Development of a fluorescence stereomicroscope and observation of Bong-Han corpuscles inside blood vessels

Byung-Cheon Lee1,2, Jung Sun Yoo1, Ku Youn Baik1, Baeckkyoung Sung1, Jawoong Lee* & Kwang-Sup Soh†

1 Biomedical Physics Laboratory, Department of Physics and Astronomy, Seoul National University, Seoul, Korea
2 Research Institute of Basic Sciences, Seoul National University, Seoul, Korea

A fluorescence stereomicroscope system was developed in order to observe in situ the distribution of nuclei in intravascular Bong-Han ducts and corpuscle tissues by injecting acridine orange, which stained specifically nuclei. Intravascular Bong-Han corpuscles, connected with Bong-Han ducts could be detected in the aortas of rats, mice, and rabbits.

Keywords: Acupuncture, Blood vessel, Bong-Han corpuscle, Bong-Han duct, Fluorescence stereomicroscope, Hematopoiesis

Acupuncture is one of most effective complementary and alternative medical practices. Needling at certain points in human or animal skin provides therapeutic effects for various acute or chronic diseases. These specific points are called acupoints, and the lines connecting them are called acupuncture meridians. Twenty-four major meridian lines in the human body are thought to control the functions of internal organs.

Even though acupuncture has been practiced for thousands of years as a traditional medicine, the nature of acupoints and meridians remains a mystery. It is not known whether they are real and physical structures or an imaginary and functional complex of the nervous system. Therefore, the announcement in the early 1960’s by Bong-Han Kim of North Korea of the discovery of anatomical structures corresponding to the acupoints and the meridians was considered a surprising and epoch-making breakthrough. He claimed that ducts through which a liquid flowed (Bong-Han duct) and corpuscles (Bong-Han corpuscle) existed at classical meridians and acupoints, respectively, in the skin of animals. Further, he discovered that the ducts and the corpuscles formed a network distributed throughout an animal’s body and that they existed even inside large blood vessels and lymph vessels.

Unfortunately, his work was ignored for a long time and was not confirmed by other groups. The only exception was a Japanese anatomist, Fujiwara, who was able to reproduce the observation of Bong-Han ducts and corpuscles in rabbits, yet his work did not get attention in Western medicine either. Only recently has the Bong-Han theory been re-illuminated by several groups. Intravascular Bong-Han ducts (BHDs) were observed inside the blood vessels of rabbits, rats, and pigs, and on the surfaces of the internal organs of rats and rabbits. Intravascular BHDs were also detected inside lymphatic vessels by using the staining materials such as Janus Green B, fluorescent nanoparticles, and Alcian blue.

In previous studies, intravascular BHDs in blood vessels were obtained by using a local perfusion method. Thus, in situ observation was not possible, and the Bong-Han corpuscle (BHC) was hard to detect. In the present study, a new system has been developed to observe in situ intravascular BHDs in the blood vessels of rabbits, rats, and mice. A homemade fluorescent stereomicroscope (FSM) system has allowed us to detect the sparsely located BHCs by injecting a fluorescent dye, acridine orange, into the blood vessels. Construction of the FSM system and on its use to observe BHCs is reported here.

According to Kim’s work, which has yet to be investigated, the physiological function of the BHD and BHC was hematopoiesis. Another function is to transport and produce neurotransmitter hormone, like catecholamine, which was confirmed by using a
biochemical measurement of adrenalin and noradrenalin in a BHC\textsuperscript{17} and by measuring the flow speed of the liquid in BHDs on the surfaces of the internal organs of rabbits\textsuperscript{18,19}. Soh\textsuperscript{20} has proposed still another hypothesis on the role of the acupuncture meridian or the BHD: the BHD is an optical communication channel to keep coherence in the whole body by utilizing biophotons produced in cellular processes\textsuperscript{20}.

Materials and Methods

ICR mice of 6-8 weeks, Sprague-Dawley rats of 6-8 weeks, and New Zealand White rabbits of 12 weeks were obtained from the Laboratory Animal Center of Seoul National University, SCL of Japan, and Chungang Experimental Animal Center, respectively. The animals were housed in a constant temperature-controlled environment (23°C) with 60% RH. All the animals were fixed on a 12 hr light-dark cycle and had \textit{ad libitum} access to food and water. Procedures involving animals and their care conformed to institutional guidelines, which were in full compliance with current international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996).

All the animals were anaesthetized with urethane (1.5 g/kg) administered intraperitoneally, and all surgical procedures were performed under general anaesthesia. Under deep anaesthesia, the abdominal sides of the mice, rats, and rabbits were incised, and the stomachs, intestines, and perivascular fats were moved to one side. The diaphragm of each animal was opened, and the target blood vessel for this study, the thoracic aorta, was isolated as soon as possible. The isolated thoracic aorta was dipped into a phosphate-buffered saline solution (PBS; $pH$ 7.4) contained in a Petri dish. The wet thoracic aorta was incised with micro-scissors on a rubber plate.

