Effective Isolation of Primo Vessels in Lymph Using Sound- and Ultrasonic-wave Stimulation

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Available online 23 June 2014

Abstract

The effects of stimulation with sound and ultrasonic waves of a specific bandwidth on the microdissection of primo vessels in lymphatic vessels of rabbit were investigated. The primo vessels stained with alcian-blue dye injected in the lymph nodes were definitely visualized and more easily isolated by sound-wave vibration and ultrasonic stimulation applied to rabbits at various frequencies and intensities. With sound wave at 7 Hz and ultrasonic waves at 2 MHz, the probability of detecting the primo vessels was improved to 90%; however, without wave stimulation the probability of discovering primo vessels was about 50% only. Sound and ultrasonic waves at specific frequency bands should be effective for microdissection of the primo vessels in the abdominal lymph of rabbit. We suggest that oscillation of the primo vessels by sound and ultrasonic waves may be useful to visualize specific primo structure, and wave vibration can be a very supportive process for observation and isolation of the primo vessels of rabbits.

1. Introduction

The knowledge of the most important 360 spots on the body suitable for acupuncture has been handed down from one generation to the next for thousands of years. These spots lie along lines that spread through the whole body like a net. In traditional Oriental medicine, a meridian is believed to be a path through which the life energy, known as “Qui,” flows. The system of meridians forms a bridge between meridian points and makes up another circulatory system within the human body [1,2]. Managing meridian points is important for the prevention and treatment of diseases. Even though the idea of the meridian system has been
advanced and is recognized in Oriental medicine, the existence of the meridian system within the human body has not been verified anatomically using any optical instrument such as a microscope [3,4]. The current notion within Western medical circles is that meridian points and pathways are simply parts of neuronal systems. In addition, acupuncture is thought to stimulate the peripheral nervous system, resulting in the transfer of a neuronal signal to the brain, which then directs the human body to cure itself.

The discovery of the circulation of blood and lymph by Western medicine has been the main driving force behind the rapid progress in modern medicine [5,6]. Discovery of the meridian circulation system can bring about a similar breakthrough in Oriental medicine. Together with Western medicine, Oriental medicine has been conducting research to find ways to enhance human health for thousands of years. These days, extensive studies on Oriental medicine, including acupuncture and moxibustion, in the United States and Europe have led to changes in the perspectives for research on Oriental medicine in Korea. Thus, the discovery of the meridian circulation system should accelerate the development of Oriental medicine.

Recently in Korea, novel scientific approaches to identify meridian spots and pathways have made worthwhile achievements. Around meridian spots, small tubes, the so-called “primo nodes,” are gathered and connected by a primo-vessel meridian system, the so-called primovascular system (PVS), through which primo fluid, which contains a high amount of basophilic granules, is transported throughout the human body [7,8]. The meridian system consists of the pathways in the body along which Qui circulates in parts of the skin, and primo vessels lie freely in blood vessels and on the surfaces of organs, forming a body-wide web. Within these primo vessels, basophilic granules, called “sanals,” stream in the primo fluid that flows at a rate of 0.3 ± 0.1 mm/second, and these sanals are in charge of functions such as cell reproduction, hematopoiesis, and so on [9,10].

Since 2004, primo nodes have been observed on the surfaces of organs from albino rats under a microscope. Inside the primo vessel, egg-shaped small cells, called sanals, containing DNA were discovered. Considerable research on primo nodes, primo vessels, meridian points, and the meridian system, as well as on their relationships to Qui, has shown that a large amount of DNA is contained in the sanal. Biophotons are known to radiate from DNA; sanals have been observed to emit biophotons in the same manner. In accordance with this theory, Qui may be projected as quantum information in a biophoton complex emitted from a sanal [11].

