Kawasaki Journal of Medical Welfare Vol. 12, No. 1, 2006 13–18

Original Paper

Effects of Ultrasonic Radiation on Cutaneous Blood Flow in the Paw of Decerebrated Rats

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(Accepted May 17, 2006)

Key words: ultrasonic radiation, cutaneous blood flow, alpha-adrenergic antagonist

Abstract

This study investigated the effect of ultrasonic radiation to the femoral region on cutaneous blood flow in the paw of decerebrated rats. Cutaneous blood flow of the right forepaw and hindpaw was measured with a laser-Doppler flowmeter in 8 male Sprague-Dawley rats. Ultrasonic radiation of 1.5W/cm^2 was applied to the ipsilateral femoral region at 1 MHz for 6min. Cutaneous blood flow (ml/min/100g) in the forepaw was 37.17 ± 14.74 at rest and 37.82 ± 14.29 during ultrasonic radiation. No significant change was observed. However, cutaneous blood flow in the hindpaw was 35.34 ± 14.77 at rest, and it increased significantly up to 39.28 ± 11.92 during ultrasonic radiation. The increased blood flow continued even after the alpha-adrenergic receptor antagonist, phentolamine, was administered. These results suggested that the increase in cutaneous blood flow elicited by ultrasonic radiation was independent of the sympathetic nervous system. Factors considered for the increased blood flow were vasodilation due to an axon reflex and/or chemical mediator release by thermal effects of the ultrasonic radiation.

Introduction

Ultrasonic therapy is applied to arteriosclerosis obliterans. However, in terms of the effects produced by ultrasonic radiation to affected regions, there is currently insufficient biophysical evidence for the clinical use of therapeutic ultrasound. One result of ultrasonic radiation is reported to be a rise in tissue temperature [1, 2, and 3]. These thermal effects are generally known to increase collagen tissue extensibility, metabolic activity and local blood flow [3]. In human experiments, effects on local blood flow are still controversial. The study of Paul and Imig [4] indicated that the administration of ultrasonic radiation to the skin of an extremity increased blood flow when measured by venous occlusion plethysmograpy. However, Robinson and Buono [5] reported that ultrasonic radiation to the forearm did not increase blood flow using the xenon–33 washout technique. In another experiment using anesthetized rats, ultrasonic radiation to the region of the biceps femoris decreased muscle blood volume by acting on the adrenergic receptors of blood vessels [6]. In an experiment using anesthetized dogs, Imig [7] reported that the administration of ultrasonic radiation to the region of the gastrocnemius muscle increased blood flow in the femoral artery as measured by an electromagnetic flowmeter. Up to now, the effects of ultrasonic radiation on skeletal muscle blood

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flow and/or cutaneous blood flow have not been identified.

The sympathetic nervous system plays an important role in the regulation of skeletal muscle vasomotor tone. Our understanding of sympathetic neural control of muscle blood flow has been enhanced by animal studies using mainly invasive techniques such as the perivascular Doppler ultrasound flowmeter ([8]–[11]). In this study, we investigated the effect of ultrasonic radiation to the femoral region on cutaneous blood flow to the paw of decerebrated rats using a Doppler ultrasound flowmeter. Moreover, the question of the effect of ultrasonic radiation is mediated by alpha-adrenergic receptors was also investigated.

Materials and Methods

Animal care was in accordance with the guidelines of the Physiological Society of Japan. Eight male Sprague-Dawley rats (10–weeks-old, 310–350 g) were used. Each animal was anesthetized with an intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg), and a tracheal cannula made from polyethylene tubing was inserted. They were then placed in a stereotaxic apparatus, and a middle incision was made in the skin to expose the skull. The upper skull and dura mater were removed, and the brain was then sectioned vertically at the precollicular level using a hand-held blunt spatula. Small pieces of cotton gauze were set in the cranial cavity to arrest bleeding. No additional anesthesia was administered after the decerebration. The left femoral vein was cannulated for the intravenous injection of saline and drugs, and the left femoral artery was cannulated for the recording of arterial pressure. Body temperature was maintained at about 37 ℃ using a heating pad and monitored with a rectal probe transducer.

