In vivo Analysis of Skin Microcirculation and the Role of Nitric Oxide During Vibration

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Abstract

Studies in healthy volunteers and patients with renal failure have shown that vibration, applied with a frequency of 47 Hz and a vibrational intensity of 600 mVpp, increases microcirculation of blood in the skin. This controlled, in vivo, experimental study was conducted to further evaluate the effect of vibration on skin microcirculation and to ascertain whether administration of a nitric oxide (NO) synthase inhibitor (NG-nitro-L-arginine methyl ester [L-NAME]) diminishes the effect of vibration on skin blood flow. Using a mouse microcirculatory model, 12 animals were prepared for study (six in the control and six in the experimental group). In the experimental group, vibrations were applied horizontally for 15 minutes. The control group received no vibration. Venular blood flow was measured using intravital videomicroscopy at baseline and at 0, 5, and 15 minutes after the application of vibration. Vibration significantly increased the blood flow at 5 and 15 minutes after application (P = 0.002 and P = 0.046, respectively). Differences between the control and experimental group also were statistically significant (P = 0.0017 and P = 0.046, respectively). In the second study, all animals (seven in each group) received an intraperitoneal injection of NO synthase inhibitor L-NAME before vibration application. When NO synthase inhibitor L-NAME was administered, the increase in blood flow in the vibration group was minimal after 5 and 10 minutes, and nonexistent after 15 minutes. No significant differences between the control and experimental group were observed. Because NO synthase inhibitor L-NAME inhibits NO production in vivo, these findings imply the involvement of NO in the observed blood flow increase during vibration. Future clinical trials to establish evidence as to the beneficial effects of vibration are warranted.

Keywords: hairless mouse, wound healing, vasodilation, vibration, intravital videomicroscopy

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the finding suggests that NO plays a significant role in the mechanism of this response.

Following this standard experimental method, an in vivo study was conducted to evaluate the effect of vibration on skin microcirculation and to ascertain whether the administration of a L-NAME diminishes the effect of vibration on skin blood flow.

Background

Microcirculatory vibration. Two clinical studies using the specially developed vibrator have been conducted.\(^3\)\(^4\) In both studies, the vibrator was placed beneath the mattress under the lower leg of healthy volunteers and patients (see Figure 2). The vibrator measures 616 mm x 182 mm x 114 mm (length x width x height); intensity, amplitude modulation cycle, and vibration time were adjustable using the attached controller. The frequency and horizontal vibration acceleration of the vibrator used in the studies was 47 Hz and 1.78 m/s\(^2\), respectively. These settings are within the allowable safety range as described in the Recommendations of Occupational Exposure Limits.\(^5\)

In the first study, within-group blood flow in the calcaneal region of 29 healthy volunteers was compared between the treated (vibration) and untreated leg.\(^3\) Sectional blood flow in the calcaneal region significantly increased compared to baseline in the experimental group (0.09 ± 0.01 cm\(^2\)) and as compared to the control group (0.02 ± 0.11 cm\(^2\)) (\(P = 0.0303\)). These results suggest that vibration in the lower leg improves blood flow in the calcaneal region of healthy volunteers.

The purpose of the second controlled study was to ascertain the effect of leg vibration on cutaneous microcirculation during hemodialysis.\(^6\) Eight patients with chronic renal failure were enrolled and transcutaneous oxygen pressure (TcPO\(_2\)) on the dorsum of the foot during hemodialysis was compared between the treatment (vibration) and control leg. The change from baseline in TcPO\(_2\), 60 minutes after vibration was significantly greater (6.9 ± 13.2 mm Hg, \(P = 0.03\)) than the change during the same time interval in the lower limb without vibration (-1.2 ± 11.4 mm Hg). The results suggested that vibration of the lower leg improves foot TcPO\(_2\) for at least 60 minutes after treatment in patients during hemodialysis.

Based on the observed clinical results, the authors of the current study experimentally showed that vibration enhanced the skin microcirculatory blood flow.\(^6\) However, these studies did not elucidate the exact mechanism by which vibration effects skin microcirculation.