Furthermore, tubes with thicknesses of 30–50 μm were discovered in animal experiments using scanning electron microscopy, nanoparticles, and fluorescent pigments. These tubes, which have been reported to have an unusual vascular bundle structure, were previously unknown in Western medicine. However, these tubes are not easy to find because they are transparent and because distinguishing them from blood clots is difficult. In addition, these tubes act like dense plant roots; a fluorescent dye injected into such a tube flows at a rate slower (0.3 mm/minute) than that of blood flow. Immune cells and hormones have also been found in these tubes [12,13]. In anatomical studies on rats and rabbits, primo vessels have been found at three places: on organ surfaces, in blood vessels, and in lymph vessels.

Now, the primo fluid circulation path is being traced from acupuncture points of skin to points inside the organs of the human body. If this spider-web network of primo vessels in an animal’s body can be completely defined, soon it will also find applications to the human body. Blood vessels contract if people feel nervous, because nervousness activates the sympathetic nervous system and the blood vessels. In other words, being nervous affects not only the sympathetic nervous system, but also the lymphatic system. In this study, finding and characterizing primo vessels were our major goal. However, when a rabbit is nervous, finding primo vessels is difficult, and so the sound waves, including ultrasonic waves, were used to relieve the rabbit’s anxiety.

In the case of sound-wave therapy, a sound wave is used to restore mental and physical health and improve the immune system. Especially, sound-wave therapy is helpful to people who have a disability or disease, because it alleviates pain and reduces the loss of function [14]. In this study, a sound-wave vibration apparatus and a digital audio player, which are thought to be useful for finding primo vessels easily, were used to locate primo vessels inside lymph vessels. In the experiments in which the sound-wave vibration apparatus delivered sound waves at frequencies of 3–50 Hz to the body, blood, and lymph circulation were improved, and bone density and muscle strength were increased without any additional stress to joints and/or ligaments.

In this study, primo vessels were isolated easily by injecting a dye into the lymph vessels of rabbits and using a vibrating apparatus to apply sound and ultrasonic waves to the bodies of rabbits; the results gave new insights into the diagnoses of modern Oriental medicine. The rabbits were exposed to sound and ultrasonic waves, produced using a vibrating apparatus and device, of various frequencies and intensities for 30 minutes, while music that the rabbits liked was being played. When sound and ultrasonic waves were applied to a rabbit, the structure of the primo vessel could be determined directly and easily because lymph circulation had been activated. The experiments on the rabbits yielded the probability of discovering a primo vessel inside a lymph vessel and its morphological characteristics, such as its diameter, length, and shape.

2. Materials and methods

This anatomical experiment on rabbits followed the Code of Animal Tests Ethics and was approved (approval code: 2013-1) by the Animal Ethics Committee (IACUC) of Sangji University, Wonju, Korea. Prior to starting this experiment, the following were prepared: laboratory rabbits, anesthetic drugs, alcohol, saline, electronic scales, an electronic microscope, microtweezers, laboratory scissors, laboratory forceps, microtubes, and so on. An anesthetic drug, a mixture of zoletil (0.5 mL) and rompun (2.5 mL), was used. Alcian-blue powder (50 mg) was suspended in 5 mL of phosphate buffer solution (PBS, distilled water and saline at a 1:100 ratio) by shaking well for 5 minutes and...
was then filtered with a microfilter \cite{15,16}. Rabbits were fasted for 1 day or 2 days in order to clean the lymphatic system in the abdominal area. The rabbits, 10-week-old females weighing 1.5–1.8 kg, were imported from New Zealand and were supplied by Dae Han Bio-link Company (Chungcheongbuk-do, Korea). The following procedures were followed: (1) periodically, saline at about 40°C was poured evenly over the organs of the rabbits to help the circulation of fluids; (2) when bleeding occurred, the wound area was packed with gauze; and (3) care was taken not to touch the blood vessels when removing the cell membrane with tweezers.

In order to observe the primo vessels during the entire experiment, the experiment was carried out in the following seven major stages.

