Effect of vibration on skin blood flow in an *in vivo* microcirculatory model

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SUMMARY **The effect of vibration on skin microcirculation was studied to investigate the possibility of clinical use of vibration to prevent and treat pressure ulcers. Vibrations at a vibrational intensity of 600, 800, or 1,000 mVpp with a fixed frequency of 47 Hz were applied horizontally to the ear of male hairless mice (n = 6 for each group) under inhalation anesthesia. The control group (n = 6) received no vibrations. Venular blood flow was measured by an intravital videomicroscope at the baseline and at 0, 5, and 15 min after the application of vibrations. A significant increase was observed in the 600 mVpp group 5 and 15 min after vibration in comparison to the control group (P = 0.002 and P = 0.046, respectively). We also detected increased blood flow in the 800 mVpp group (P = 0.028) and the 1,000 mVpp group (P = 0.012) 5 min after vibration; however, these increases attenuated after 15 min. These results indicate that direct skin vibration at a frequency of 47 Hz improves skin blood flow. The present study gives further support to the role of vibration on a short-term increase in skin blood flow.**

Key Words: Microcirculation, chronic wound, vasodilation, intravital videomicroscope

Introduction

Prevention and treatment of pressure ulcers caused by tissue ischemia is a priority in an aged society (*1*). Many efforts have been made to reduce the incidence and increase the healing rate of these wounds, but some remain intractable due to poor circulation (*2*). This type of wound may not be healed by the use of pressure redistribution devices to reduce the tissue ischemia caused by compression (*3*). The crucial strategy in preventing or treating such pressure ulcers is to actively increase the circulation in the affected regions. Microcirculation of the skin has been widely investigated, and various methods to promote blood flow have been developed, including the use of vasoactive drugs such as prostaglandin (*4*), foot-baths (*5*), and various methods of promoting angiogenesis (*6*). However, these methods are sometimes invasive and time-consuming and can cause side effects, causing difficulties when applying them to vulnerable skin predisposed to ischemic wounds or wounded areas. Thus, the current study focused on vibration, which has been reported to affect circulation of skin in a non-invasive manner (*7*). Although previous studies have demonstrated the effect of vibration on blood flow, they evaluated the blood flow indirectly using near-infrared spectroscopy (*8*), plethysmography (*9,10*), thermography (*11*), or histological examination (*12*). The methods of blood flow evaluation employed in those studies are based on indirect quantification such as using the change in the hemoglobin concentration *via* near-infrared spectroscopy, or the change in the strain derived from the limb volume change caused by vibration. Additionally, the effect of vibration on

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the blood flow cannot be estimated using histological examination. These methods cannot determine to what extent vibration has an impact on microcirculation, so the current authors thus employed a microcirculatory model using the ear of the hairless mouse (*13*). This model uses an intravital microscope to quantitatively visualize the skin microcirculation (*14*). The current study employed a vibrational frequency of 47 Hz since the most effective frequency had been determined in a previous experimental study (*15*). The purpose of the study was to explore the effect of vibrations of varied intensity on skin microcirculation in order to investigate the possibility of clinical use of vibration to prevent and treat pressure ulcers.

Materials and Methods

Experimental animals

Twelve male homozygous (hr/hr) hairless mice (Saitama Experimental Animals Supply Co. Ltd, Japan) were used. The animals were given a standard diet ad libitum. The experimental procedures conformed to the Guidelines for Animal Studies at Saitama Medical University.

Vibration profiles and experimental set-up

To apply vibrations, a vibrator consisting of a box-shaped vibration source including an electric motor and a virgulate vibration exciter 5 mm in diameter was developed (Figure 1). With this vibrator, changes in the intensity of vibration were achieved by changing the voltage of the power supply. The vibrator was mounted on a triaxially mobile manipulator.

Experimental procedures

Before vibrations were applied, anesthesia was induced and maintained by continuous inhalation (isoflurane; 1.5-2.0%, air; 200 mL/min). The ears of hairless mice were prepared as described in previous reports (*13,14*). Two distal regions of the auricular margin were sewn with nylon thread to spread and fix the ear on the light source in order to measure blood flow. A thermistor probe was sandwiched between the ear and the light source to monitor the local temperature (Figure 2).