Cutaneous blood flow of the right forepaw and the hindpaw was measured with a laser-Doppler flowmeter (ALF–21, Advance) using a type-C probe. The right femoral region was shaved, ultrasound gel was applied to the shaved area, and an ultrasound probe was attached and moved over the area. After 5 min of baseline data collection, ultrasonic radiation at 1.5W/cm^2 was applied for 6 min using a continuous wave ultrasound (SUS–7P, SAKAI Medical.) at 1 MHz. Subsequently, an alpha-adrenergic receptor antagonist, phentolamine, was administered intravenously (1mg/kg) at a rate of 0.25ml/min. Ultrasonic radiation was applied 15 min after the injection. At the end of the experiment, rats were killed by an overdose of anesthetic.

All measured variables were displayed continuously on a computer monitor and date, were stored on a hard disk through analog-digital conversion (Powerlab/16s, AD Instruments) at a 2–kHz sampling rate. The data were expressed as means ± SD. Mean blood flow before ultrasonic radiation was compared with the last minute during ultrasonic radiation using the Wilcoxon signed-ranks test. Mean blood pressure before phentolamine administration was compared with 20 minutes after application using one-way analysis of variance (ANOVA) followed by post hoc Bonferroni/Dunn. The level of statistical significance was set at $P < 0.05$ unless otherwise noted.

Results

Changes in mean blood flow by ultrasonic radiation

Cutaneous blood flows were measured at least 1 hr after decerebration. Cutaneous blood flows of the forepaw and hindpaw at rest were 37.17±14.74 and 35.34±14.77 ml/min/100g, respectively. After ultrasonic radiation was delivered for 6 min, no obvious change was observed in forepaw cutaneous blood flow during application (Fig. 1A). However, cutaneous blood flow in the hindpaw gradually increased during radiation, and the increase was maintained for several minutes after radiation was completed (Fig. 1A).

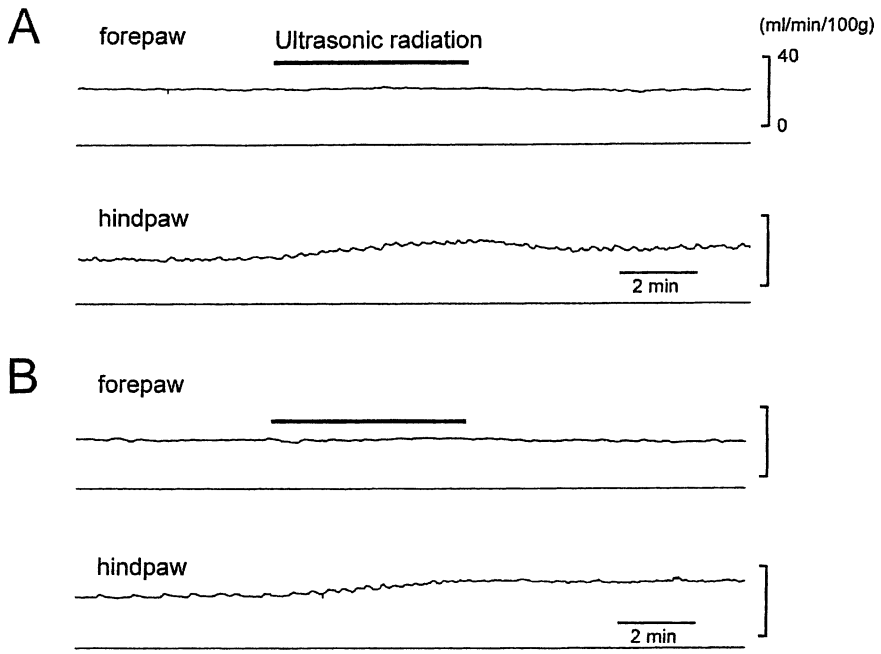

Fig. 1 Changes in cutaneous blood flow in the forepaw and hindpaw evoked by ultrasonic radiation to the femoral region before (A) and after the administration of phentolamine (B).

Cutaneous blood flow of forepaw and hindpaw during ultrasonic radiation was 37.82±14.29 and 39.28±11.92 ml/min/100g, respectively. The mean blood flow for 1 minute before ultrasonic radiation (control) was compared to the last minute during radiation (US-radiation). No significant difference was found between control and US-radiation in the forepaw (Fig. 2A), but hindpaw cutaneous blood flow increased significantly during ultrasonic radiation ($p<0.05$, Fig. 2A).

Changes of mean arterial blood pressure by phentolamine administration

To evaluate the contribution of the sympathetic nervous system to increased blood flow during ultrasonic radiation, the alpha-adrenergic receptor antagonist, phentolamine, was administered intravenously. Figure 3A shows changes in blood pressure and cutaneous blood flow following phentolamine administration. Cutaneous blood flows increased in both forepaw and hindpaw after phentolamine administration, and recovered after several minutes (Fig. 3A). Blood pressure decreased rapidly, and slowly recovered to the baseline (Fig. 3A). The significant decrease after phentolamine administration continued for over 20 min ($p<0.05$, Fig. 3B).