For mice and rats, a homemade fluorescence stereomicroscope (FSM) was used to search for corpuscular structures after staining the incised aorta by 0.1% (v/v) acridine orange (fluorescence stereoscopic method). Acridine orange selectively stains DNA in the tissue and emits a green light of 520 nm when it is excited by blue light at 487 nm. In order to find the novel corpuscular structures in rats and mice, this DNA-staining fluorescence dye was used. For this experiment a fluorescence stereomicroscope was developed by attaching a light source designed for the experimental purposes to a typical stereomicroscope. Figure 1 is a schematic of the constructed fluorescence stereomicroscope. The zooming stereomicroscope multiplies the image of a sample from 10× to 200×. The excitation light source consists of a mercury arc lamp of 200 W and a band pass filter, which permits the passage of wavelengths from 450 to 490 nm. An emission filter was attached to one side of the objective to allow the passage of fluorescent green light [emissions] from the sample through the objective, but to reject the excitation light scattered from the sample. The emission filter is a long-wavelength pass filter whose pass-on wavelength is 515 nm and whose rising edge interval is less than 10 nm. The excitation light from the source was guided to the sample by using an optical glass fiber bundle and coupling optics. An optical fiber bundle of 3 mm in diameter is covered by 0.5 mm-thick stainless-steel [tubing], creating a stiff tip of 60 mm in length at the side of sample. The tip is mounted on a micro-manipulator in order to control the direction and the position of the illuminating light. The delicately controllable localized illumination through the miniature optical fiber tip is more efficient than epi-fluorescence illumination through the objective when very complex three-dimensional \textit{in vivo} samples were investigated.

Results

A homemade fluorescence stereomicroscope (FSM) system that was specifically suitable for observation of a Bong-Han corpuscle (BHC) inside blood vessels of mice and rats was developed. The stereomicroscope was augmented with an emission filter (520 nm) that suited the green light emitted from the staining dye acridine orange (Fig. 1). The illumination system was a miniature fiber tip controlled by a micromanipulator, and the light source was a mercury lamp, which was equipped with an excitation filter (487 nm) and excited the acridine orange. The filtered blue light was coupled to the optical fiber of the illumination system. This FSM made it possible to observe BHCs \textit{in situ} inside blood vessels.

Figure 2 is an image of two corpuscles (arrowheads) in a mouse aorta that was layed open on a black plate after it had been taken from the body. The corpuscles were connected by a Bong-Han duct (BHD; indicated by arrows). The incised boundaries of the aorta are shown by the thick arrows. The contrast of the BHC and the BHD against the inner surface of the aorta was not good because the fluorescence dye (acridine orange) also stained the
However, the BHC and the BHD were discernible from the background. The diameters of the BHCs were about $110 \times 65$ µm and $55 \times 40$ µm, respectively, and the diameter of the connecting BHD was $10 \mu m - 20 \mu m$.

Similarly, a BHC was observed in the aorta of a rat, (Figs 3A and B). The FSM image and the confocal laser scanning microscopic images in Figs 3A and 3B, respectively, show BHCs with sizes of $1010 \times 510$ µm and $1100 \times 400$ µm. The BHCs were connected with Bonghan ducts (arrowheads) at both ends.
A BHC was also detected inside a rabbit thoracic aorta without using the FSM. After local perfusion, the BHC looked like a blood-coagulated body (Fig. 4A). This structure was examined after Janus Green B staining dye had been applied, and its outer boundary was stained (Fig. 4B). A confocal laser scanning microscopic image of a section of the same sample shown in Figs 4A and 4B, which had been stained with propidium iodide (PI), is presented in Fig. 4C. Rod-shaped nuclei (arrows) were present, as were many sinuses.

Discussion
The presence of intravascular Bong-Han ducts (BHD) has been confirmed in the blood vessels of rabbits3-5, rats6, and pigs7, and in the large lymphatic vessels of rats8,9 and rabbits10,11. In these works, a perfusion technique was used, so no in-situ observation of the intravascular BHD was possible. The significance of the present work is the development of a fluorescence stereomicroscopic system with which in situ detection of the intravascular BHD is possible. In addition, the present work focused on the observation of the Bong-Han corpuscle (BHC), a voluminous oval-shaped body connected to BHDs at both its ends, which had not been studied in previous works3-7. The existence of such BHCs sparsely distributed along the BHD is one of the characteristic features of the intravascular BH system, as described in Kim’s earlier paper1. Hence, the present work can be considered to be strongly supportive of the BH theory, which is still not widely recognized after its long neglect.

A frequent question about the intravascular BHD and BHC is why surgeons have not noticed such objects. The answer is the indistinguishability between a BHD and the fibrin strings formed during blood coagulation. They look so similar that they are not discernible, not even under a microscope. Thus without careful examination, surgeons assume any threadlike structure to be fibrin strings. Earlier a method using a fluorescent DNA-staining dye, acridine orange, that distinguished the BHD from the...
Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R0A-2003-000-10371-0) and by the Ministry of Science and Technology through the Cavendish-KAIST Cooperative Research Program. The authors are thankful to Miss Eun-Jung Kang, The National Center for Inter-University Research Facilities for clear confocal laser scanning microscopy.

References