In the experimental group (n = 6), the virgulate vibration exciter was placed in contact with the proximal base of the ear, vertical to the auricular axis, to vibrate the whole ear for 10 min. Intensities of 600 mVpp, 800 mVpp, and 1,000 mVpp were used with a fixed frequency of 47 Hz based on a preliminary study (*15*). Control animals (n = 6) were submitted to the same exact conditions, except for energizing the vibrator. The exciter was placed in contact with the part of the ear as in the experimental group. Use of the same mouse for experiments was avoided for 1 week. All experiments were conducted in a thermoneutral laboratory with a room temperature of 24ºC.

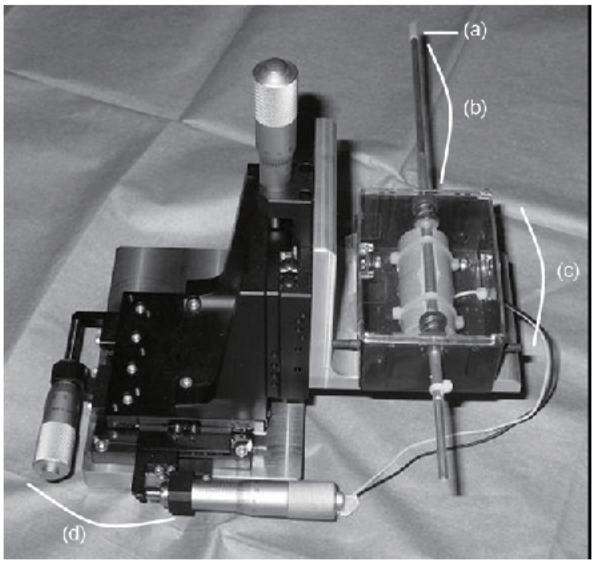

Figure 1. External view of the vibrator mounted on a triaxially mobile manipulator. (a) vibration applicator in contact with the ear. (b) virgulate vibration exciter. (c) vibration source including electric motor. (d) triaxially mobile manipulator.

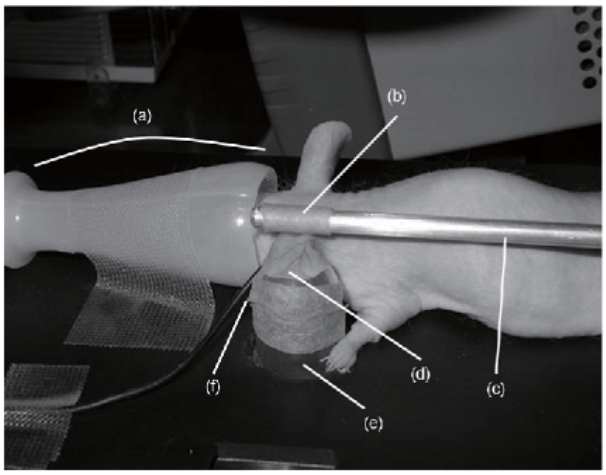

Figure 2. Experimental set-up for application of vibrations to the ear. (a) inhalation anesthesia. (b) vibration applicator in contact with the ear. (c) virgulate vibration exciter. (d) ear. (e) halogen lamp. (f) thermistor thermometry.

Observation and recording of the microcirculation

The experimental apparatus used for blood flow monitoring has been described elsewhere (*16*). Briefly, it included a function generator (33220A 20 MHz; Agilent Technologies International Japan, Ltd., Japan), a mounted vibrator, a halogen lamp (PCS-UMX250; Mejiro Precision Inc., Japan), and a charged-coupled device camera (DXC-107a; Sony Corporation, Japan). Images of microcirculation were recorded on a hard disk video recorder (Rec-On; I-O data, Japan) together with time and frame counts (VTG-33; FOR.A, Japan) for later analysis.