Changes of mean blood flow due to ultrasonic radiation after phentolamine administration

Blood pressure was significantly decreased after phentolamine administration, indicating that the blocking effect of the alpha-adrenergic receptor continued for at least 20 min. Thus, the effects of ultrasonic radiation on blood flow were tested 15min after the administration. Changes in cutaneous blood flow in the hindpaw and forepaw due to ultrasonic radiation 15min after phentolamine administration are illustrated in Fig. 1B. No obvious change was observed in the forepaw during radiation, but a gradual increase was recorded in the hindpaw. This increase continued even after radiation was stopped. No significant difference was observed in the forepaw between control and US-radiation (Fig. 2B), but ultrasonic radiation produced a significantly greater blood flow in the hindpaw even after phentolamine administration ($p<0.05$, Fig. 2B).

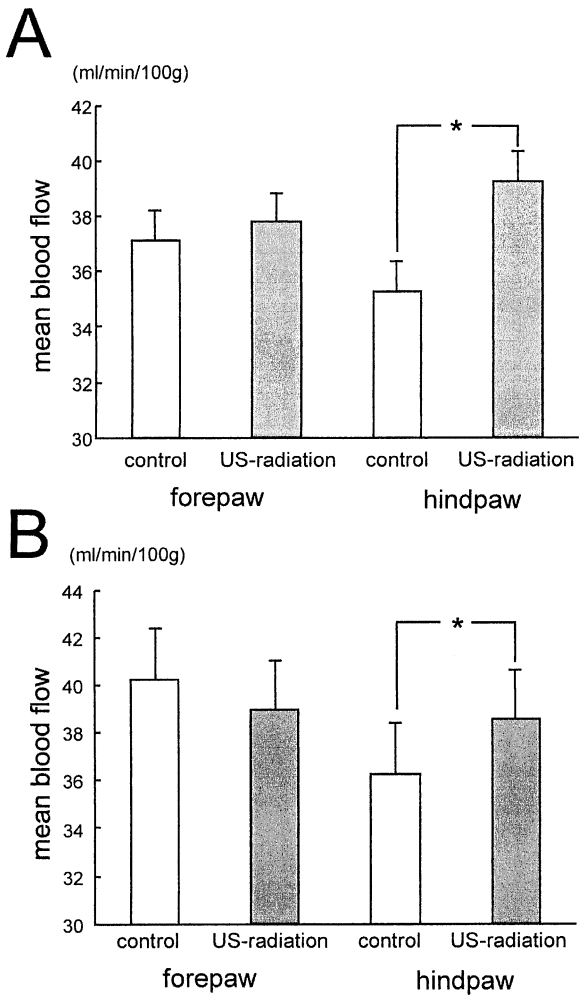

Fig. 2 Changes in mean cutaneous blood flow in the forepaw and hindpaw evoked by ultrasonic (US) radiation. A: control, B: 15min after the phentolamine administration. * Significant difference ($p<0.05$, Wilcoxon signed-ranks test)

Discussion

Ultrasound penetrates skin, and subcutaneous fat and blood vessel with minimum attenuation [3]. The mechanism of physiological changes produced by ultrasonic radiation has not been well investigated, however, ultrasonic radiation is known to produce thermal effects. In human experiments, Paul and Imig [4] have reported that ultrasonic radiation for 20 minutes at intensities of $2W/cm^2$ produced more than a 25% increase in blood flow in the femoral artery. They concluded that the increased flow in the treated limb was due to local vasodilation resulting from tissue hyperthermia [4]. A blood flow increase by ultrasonic radiation with an intensity above $1W/cm^2$ for 15 min has also been reported in anesthetized dogs [7]. In the present study using decerebrated rats, ultrasonic radiation at an intensity of $1.5W/cm^2$ for 6 minutes produced a significant increase in cutaneous blood flow. Thus, it is possible that the blood flow increase was elicited due to local tissue hyperthermia. On the other hand, Robinson and Buono [5] reported that ultrasonic radiation at 1.5 W/cm^2 for 5 minutes to the forearm did not influence cutaneous and muscular blood flow in humans. The amount of heat produced is considered to depend on the intensity and frequency of ultrasound, as well as the duration of exposure and tissue constitution. High collagen tissue is reported to absorb a large amount of the ultrasonic beam [2, 3]. Unfortunately, the causes for differences in ultrasonic effects are not well understood.

