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BRIEF REPORT

Primo-vessels and Primo-nodes in Rat Brain, Spine and Sciatic Nerve

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Abstract
We report a method using Trypan blue staining to detect primo-vessels in the nervous system on internal organs or in the skin of rat. We applied this technique to visualize the primo-vessels and primo-nodes in the brain, spinal cord and sciatic nerve of a rat. Primo-vessels and primo-nodes were preferentially stained at nerves, blood vessels, or fascia-like membranes and turned blue after the spread and washing of Trypan blue. The physiological role of the primo-vessels within the nervous system is an important question warranting further investigation.

1. Introduction

Primo-vessels (Bonghan ducts) and primo-nodes (Bonghan corpuscles) are anatomical structures, which were first observed by Bong-Han Kim in the early 1960s [1], but these structures have been neglected since then as no one has been able to reproduce observations made by Bonghan Kim. By applying modern technology, primo-vessels and nodes have recently been rediscovered on the surfaces of abdominal organs [2–8], in the blood vessels [9,10], lymph vessels [11,12] and brain ventricles [13] of mice, rats, and rabbits. According to Bong-Han Kim's claim [1], and our own previous experience [2–13], the primo-vascular system was expected to exist in the perineurium and the epineurium of a nerve or in the meninges of the brain and the spine.

We could only confirm this hypothesis using Trypan-blue staining that specifically revealed the primo-vascular system from among blood or lymph vessels, nerves, muscles, and various membranes [5,8]. In the present brief report, we describe the method used to visualize the primo-vessels and nodes in the arachnoid mater of the brain, the perineurium of the spinal cord, and the perineurium and the endoneurium of the sciatic nerve of a rat by using Trypan blue. Considering the close relationship between acupuncture and the nervous system, in terms of either pain [14] or hypertension [15], a primo-vascular system in the fascia wrapping nerve tissues

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leads to a range of questions regarding its physiological role in acupuncture treatment.

2. Materials and Methods

Male Wistar/Sprague-Dawley rats (200 g; Jung-Ang Laboratory Animal Co., Seoul, Korea) were housed at 23°C and 60% relative humidity under a 12-hour light/dark cycle with *ad libitum* access to food and water. Animals were handled in accordance with the Guidelines of the Laboratory Animal Care Advisory Committee of Seoul National University.

To visualize primo-vessels and primo-nodes, we injected 0.2% Trypan blue (TB; Sigma-Aldrich Co., St-Louis, MO, USA) into the brain, spinal cord and sciatic nerve of a rat. For injection of TB into a live brain and spinal cord, we removed the rat brain and, as soon as possible, injected about 0.2 mL of 0.2% TB into the brain stem and cervical spinal cord. For TB injection into the sciatic nerve, we cut across a part of the sciatic nerve and injected about 0.2 mL of 0.2% TB into the cut portion of the sciatic nerve and washed it twice with saline injection.

After staining the brain, spinal cord and sciatic nerve with TB, these structures were carefully dissected under a stereomicroscope (SZX-12; Olympus Co., Tokyo, Japan). The primo-vessels and primo-nodes in the brain, spinal cord and sciatic nerve were found to be TB stained, and their images were taken by using a CCD camera (DP 70, Olympus Co.). We isolated the TB-stained primo-vessels and primo-nodes for microscopic examination. After staining the isolated primo-vessels and primo-nodes with phalloidin (Molecular Probe, Eugene, OR, USA) and DAPI (Molecular Probe), we examined them by using fluorescence microscopy (Olympus Co.) and confocal laser scanning microscopy (LSM 510; Carl Zeiss Inc., Oberkochen, Germany).