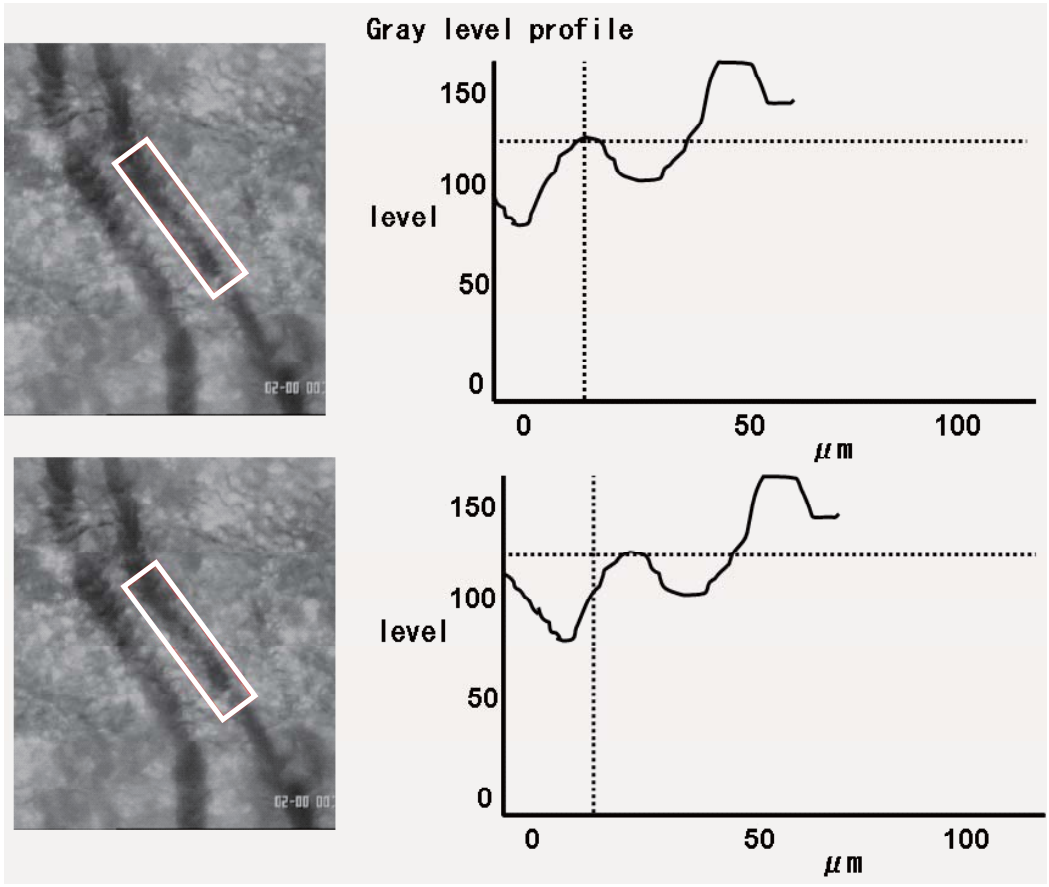

Figure 3. Blood flow velocity measurement using the CapiScope. (Above) The gray-level pattern along each line is measured. (Below) The pattern is compared with that from the next field (or several fields later for very low velocities). The velocity can be calculated based on the distance that the correlated pattern has traveled between the two gray-level profile measurements.

Measurement of blood flow

Blood flow was measured at the baseline and at 0, 5, and 15 min after the application of vibration. To determine the blood flow in the vessels, the blood velocity (μm/sec) and diameter of individual venules (μm) were measured. Venules with a diameter of 30-60 μm were selected for measurement.

The blood velocity through individual vessels was determined by a spatial correlation technique using recently developed image acquisition and analysis software (CapiScope II; KK Technology, UK) (*17*). This software detects the gray-level profile along a given vessel for each field of recorded images and compares this pattern with that of the next field (or several fields later for very low velocities). The comparison is performed by calculating the correlation coefficient for every possible shift in the previous gray-level profile relative to the new profile. Since the time lapse between the two gray-level profiles is known (*i.e.* 1/60th second for NTSC-based systems), the velocity can be calculated based on the distance that the correlated pattern has traveled between the two gray-level profile measurements (Figure 3).

Venule diameters were measured on acquired images using the calibrated ruler function of image-analyzing software (Beta 4.0.3 of Scion Image; Scion Corporation, MD, USA). Using the velocity (v) and the radius (r) of each venule, the blood flow (F) was calculated as:

$$F = \pi r^2 v$$

Statistical analysis

Relative blood flow (%) 0, 5, and 15 min after vibration was calculated using the baseline values as a reference for each intensity of vibration with the following formula: relative blood flow (%) = value at each time point/baseline value × 100. All data are represented as means with standard deviation. To detect differences between the experimental and control groups, analysis of covariance was employed using the baseline data as covariates. All statistical analyses were performed using Statistical Analysis System ver. 9.1.3 (SAS Institute Inc, Cary, NC). The level of significance was set at $P = 0.05$.

Results

The representative microscopic appearance of a venule in the 600 mVpp group is shown in Figure 4.