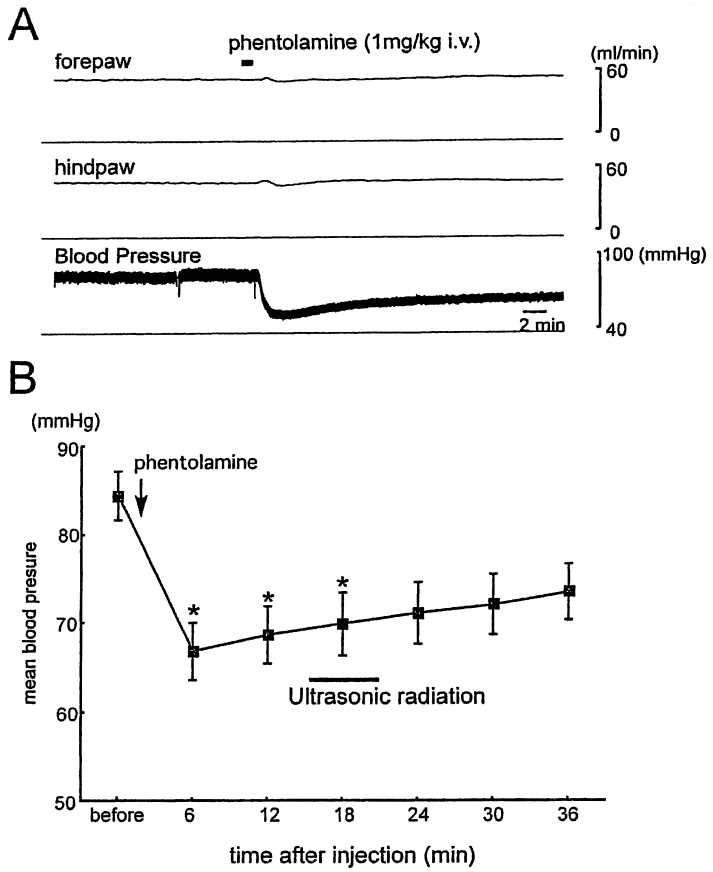

Fig. 3 A: Effects of phentolamine administration on cutaneous blood flow and arterial blood pressure. B: Changes in mean arterial blood pressure after phentolamine administration. * Significant difference ($p<0.05$, ANOVA)

The mechanism for the physiological changes caused by ultrasonic radiation may be thermal or a combination of factors. In the case of thermal effects of ultrasonic radiation, a local spinal-cord reflex should be considered. This reflex results in a decrease in postganglionic sympathetic nervous activity innervating the smooth muscles of blood vessels [12]. The vasodilative effects of this reflex response are not limited to just the area heated, but can be maintained in areas remote from the site of application. Thus, it was decided to investigate the effect of an alpha-adrenergic receptor antagonist on the blood flow increase elicited by ultrasonic radiation. Arterial blood pressure was significantly decreased for 20 min after phentolamine administration, which was probably due to vasodilative effects. Nevertheless, ultrasonic radiation produced a blood flow increase when phentolamine was presumably acting (Fig. 3B). It was speculated that vasodilation of the femoral artery in areas where ultrasonic radiation was applied could be due to two factors, an axon reflex and the release of chemical mediators. Excitation of cutaneous thermoreceptors by ultrasonic radiation produces afferent impulses. These impulses are carried antidromically through branches toward skin blood vessels, and a vasoactive mediator such as substance P is released [13]. Vasodilation without an adrenergic nerve effect might be produced by substance P. Heat produces a mild inflammatory reaction [12]. Chemical mediators including histamine and bradykinin are released in the area of the inflammation, and results in the discharge of an endothelium derived relaxing factor, namely nitric oxide. Nitric oxide is well known to relax the smooth muscles of blood vessels [14].

Our results indicated that cutaneous blood flow was significantly increased by ultrasonic radiation under the presence of an alpha-adrenergic receptor antagonist. This result suggested that ultrasonic therapy can be useful for treating patients with problems of the peripheral vascular system. However, further

physiological evidence should be collected for the clinical use of ultrasonic therapy.

Acknowledgments

This study was supported by grants-in-aid for Project Research from Kawasaki University of Medical Welfare in 2000.

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