3. Results

Figure 1 demonstrates primo-nodes and primo-vessels on the arachnoid mater in the brain of a rat. The primo-node was also visualized in the fourth ventricle of the rat. Networks of primo-vessels on the surface of the cerebellum of the rat were also

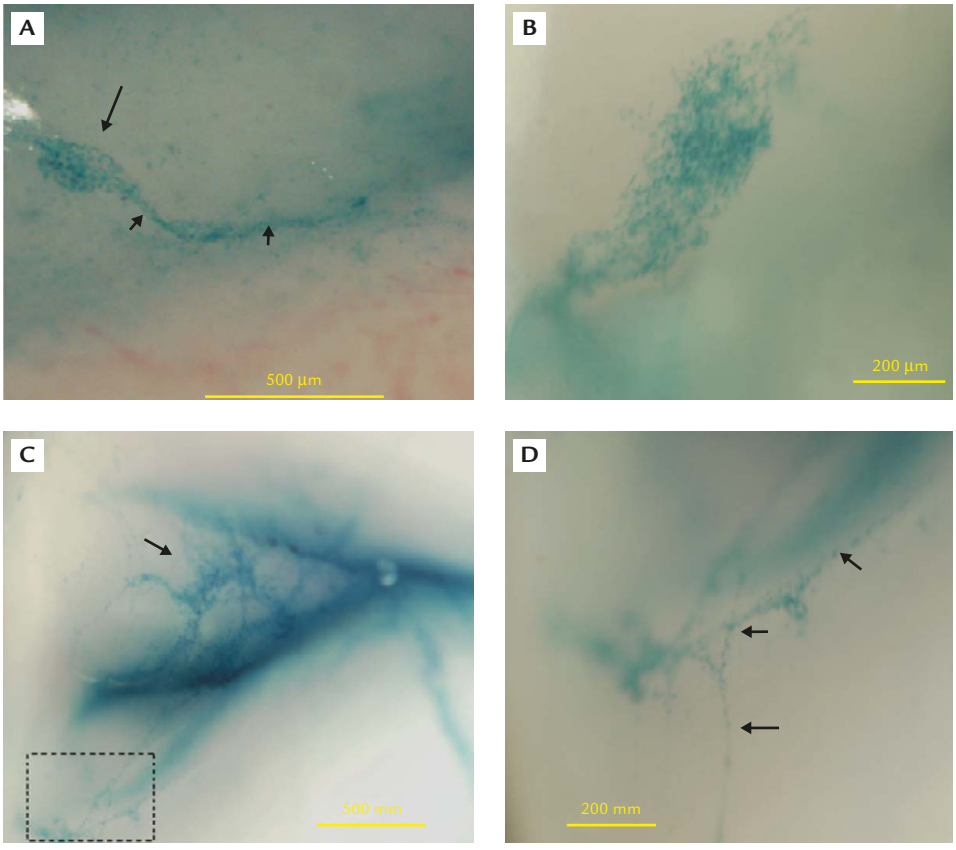

Figure 1 Visualization of primo-nodes and primo-vessels in the rat brain. (A) Primo-nodes (thick arrow) and primo-vessels (two thin arrows) were visualized in the arachnoid mater. Capillaries below these primo structures were notably not stained. (B) A primo-node was visualized in the fourth ventricle. (C) Networks of primo-vessels (arrow) on the surface of the cerebellum. (D) One of the networks (dotted rectangle) is magnified and reveals that primo-vessels form fine networks (arrows).

noticeable. We also noticed that blood vessels under the arachnoid mater were not stained by TB.

As shown in Figure 2 primo-nodes and primo-vessels stained by TB also emerged in the rat spinal cord. A small primo-node with primo-vessels and a large primo-node were visualized around the perineurium in the spinal cord.

Figure 3 shows primo-vessels in the epineurium, perineurium and the endoneurium of a rat sciatic nerve. Primo-vessels were visualized in the epineurium and perineurium of rat; however, blood capillaries inside the perineurium were noticeably not stained by TB. The primo-vessel around the endoneurium was also visualized.

To distinguish primo-vessels from nerve bundles, we isolated primo-vessels from the sciatic nerve of rat after TB injection and stained them using phalloidin for F-actins and 4′,6-diamidino-2-phenylindole, or DAPI for nuclei. Figure 4A shows a fluorescence microscopic image of DAPI-stained nuclei of the sciatic nerve and the primo-vessel. The inset also shows that the primo-vessel contains F-actins (green fluorescence) stained by phalloidin; however, the sciatic nerve has no F-actin signal. Figure 4B presents a confocal laser scanning microscopic image showing that one fine primo-vessel in the sciatic nerve of a rat consists of a bundle of F-actin with nuclei.