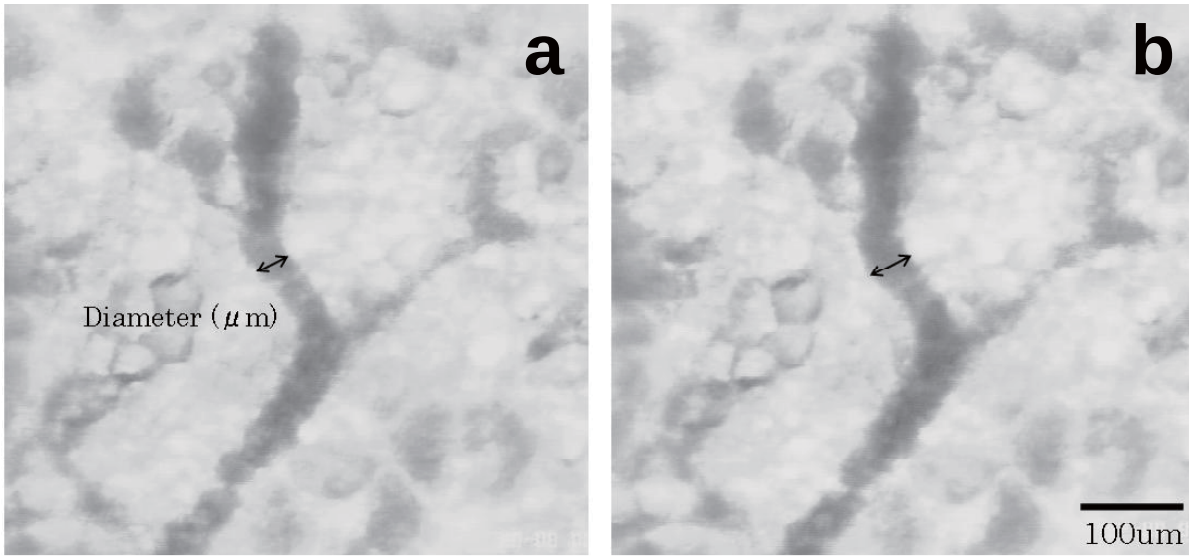

Figure 4. Visualization of venules with an intravital videomicroscope. (a) before the application of vibration. (b) 5 min after vibration. Compared to venules prior to vibration, increases were observed in the density of red blood cells and vessel diameter.

Table 1. Summary of relative blood flow velocity and vessel diameter.

	pre	0 min	5 min	15 min
Relative blood flow velocity (%)				
600 mVpp (*n* = 6)	100	99.8 ± 7.2	103.1 ± 8.3	102.8 ± 13.4
800 mVpp (*n* = 6)	100	103.3 ± 11.6	104.9 ± 11.8	102.8 ± 16.0
1,000 mVpp (*n* = 6)	100	104.4 ± 9.4	108.9 ± 11.6	107.2 ± 15.5
Control (*n* = 6)	100	104.3 ± 6.9	103.3 ± 7.4	108.6 ± 9.8
Relative vessel diameter (%)				
600 mVpp (*n* = 6)	100	104.7 ± 4.3	113.2 ± 6.1	112.4 ± 5.4
800 mVpp (*n* = 6)	100	103.0 ± 5.3	106.2 ± 5.0	107.5 ± 5.1
1,000 mVpp (*n* = 6)	100	102.4 ± 6.3	103.7 ± 4.6	100.1 ± 4.7
Control (*n* = 6)	100	100.3 ± 6.0	97.3 ± 6.2	97.7 ± 3.0

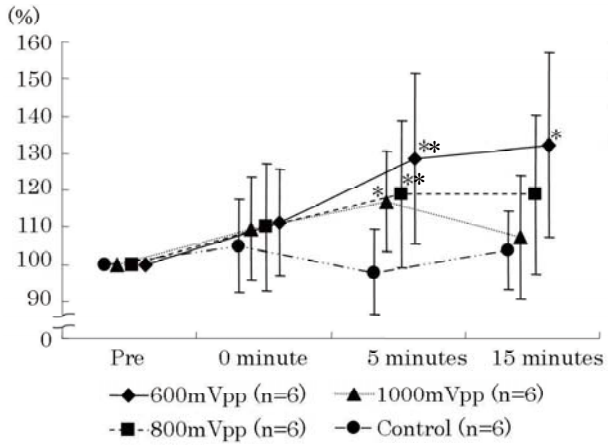

Figure 5. Time course of blood flow change. Means with SD are presented. The most significant increase was observed in the 600 mVpp group in comparison to the control. **P* <0.05, ***P* <0.01 compared with the control group.