First, the rabbits were placed in a lying position in a box on the sound-wave vibration apparatus, and sound waves at various frequencies and intensities were applied to the rabbits. Second, an anesthetic drug, a mixture of zoletil (0.5 mL) and rompun (2.5 mL), was injected into the abdominal vein of the rabbit for euthanasia. The entire process as described above from animal preparation to euthanasia is summarized in a flowchart in Fig. 1.

According to previous observations made by the inventor of the sound-wave vibration equipment, Wonju Medical Equipment Industry and Technology Park, Wonju-si, Gangwon-do, Korea, located in Sonic World, sound waves of frequencies 7–9 Hz can affect an electromyogram because of changes in the abdominal blood circulation and lymph circulation \cite{17,18}. Interestingly, the bandwidth of the sound wave can stimulate and activate the lymphatic circulation system, even though the conditions differ somewhat depending on the subjects, their postures, and the type of anesthesia used.

Fig. 2A and B show the supplication of sound and ultrasonic waves to a rabbit. Prior to anesthesia, the rabbit is exposed to music and the sound wave simultaneously. Sound and ultrasonic waves are applied to different areas of the rabbit’s body, such as bones, fractures, auditory

![Flowchart of the experiment](Image)

Figure 1 Total surgical anatomical procedure for observation, isolation, and extraction of a primo vessel from inside a rabbit’s lymph vessel using sound- and ultrasonic-wave stimulation. PVS = primovascular system.
nerve, and muscles. Fig. 2D and E show the apparatus and the device that control the intensity and frequency of the sound wave and provide the music that the rabbits liked. Fig. 2B shows an anesthetized and shaved rabbit in a cage to which an ultrasonic wave of appropriate intensity and frequency is being applied. In the case of sound waves, as shown in Fig. 2A and D, waves of frequencies 3–50 Hz were delivered to the body without any stress on the joints and/or ligaments, in order to improve blood and lymph circulation, strengthen muscles, and increase bone density [14,17–19]. In addition, this apparatus can be used to simulate the effect of not only a full-body workout, but also a workout focusing on each part of the body, even in a supine position, using sound waves of frequencies in the range 3–50 Hz. Fig. 2D shows the sound-wave vibration apparatus. The vibration apparatus used the principle of conventional speakers produces a sound wave by adjusting the frequency and the intensity delivered to the organs of the rabbit’s body, which is placed on the plate. As Fig. 2E shows, the ultrasonic generator is designed to produce ultrasonic waves at an intensity of 60 W/cm² and a frequency of about 2 MHz. Recently, such waves were shown to affect the circulatory, endocrine, and musculoskeletal systems, and so they have widely been applied when working out indoors [19].

3. Results

In this experiment, experimental groups were classified based on the frequency and intensity of sound prior to anesthesia and whether the rabbits were exposed to music or not. Following the administration of anesthesia, the rabbits were exposed to sound waves and music for 10 minutes. Throughout this experiment, the diameter and length of a primo vessel could be observed. In this experiment, even though a primo vessel was not always found, the primo vessels observed were of different types.

Fig. 3 shows the typical six steps required to separate primo-vessel bands near veins. First, Fig. 3A and B show bands like lymph nodes, where the largest veins were found, and the syringe needed to be inserted carefully near these spots. The alcian-blue dye was injected into the lymph nodes, which quickly spread throughout the lymphatic system (Fig. 3C). Fig. 3D shows primo and lymph vessels dyed with alcian-blue. In right side, we can see floated primo vessel (blue dotted lines) in lymph vessel (long, white broken lines). The primo vascular structure could be visualized because the dye attached itself to the wall of the vessel and did not interfere with the flow of the lymph fluid. Because of this floating dye in the lymph vessels, the primo vessel could be observed easily [20,21]. Fig. 3E and F show the extraction and isolation of the carefully collected, dyed primo vessels, respectively. These experiments showed that primo vessels were connected to lymph nodes and could be stored in PBS. Using ultrasound and microtweezers, we were able to remove the primo vessel from the lymph vessel, and investigate the physiological and the pathologic features of the primo vessel [22,23].