4. Discussion

A visualization method using TB was developed to reveal the primo-vascular system in the rat nervous system. The primo-vessels and primo-nodes were found on the arachnoid mater of the brain, on the surface of the cerebellum, in the perineurium of the spinal cord, and in the perineurium and the endoneurium of the sciatic nerve.

The locations of the nerve primo-vessels and primo-nodes we observed are in agreement with those that Bong-Han Kim claimed to have found [1]. In broad terms, the location is in the fascia, which

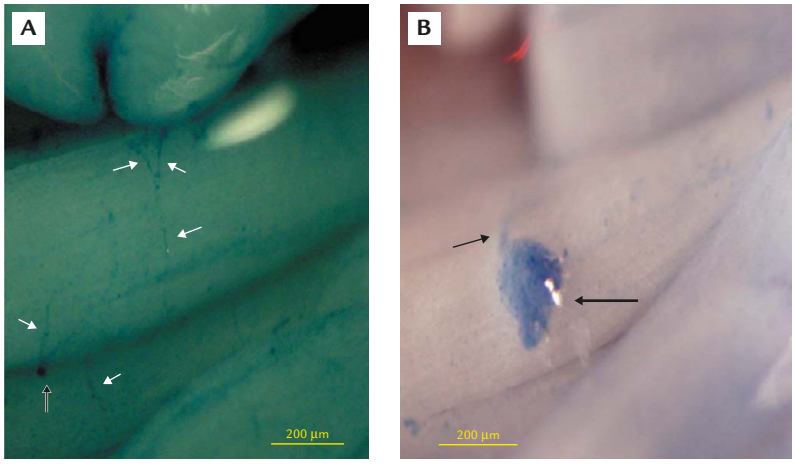

Figure 2 Visualization of primo-nodes and primo-vessels in the rat spinal cord. (A) A small primo-node (black arrow) with primo-vessels (white arrows) was visualized around the perineurium of the spinal cord. (B) A large primo-node (thick arrow) with a primo-vessel (thin arrow) was also found around the perineurium of the spinal cord.

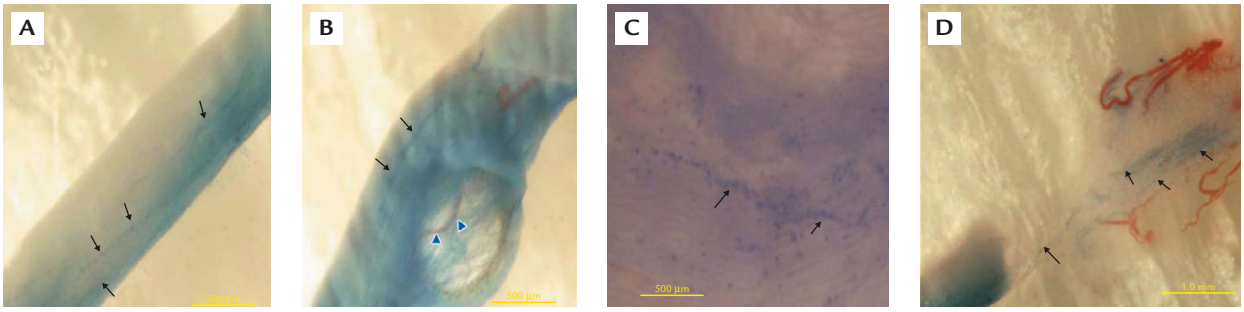

Figure 3 Visualization of primo-vessels in the perineurium, endoneurium and epineurium of a rat sciatic nerve. (A) primo-vessels (four arrows) were visualized in the perineurium. (B) A primo-vessel (two arrows) was visualized; however, a blood capillary (two triangles) inside the perineurium was not stained by Trypan blue. (C) A primo-vessel (two arrows) was visualized around the endoneurium of the sciatic nerve. (D) Primo-vessels (four arrows) were visualized in the rat epineurium. Blood capillaries were not stained by Trypan blue.