Compared to venules prior to vibration, increases were seen in the density of red blood cells and vessel diameter. Blood flow velocity and vessel diameter are summarized in Table 1 and blood flow is shown in Figure 5. In the 600 mVpp group, the relative blood flow 5 and 15 min after vibration was 128.6 ± 22.9% and 132.2 ± 24.9%, respectively. These values were significantly higher than those in the control group at the same point in time ($P = 0.0017$ and $P = 0.046$, respectively). In the 800 and 1,000 mVpp groups, the values 5 min after vibration significantly increased to 119.0 ± 19.9% ($P = 0.028$) and 117.0 ± 13.6% ($P = 0.0012$), respectively, in comparison to the control group. However, the increases in the 800 and 1,000 mVpp groups attenuated 15 min after vibration. There were no changes in skin temperatures during the experiments.

Discussion

The present study first demonstrated the short-term effect of vibration on skin blood flow. These results, based on a newly developed experimental method, can aid further studies in elucidating the mechanism of vasodilation of venules by vibration.

A significant increase in blood flow was observed in the 600 mVpp group 5 and 15 min after vibration in comparison to the control group. Increased blood flow in the 800 and 1,000 mVpp groups was also detected 5 min after vibration. These results indicate

that direct vibration of the skin at a frequency of 47 Hz increases skin blood flow. A study by Kerschan-Schindl using a vibration frequency of 26 Hz demonstrated an increase in muscle blood volume (*18*). Bovenzi reported a decrease with time after stopping vibrations at a frequency of 31.5 Hz (*9*). Skoglund showed that low-amplitude, high- frequency vibration induced vasodilation in human skin (*11*). These facts may point to a relationship between the intensity of vibration and blood flow rather than frequency.

Out of several pathways for vasodilation demonstrated in previous investigations, two main mechanisms were considered to be responsible for the increase in blood flow with vibration. Vibrations in the applicator at 47 Hz may be transmitted to the tissue (*12*), leading to the production of mechanical stresses including shear stress, compression, and stretching of endothelial cells (*19*,*20*). These induce vasodilation of venules via mechanotransduction, which is mainly regulated by nitric oxide (NO). Many studies have reported a relationship between NO production or NO synthase (NOS) expression and mechanical stress created by flow stress (*21*) or exercise (*22*); however, the present study shows that vibration may induce vasodilation by mechanical stress. Experiments using an NOS inhibitor such as N^G-nitro-L-arginine are required to elucidate the mechanism of vasodilation through NO production (*23*).

The second mechanism of vasodilation observed in the present study was nerve axon reflex-related microvascular vasodilation (*24*). Vibration may induce impulses *via* polymodal receptors widely distributed on the skin surface, resulting in the release of neuropeptides such as substance P and calcitonin gene-related peptide that dilate the blood vessels (*25*). The 800 and 1,000 mVpp groups showed attenuation of the blood flow increase 15 min after vibration. These results indicate negative feedback, suggesting that caution should be exercised in selecting the intensity of vibrations for clinical use. The attenuation observed may be explained by the habituation of polymodal receptors sensing the vibrations (*26*).

Although there have been reports that type IIa fibers in muscle tissues have typical contraction rates in the range 20-60 Hz (*27*), the ear does not consist of muscle tissue, and hence muscle pump activity cannot be involved in the blood flow increase in the present study (*28*). Since there were no changes in skin temperature during vibration, vasodilation was also not a heat-induced phenomenon (*29*).

The vasodilation achieved by skin vibration may assist the healing of wounds caused by tissue ischemia such as pressure ulcers. The use of vibration to prevent or treat wounds may require that appropriate settings for the intensity of vibration be determined.

The present study does not reveal the detailed mechanism for vasodilation by vibration. In addition, the long-term effect of vibration on skin microcirculation was not determined. Further experiments are needed to address these concerns using an NOS inhibitor to reduce the effect of NO or local anesthesia to block the axon reflex for longer experimental periods.

In conclusion, this study demonstrated that vibration accelerates blood flow *via* venule vasodilation. Accelerated blood flow may be beneficial not only for prevention but also for treatment of cutaneous wounds caused by ischemic complications. Further studies are needed to elucidate the mechanism of vasodilation by vibration.

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