Prior to extracting the primo vessels using the sound-wave apparatus, morphological structural characteristics of the primo vessels, such as their thickness and length, that were connected to the suspected primo node (red arrow in Fig. 4A) could be obtained. Some representative photos of long primo vessels are shown in Fig. 4. Typically, several primo vessels were observed in rabbits that were exposed to sound and...
ultrasonic waves of frequencies of 7 Hz and 2 MHz at intensities of 50 W/cm² and 60 W/cm², respectively. To investigate the primo vessels in an abdominal lymph vessel, we injected alcian-blue into two inguinal lymph nodes. Immediately, the alcian-blue solution flowed into the lymph node and slowly exited at an abdominal lymph vessel. This indicates that the primo vessels may form a vascular system circulating from one abdominal lymph node to another lymph node, as shown in Fig. 4. Long lymph vessels of a few millimeters in length and small lymph vessels of a few hundred micrometers were observed. Fig. 4A shows two primo vessels and one suspected primo node (red arrow), Fig. 4B one primo vessel, and Fig. 4C several suspected primo nodes (red arrows) branching from one primo vessel prior to its isolation from the lymph vessel. The extracted and isolated primo vessels were, on average, about 40 mm long.

In this study, to find out the characteristics of the primo vessels with nuclear vascular structures, we washed and placed them on a slide for staining with acridine orange pigment and 4',6-diamidino-2-phenylindole dihydrochloride, in order to make observations using confocal laser scanning microscopy. The nuclear images obtained were analyzed to determine the size and the shape of a typical primo vessel [15,16].

Figure 3 Six-step process for selecting a lymph node for the isolation of a primo vessel: (A) rabbit’s lymph node prior to alcian-blue injection, (B) lymphatic primo vessels stained with alcian-blue and floating in a lymph vessel, (C) injection of alcian-blue dye, (D) observation of a lymph vessel (long, white broken lines) and a primo vessel (blue dotted lines), (E) extraction of a primo vessel, and (F) isolation of a primo vessel.
Figure 4  Observation of several typical primo vessels from a rabbit after applying sound and ultrasonic waves of frequencies of 7 Hz and 2 MHz and intensities of 50 W/cm² and 60 W/cm², respectively: (A) two primo vessels and one suspected primo node (red arrow), (B) one primo vessel, and (C) several suspected primo nodes (red arrows) branching from one primo vessel prior to isolation of the primo vessel from the lymph vessel. A long lymph vessel having a size of a few millimeters and a small lymph vessel having a size of a few hundred micrometers are seen. The primo vessel exists inside a lymph vessel of the same length.

Figure 5  (A) Optical microscopic image of an abdominal lymph vessel in the neighboring region of a rabbit’s caudal vena cava after treatment with sound and ultrasonic waves. This image shows rabbit lymph bundles prior to alcian-blue injection. (B) A magnified optical microscope image of the same specimen (shown in the inset in Fig. 5A), shows a strand-like microtubular PVS stained with alcian-blue floating inside a lymph vessel. (C) Image obtained using acridine orange and DAPI nuclear staining. The sample taken from the lymph vessel was placed on a slide after having been washed with PBS. DAPI = 4′,6-diamidino-2-phenylindole dihydrochloride; PBS = phosphate buffer solution; PVS = primovascular system.
As mentioned earlier, not much can be said about the structural characteristics of the primo vessels could not be identified for certain. However, whether any frequency band had an effect could be stochastically determined. Structural characteristics of the primo vessels may change depending on the condition of the rabbit. As seen in Table 1, in experiments 1 and 2, a stimulation intensity of 20 W/cm² was used. Primo vessels were found at 7 Hz and 14 Hz, but not at 4 Hz and 30 Hz. The first experiment was the same as the second one, with a frequency of 7 Hz and an intensity of 50 W/cm². Primo vessels were found in the first and second experiments. In conclusion, primo vessels were observed when sound waves in the frequency band of 7–14 Hz were applied to the rabbits, and frequencies of 7 Hz, 11 Hz, and 14 Hz were found to be beneficial for finding and extracting the primo vessels.