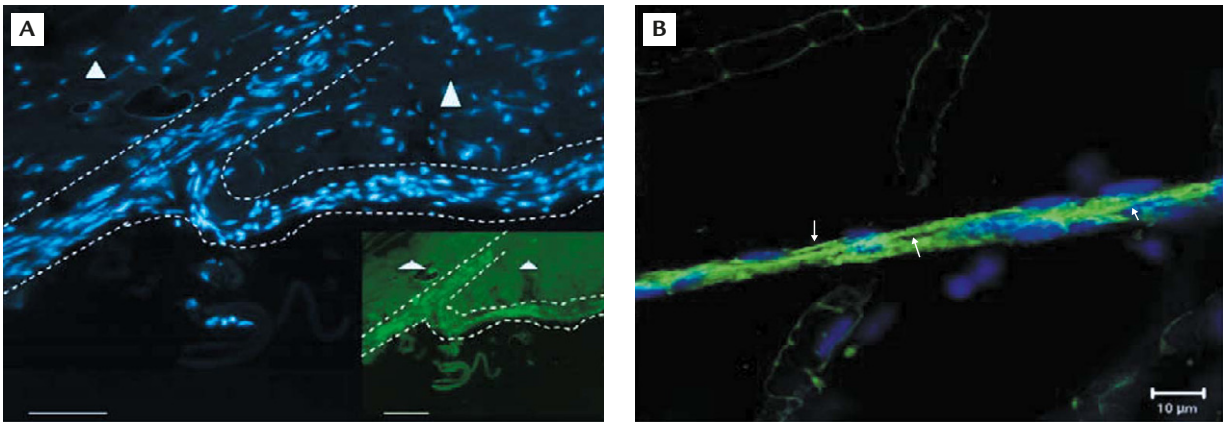

Figure 4 Distribution of F-actin and nuclei of primo-vessels in rat sciatic nerve. (A) Fluorescence microscopic image of 4′,6-diamidino-2-phenylindole, or DAPI-stained nuclei of the sciatic nerve (triangles) and a primo-vessel (between dotted lines). The inset shows a primo-vessel (between dotted lines) containing F-actin (green fluorescence) stained by phalloidin; however, the sciatic nerve (triangles) has no clear F-actin signal. (B) Confocal laser microscopy clearly demonstrates a fine primo-vessel consisting of a bundle of F-actin (arrow, green fluorescence) with DAPI-stained nuclei (blue fluorescence) in the sciatic nerve of a rat. Nerve fibers scattered around the primo-vessel emit a weak signal due to F-actin on its boundary.

is the fibrous connective tissue in the membrane wrapping various organs, in our case the nerves. Acupuncture meridians were considered as a part of fascia in anatomy [16], and "fascialogy" was proposed as a generalized acupuncture-related physiology [17]. A control function of the fascia on the human body has been proposed by Becker [18], suggesting a duality of the nerve and perineurium systems; Oschman [19] also concurs. Acupuncture treatment of pain [14] or hypertension [15] is understood to occur via nerves. In view of our observation we propose that the primo-vascular system may be involved in the acupuncture effects on the epineurium, perineurium and endoneurium, especially as Bong-Han Kim claimed that the primo-vascular system is the anatomical substance of acupuncture meridians [20]. Interestingly, Lee and Soh [21] very recently insisted on a novel model, "Bonghan-Fascia Model" for understanding the acupuncture meridian system, which is that almost all primo-vascular system is embedded in fascia loose connective tissues. Further investigations are required to test his claims.

The primo-vessels and primo-nodes in rat nerves have similar characteristics to those in rabbit brain ventricles [13], on the surfaces of rabbit, rat and mouse internal organs [2–6], on fascia wrapping cancer tissue [7], and in the hypodermis of rats [8]. Primo-vessels and primo-nodes are preferentially stained by TB and their nuclei are rod-shaped and aligned in broken-lines. Primo-vessels and primo-nodes also have bundles of actin-containing structures.

This current work focused on the visualization method used to reveal a novel structure within the nervous system, which is unknown in Western medicine. Finding its function requires immediate investigation. The presence of actin, for example, suggests the structure's contractibility, which is necessary for the flow of fluid, and this can be tested with an electrophysiology study. Another point of interest is that the dual function of the perineurium suggested by Becker [18] and Oschman [19] may be related to the primo-vascular system in the perineurium. A limitation of the current work is the lack of a histological method to detect the primo-vessels or primo-nodes without TB staining.

Acknowledgments

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