の潰瘍化抑制効果）

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Pressure ulcers (PUs) are common complications in hospitals and nursing homes. Severe PUs are more devastating than superficial PUs, as they are associated with infection-related mortality, reduced of quality of life, and high cost burden. In Japan, the proportion of severe PUs is still high, in spite of many attempts by wound care experts, Japanese Society of Pressure Ulcer, and government to improve the treatment of PUs in the clinical setting. Previous study strongly suggested that part of the severe PUs in Japan might be due to deep tissue injury (DTI).

Deep tissue injury is a type of PUs which is recently categorized. By contrast with usual PUs that is conventionally considered to develop from superficial skin and progress toward the deep tissue (top-down direction), DTI formation originates from deep tissue and develops from deep tissue to superficial (bottom-up theory).

Based on the previous study, there were two types of DTI: a healing type that will heal with standard treatment for PUs; and a deteriorating type that rapidly deteriorates to a severe PU despite optimal treatment. Although the prevalence study for DTI in Japan has not been conducted yet, it is strongly suggested that a high proportion of severe PUs in Japan might be due to DTI deterioration. The efforts to reduce the number of the deteriorating type of DTI cases such as prediction and prevention of formation of DTI will be effective for the improvement of the current critical situation in Japan. However, most cases of DTI in which the patients undergo a multi-hour surgery, and protracted unconsciousness due to cerebral infarction or excess drug intake are unavoidable.

Deteriorating type of DTI rapidly progresses into a severe PU although it only appears a bruise or dark tissue in the superficial skin at the initial phase. Therefore, clinicians and researchers developed methods to detect DTI formation, such as by high frequency ultrasound, exudate creatine kinase, and urine myoglobin.

Although the recent progress of the research already used development of technologies to detect the formation of DTI as mentioned above, nevertheless, we do not have any choice to treat the deteriorating type of DTI.

In order to solve and consider the best strategy of the problem of DTI, it is needed to focus on its deterioration. However, up to the present, the study about DTI deterioration is very limited. Moreover, there is no study about the strategy for DTI deterioration. One of the most important strategies for DTI management is the prevention of its deterioration into severe PUs, which can reduce the risk of morbidity of lethal complications and mortality due to infection in patients. To establish a prevention method of DTI deterioration, mechanisms of DTI deterioration should be investigated first. Therefore, the purposes of my study was to elucidate the mechanism involved in DTI deterioration, and then, based on those mechanisms, to establish the prevention a means of preventing DTI deterioration.

Development of a prevention method for DTI deterioration requires elucidation of its mechanisms. Recently, we reported deep-muscle hypoxia in the DTI formation which was made by modified previous PU rat model. In this model, we observed the expanding tissue damage in deep muscle tissue on post-wounding day (PWD) 3, although there was no ulceration. Nuclear localization of HIF-1α was frequently observed and enhanced on PWD 3 compared to PWD 1. This result suggested that hypoxia is a key mechanism for DTI deterioration.

Conceptually, DTI deterioration originates from deep muscle layer and then expands to dermis tissue, therefore DTI deterioration should involve degradation of muscle tissue and dermis collagen matrix. In this study, I focused on cellular apoptosis, and activities of MMP-2 and -9, since they could be induced by hypoxia, and can degenerate muscle and/or dermis tissue. Oxidative stress is also a candidate mechanism of DTI deterioration, because hypoxia can enhance production of reactive oxygen species that can cause apoptosis and MMPs activation. I hypothesized that elevated hypoxia and/or oxidative stress induce cellular apoptosis in the muscle tissue and increased activities of MMP-2 and -9 in dermis layer, resulting in degradation of muscle and dermis in the deteriorating site.

To elucidate my hypothesis for the mechanism of DTI deterioration, an appropriate animal model was required. However, an animal model for DTI deterioration had not been reported. At first, I established the novel DTI deterioration rat by improving the conventional PU rat model. Analysis by finite element method was performed to estimate distribution of shear stress and pressure stress within skin tissue. Based on this analysis, I established the novel rat model for DTI deterioration by the use of round-shaped prominence on metal plate, the soft cushion, and the wide indenter.