Nitric oxide. NO is synthesized by three distinct isoforms of NO synthase (NOS). One of these forms (referred to as iNOS) is inducible only in response to allergic or inflammatory challenges and produces large amounts of potentially cytotoxic NO in inflammatory cells. The other two types of NOS isoforms normally are active in many cells; they are referred to as constitutive. Of the constitutive enzymes, the first was originally discovered in brain and neural tissues and is referred to as nNOS. This form acts as a neurotransmitter or neuromodulator. The second constitutive enzyme was the last to be discovered and was found to be largely localized in endothelial cells. This form, endothelial NOS (eNOS), not only plays a major role in the control of vascular tone, but it also affects the adhesion of leukocytes and platelets to the blood vessel walls.\(^7\)

NO is produced from L-arginine, and many L-arginine analogs are available for use as competitive inhibitors of NO synthases. Among the nonselective inhibitors, the most widely used are NG-monomethyl-L-arginine (L-NMA), NG-nitro-L-arginine (L-NA), and L-NAME. The latter is preferred for in vivo experiments because of its good aqueous solubility and ease of systemic administration.\(^7\)

L-NAME is a competitive NO synthases inhibitor and known to inhibit NO production in vivo. The authors hypothesized that NO plays a significant role in microcirculation because NO has a tonic systemic vasodilator effect critical in vascular biology; in addition, NO production from endothelial cells is known to be stimulated by a variety of mechanical forces.\(^4\) Vibration is likely to be one of the mechanical stresses that influences NO release. Generally, when a physiological phenomenon is inhibited by
blocking NO production, the physiological event is associated with NO.

Materials and Methods

Study design. This controlled, in vivo, experimental study using a mouse microcirculatory model consisted of two sets of measurement. The first experiment estimated the effect of vibration on skin microcirculation by comparing the results of the experimental (vibration, n = 6) to a control group (n = 6). The second study measured microcirculatory changes caused by vibration after administration of NO synthase inhibitor L-NAME. This investigation consisted of two groups: a NO synthase inhibitor with vibration group (n = 7) and a NO synthase inhibitor without vibration group (n = 7).

Materials. 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. To apply vibration, a vibrator was developed consisting of a box-shaped vibration source including an electric motor and a 5-mm (diameter) virgulate (ie, rod-shaped) vibration exciter. Changes in the vibration intensity were achieved by changing the voltage of the vibrator’s power supply. The vibrator was mounted on a triaxially mobile manipulator.

Methods. All experiments were conducted in a thermoneutral laboratory with a room temperature of 24˚C. Before the experiment, all animals were anesthetized by continuous inhalation (isoflurane: 1.5% to 2.0%, air: 200 mL/minute) to prevent movement. The ears were prepared by stringing two distal regions of the auricular margin with nylon thread to spread and fix the ear on the light source for the measurement of blood flow. The thermistor probe was situated between the ear and the light source to monitor the local temperature (see Figure 3).

Effect of vibration on skin microcirculation. In the six animals in the experimental (vibration) group, the virgulate vibration exciter was placed in contact with the proximal base of the ear, vertical to the auricular axis. The whole ear was exposed to vibration for 10 minutes using an intensity of 600 mVpp and a frequency of 47 Hz based on the findings of the preliminary study.4 The six animals in the control group were prepared in the same way as the experimental group and the exciter was placed in contact with the part of the ear as in the vibration group but no vibration was applied.

Figure 2. Application of the vibrator. The device was placed beneath the lower leg of study volunteers. The mattress was inserted between the lower leg and the vibrator in the actual situation.

Figure 3. Preparation of the experimental model and set-up. A) Appearance of the hairless mouse ear. The auricular margin was spread and fixed with nylon thread on the light source; B) Experimental set-up for the application of vibration to the ear.
Microcirculatory changes caused by vibration after administration of the NO synthase inhibitor (L-NAME). To estimate the effect of blocked NO production on microcirculatory changes caused by vibration, the NO synthase inhibitor L-NAME was injected intraperitoneally before measurement. Animals were prepared in the same manner as in the skin microcirculation experiment. In the experimental group (n = 7), vibration was applied to the ear for 10 minutes at an intensity of 600 mVpp and a frequency of 47 Hz. In the control group (n = 7), NO synthase inhibitor L-NAME was injected but no vibration was applied.

Blood flow measurement and application of vibration started 15 minutes after administration of NO synthase inhibitor L-NAME.

**Measurements.** The experimental apparatus used for blood flow monitoring 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 (DWC-107a; Sony Corporation, Japan). The microcirculation images were recorded on a hard disk video recorder (Rec-On; I-O data, Japan) together with the time and frame counts (VTG-33; FOR.A, Japan) for later analysis. Blood flow was measured at baseline and at 0, 5, and 15 minutes after vibration was applied.

Blood velocities and individual venules with a diameter of 30 to 70 μm were measured to assess blood flow in the vessels. 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 using the formula: \( F = \pi r^2v \).

The blood velocity through individual vessels was determined using a spatial correlation technique and a recently developed image acquisition and analysis software (CapiScope II; KK Technology, UK). 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. Because the time lapse between the two gray-level profiles is known (ie, 1/60th of a 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 (see Figure 4).