As mentioned earlier, not much can be said about the experimental reproducibility. At frequencies of 7–11 Hz, primo vessels were found, but more objective tests are needed. Although the number of experiments was not large, the use of frequencies of 7–14 Hz for isolating the primo vessels matches with the findings of the second experiment. In the second experiment, as in the first experiment, frequencies of 7–14 Hz and an intensity of 60 W/cm² were found to be the best. An ultrasonic sound wave was applied after anesthesia and shaving. The results are also shown in Table 1. The average thickness and length of the primo- and lymph-vessel tube were found to be approximately 28 μm, 39 mm and 374 μm, respectively.

We tried to determine how a vibrating sound wave acting on the abdomen could activate the lymphatic circulatory system at a certain wavelength. To specify the characteristics of the sound that affected the primo vessel, we designed more experiments and found that frequencies of 7–14 Hz increased the probability of discovering a primo vessel. When no sound wave was applied, a primo-vessel tube was discovered in five of the 12 experiments (42% probability of success). However, when sound waves of frequencies in the range 7–14 Hz were applied, the probability of successfully isolating a primo vessel increased to 90.0%.

This experiment with sound waves caused primo vessels to oscillate the increase probability of visualizing the morphological structure. For the study of this circulatory system, this method for finding a basic primo vessel is very helpful. This study, which involved these experiments on the primo circulatory system, has made a significant contribution to efforts to develop a basic diagnostic protocol in Oriental medicine.

### 4. Discussion

The existence of primo vessels was demonstrated through a rabbit dissection experiment, and the morphological structural characteristics of those vessels were observed using sound and ultrasonic waves. Prior to the dissection experiment, rabbits were placed inside of a cage that had been placed on a sound-wave vibration apparatus for treatment with sound waves at various frequencies and intensities for 30 minutes. After the rabbits had been anesthetized and shaved, they were exposed to an ultrasonic wave with an intensity of 60 W/cm² and a frequency of 2 MHz. The primo vessels were subjected to sound and ultrasonic waves, and the effect on the activity of the lymph vessels was investigated. The probability of isolating a primo vessel at a specific frequency and intensity was two times that without using a sound wave. In other words, the

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**Table 1** Morphological structure and observation of the primo vessel after treatment with sound and ultrasonic waves.

<table>
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<th>Subject number</th>
<th>Weight (kg)</th>
<th>Sound wave intensity (W/cm²)</th>
<th>Frequency (Hz)</th>
<th>Ultrasonic wave intensity (W/cm²)</th>
<th>Frequency (MHz)</th>
<th>Primo vessel diameter (μm)</th>
<th>Lymph vessel diameter (μm)</th>
<th>Lymph vessel length (mm)</th>
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$L_D$ = diameter of the lymph vessel; $P_D$ = diameter of the primo vessel; $P_L$ = length of the primo vessel.

Fig. 5 shows an optical microscopic image of an abdominal lymph vessel in the region of a rabbit’s caudal vena cava after treatment with an ultrasonic wave. Fig. 5B, which is a magnified optical microscopic image of the same specimen (shown in the inset of Fig. 5A), shows a strand-like microtubular PVS stained with alcian-blue and floating inside a lymph vessel. The magnified image of the inset in Fig. 5A after nuclear staining with acridine orange and 4’,6-diamidino-2-phenylindole dihydrochloride is shown in Fig. 5C. The sample taken from the lymph vessel was placed on a slide after having been washed with PBS. The data for the primo vessels are presented in Table 1. These results demonstrated that the use of sound and ultrasonic waves improved our ability to isolate primo vessels.
probability of observing a primo vessel was increased to 90%. Therefore, the use of sound- and ultrasonic-wave vibration apparatuses is thought to be necessary in order to reproducibly isolate and observe primo vessels.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

Acknowledgments

This research was supported by the National Research Foundation of Korea (2011-0007552).

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