



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 (200g; 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

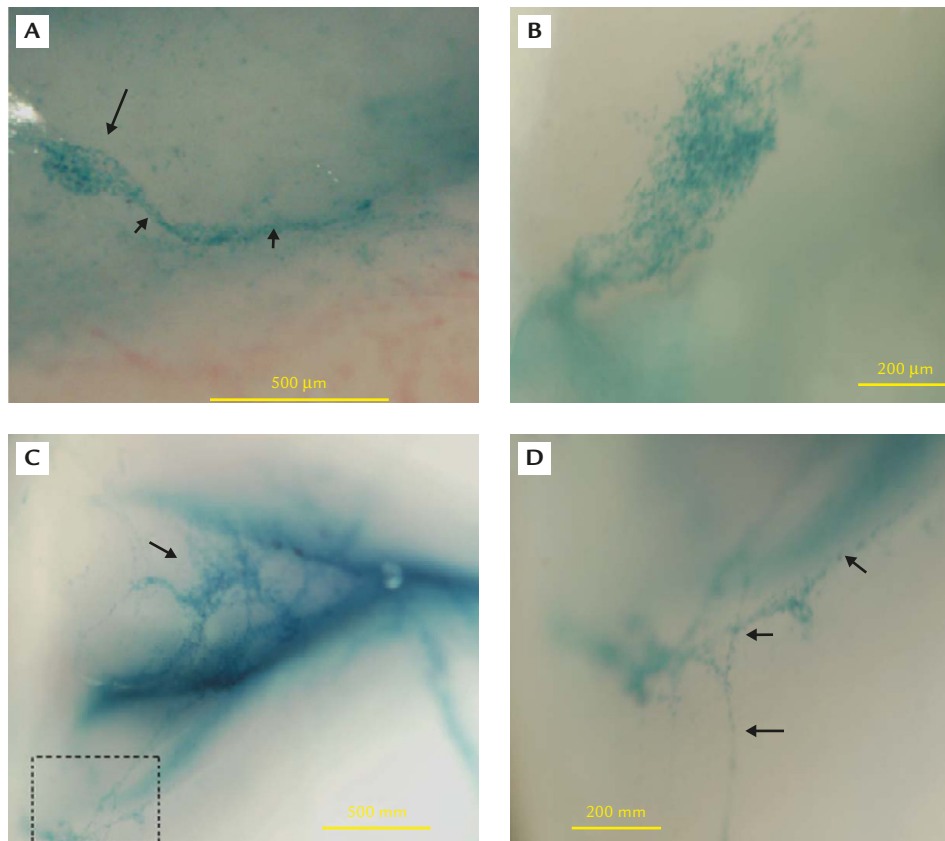


Figure 1 Visualization of primo-nodes and primo-vessels in the rat brain. (A) Primo-nodes (thick arrow) and primo-vessels (two thin arrows) were visualized in the arachnoid mater. Capillaries below these primo structures were notably not stained. (B) A primo-node was visualized in the fourth ventricle. (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).

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