To establish a novel rat model for DTI deterioration, I compared the macroscopic wound appearance, and tissue histology by H & E staining at the wound site and surrounding tissue among three groups. They were the deterioration group (metal plate with round-shaped prominence, and soft cushion were used); the prominence group (metal plate with round-shaped prominence was used but without soft cushion); and the flat group (without the prominence and without soft cushion). Flat group represented a conventional PU rat model. Hypoxia and oxidative stress were analyzed using immunohistochemistry for HIF-1α and 8-OHdG, respectively. Apoptotic cells were detected by the TUNEL method; activities of MMP-2 and -9 in dermis layer were measured by gelatin zymography. To clarify the mechanisms of DTI deterioration, I performed immunohistochemistry and gelatin zymography in the DTI deterioration and flat (conventional PU) groups.

In the deterioration group, levels of hypoxia, oxidative stress and activities of MMPs were significantly increased compared with those in the flat group on PWD 7. However, apoptotic cells were rarely observed in both groups. These results suggested that the process of DTI deterioration involved elevation of hypoxia, oxidative stress and activation of MMP-2 and -9, but not apoptosis. Taken together, this study suggested that hypoxia is the responsible mechanism for DTI deterioration, therefore it could be a target for prevention of DTI deterioration.

In the next step, I tried to reduce the hypoxia in the DTI deterioration rat model by the vibration therapy. Simultaneously, this study also could contribute to the elucidation of the causal relationship among hypoxia, oxidative stress and the MMPs activities. Previous studies reported that 30-100 Hz of vibration increased the blood flow. Our research groups also suggested that the application of vibration at lower frequency had the promoting effect on healing of PUs. We revealed that 47-Hz vibration accelerated the healing of stage I PUs, and also reduced the necrotic tissue on the wound surface of stage III and stage IV PUs. However, these previous studies did not expect the effect on deep layer since the 47-Hz vibration was considered too weak. However, it has been known that 60-80% of body component is consisted of water and the low frequency of wave can penetrate into the deep part of body such as low frequency of ultrasound. Therefore, I considered that the effect of low frequency of vibration can also affect the deep layer of subcutaneous tissue.

The rat DTI deterioration model was divided into 2 groups, the vibration and the control group. The conditions of vibration were same as the previous studies, briefly, the frequency was 45 Hz, the amplitude was 600 mV peak to peak, the duration was 15 min/a day. The rat was laid on the rubber foam during application of the vibration. The effects of vibration were analyzed on PWD 3, 7 and 13 by H & E staining. While the

immunohistochemistry for hypoxia (HIF-1α) and oxidative stress (8-OHdG) were analyzed on day 7, and gelatin zymography (activities of MMP-2 and -9) were analyzed on day 3 and 7. The control group underwent the same procedure, except for the application of vibration.

There was no difference in the macroscopical appearance between 2 groups until PWD 5. From PWD 7 to 9, the necrotic tissue rapidly decreased and granulation tissue was increased in the vibration group. Subsequently, the wound area also rapidly decreased from PWD 9 to 13 in vibration group. All of the wounds in vibration group were classified as superficial ulcer throughout the experimental period, while all of the wounds in control group were deteriorated from superficial wounds to deep ulcers. Histological analysis showed that the vibration inhibited the extent of the tissue damage in the dermis and muscle layer and promoted healing of damaged tissue.

Hypoxia was remarkably reduced, but oxidative stress was not different in the vibration group compared with the control group on PWD 7. Gelatin zymography showed significant reduction of activity of MMP-2 and expression of MMP-9 in the vibration group on PWD 7. These results revealed that the elevated activities of MMP-2 and over-expression of MMP-9 under the regulation of severe hypoxia were the main mechanisms of DTI deterioration, and suggested that the increased level of oxidative stress did not contribute to the DTI deterioration. It is suggested that the improvement of the hypoxic condition by vibration resulted in the prevention of DTI deterioration.

This is the first attempt to develop the prevention method for DTI deterioration. Since vibration therapy could reduce DTI deterioration, and is convenient and easy to use by nurses and patients themselves, it is expected that the application of vibration therapy will decrease the prevalence of severe PUs. This study can be recognized as the milestone for the research of DTI deterioration. The further studies to reveal the preventative effects of vibration in the actual patients with DTI as well as the confirmation of the adverse effect of long term application of vibration in animal study are required.