**Statistical analysis.** Each absolute blood flow value was expressed as percent value versus baseline, then averaged. The relative blood flow at 0, 5, and 15 minutes after vibration was calculated using the baseline values as a reference for each measurement. An analysis of covariance was employed using the
baseline data as covariates to detect differences between the experimental and control groups. All the statistical analyses were performed using Statistical Analysis System version 9.1.3 (SAS Institute Inc, Cary, NC). Data were expressed as the mean ± standard deviation. The level of significance was set at $P = 0.05$.

Results

In the first experiment, the average microcirculatory blood flow increased continuously to 132.2 ± 24.9% at 15 minutes over baseline following the application of vibration (see Figure 5). The relative blood flow in the vibration group at 5 and 15 minutes was significantly higher than in the control group at the same time points ($P = 0.046$) (see Figure 6).

When the NO synthase inhibitor L-NAME was administered, relative blood flow increased slightly at 0 and 5 minutes (105.4% ± 10.1% and 104.3 ± 14.1) but no significant differences were observed during the observation period between the groups at any time point (see Figure 7).

Discussion

The results of this study confirm that vibration significantly increases skin microcirculatory flow. Low-frequency vibration has been reported in clinical studies to increase the muscle blood volume in human subjects. The measurement of human skin temperature changes also has indicated that low-amplitude, high-frequency vibration regularly induces vasodilation.

Utilizing vibration to enhance blood flow, the authors developed a vibrating device that promotes lower-extremity circulation. Subsequently, this author group recently conducted a non-randomized, blinded, controlled study involving 31 older adult patients with Stage I pressure ulcers. The vibration group consisted of 16 patients with 20 Stage I pressure ulcers; the control group consisted of 15 patients with 21 Stage I pressure ulcers. In the vibration group, eight pressure ulcers (40.0%) healed; in the control group, two (9.5%) pressure ulcers healed. The number of healed ulcers was significantly higher in the vibration than in the control group ($P = 0.033$) and the healing rate during the study period was significantly higher in the experimental group than in the control group ($P = 0.018$, log rank test). The results indicated that the use of the vibrator may facilitate the healing of Stage I pressure ulcers. The results of the second experiment show that NO synthase inhibitor L-NAME administration almost completely diminished the observed effect of vibration on skin blood flow.

The authors had suspected that vibration might be one of the mechanical forces that affects the production of NO. The authors focused on the role of NO because of its ability to dilate blood vessels. To test this hypothesis, the effect of inhibiting NO synthesis on vibration-induced blood flow changes was analyzed. L-NAME inhibits NO production in vivo, suggesting the involvement of NO in the mechanism of action related to the effect of vibration on skin blood flow.

Historically, an endothelium-derived relaxing factor was first discovered by Furchgott and Zawadzki and subsequently was shown to be identical to NO by Palmer et al and Ignarro et al. Since then, it has become clear that NO is produced in response to a number of different stimuli through various mechanisms. In particular, NO production from endothelial cells is stimulated by a variety of mechanical forces such as shear stress and cyclic strain. Endothelial cells have been shown to act as mechanotransducers, mediating the effects of shear stress that control cellular structure and function, including the regulation of vascular tone and diameter, vessel wall remodeling, hemostasis, and inflammatory responses. Some of the most prominent effects of
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NO produced in response to shear stress include vessel relaxation, apoptosis inhibition, and platelet and monocye adhesion triggered by a variety of proatherogenic factors.

The current experiment revealed that the administration of NO synthase inhibitor L-NAME almost completely blocked the vibration-induced increase in blood flow. A recent study on human subjects estimated the effects of vibration at 50 Hz for 5 minutes applied to the skin of the forearm in healthy adults and patients with type 2 diabetes. The report indicated that vibration significantly increased skin blood flow and the rate of NO production in both groups. The authors proposed that the mechanism of action might involve the shear stress that is generated by vibration. The current findings are consistent with previous findings, suggesting that NO plays a significant role in the mechanism of action responsible for vibration-induced microcirculatory changes. Vibration at an optimal frequency and intensity likely acts as a mechanical force that stimulates endothelial cells to release NO. Future clinical trials are desirable to establish evidence regarding the beneficial effects of vibration.

Conclusion
An in vivo study was conducted to observe the effect of vibrations on skin blood flow and the involvement of NO in the mechanism of action. Using a hairless mouse model, vibration at a frequency of 47 Hz increased skin blood flow in a microcirculatory experimental system. Administering NO synthase inhibitor L-NAME almost completely suppressed the vibration-induced increase in blood flow. The findings suggest that NO plays a significant role in the mechanism of action responsible for vibration-induced changes in microcirculation. Vibration at an optimal frequency and intensity likely acts as a mechanical force that stimulates endothelial cells to release NO.

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References