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RESEARCH ARTICLE

Isolation and Morphological Features of Primo Vessels in Rabbit Lymph Vessels

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Abstract

Until now, even though intensive research has been dedicated to the primo vascular system (PVS) during these years, no statistical data on primo vessels and primo vessels in lymph flow have been available. Recently, the general morphological features of primo vessels in lymph vessels around the abdominal aorta were identified from microdissections of tissues from New Zealand White rabbits, and with Alcian blue staining, primo vessels in lymphatic vessels could be definitely identified under a digital microscope. The micro-dissected specimens *in situ* reveal rod-shaped nuclei stained by Acridine orange. The blue-stained nuclei, which were distributed in a broken-lined stripe, formed a tube structure of about 20 μm in diameter. The distance between the nuclei of two cells on neighboring aligned stripes, which is also the diameter of the micro tube, was measured to be about 5~10 μm . The average length of the primo vessels was 2.4 mm, with the longest being 5.6 mm. The average size of the primo vessel was 50 μm , and the average diameters of the primo and the lymph vessels were 26.0 μm and 258.5 μm , respectively. Occasionally, without the use of Alcian blue staining, milk-white transparent primo vessels were observed floating in lymph vessels. Thus, we suggest that the PVS might also have an important function connected with the lymph system. We also expect the traditional Korean meridian system to leave its invisible world during the last thousands of years and soon enter the visible scientific world.

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1. Introduction

In advanced medical history, the new discovery of a third circulating system in addition to the blood system and the lymphatic system should change the paradigm of medicine and biology. Revelation of the undisclosed structures and functions of the third circulating system would be intensely important research that could lead to a revolution in medicine, having a much bigger impact than any other medical discovery [1]. With its modes, the third circulating system, in addition to the autonomic nervous system and the hormonal system, in the human body could provide an approach to various chronic diseases such as cancer, diabetes, and hypertension [2–4].

In the perspective that the substance of pipe, which is the path of spirit, exists, the two most important two things for the human body to be are widely known to be spirit and blood. In addition, the blood, which wanders everywhere in the body through blood vessels, supplies fresh air and sufficient nutrient to cells, and the meridian system, which is comprised of invisible pathways through which Ki (Qi, Chi, life energy) circulates throughout the body, has been regarded as the basis of disease control for thousands of years. Bonghan theory, proposed by Bong-Han Kim of North Korea to the world in the early 1960s, suggested that not only was the acupunctural meridian system another anatomic structure just like the lymphatic system and the blood system, but it was also completely different from those two systems [5–7].

Now, 40 years after the initial publication of Bonghan theory, the PVS (primo vascular system) has been found connecting with acupunctural points in mammalian internal organs, such as blood vessels, lymph vessels, spinal cords, brain ventricles, fascia, and skin, by a Seoul National University research group [1–4]. Primo vessels have been found, by staining with dyes such as Alcian blue, Acridine orange, and Trypan blue, to be spread on the surfaces of all of the abdominal organs [2]. These primo vessels are not adhered to the lymph walls, but are floating in lymph fluid, and the Sanal micro-cells that flow in the primo vessels might have an executive ability in the primo function [3,4].

For this study, we selected abdominal lymph vessels distributed in the region neighboring a rabbit's caudal vena cava. This region included many PVS vessels, and those

vessels were the best to observe from among several lymphatic systems. We were able by using some microsurgical procedures on the lymphatic vessels around the abdominal aortas of rabbits during Alcian blue staining to find enough evidence to identify easily the primo vessels under a digital stereo zoom microscope [8,9].

2. Materials and methods

2.1. Animals

For the laboratory animals, ten New Zealand white female rabbits (approximately 2 kg) were purchased from Nara-Biotech Animal Company (Seoul, Korea). Two rabbits were sacrificed for anatomy experiments in the first week, and the other 10 rabbits in the first month. Each rabbit was kept in constant temperature and humidity conditions (23°C, relative humidity 60%), with a 12-hour light-dark cycle. All animals were deprived of food and water for 1 day before anatomy. All procedures conformed to the ethical regulations for animal experiments constituted by the institutional regulation board of Sangji University [approval number: 2012-1].

2.2. Anatomy

The rabbits used in the anatomical experiment were sacrificed by intraperitoneal injecting 1.5 g/kg of urethane or Zolatil into the peritoneum. The adipose tissue surrounding the inferior vena cava was then separated and removed. Next, inside the inferior vena cava, the PVS, which had been stained blue, was visualized [10]. Images of the PVS under a stereomicroscope (SZX12; Olympus, Tokyo, Japan) were captured using a charge coupled device camera (DP70; Olympus, Tokyo, Japan). In all other process of dissection was done with general circumstances of anesthesia [11].

2.3. Staining and microscopy

Alcian blue solution was prepared from 0.1 g of Alcian blue (Sigma, St. Louis, MO, USA) in 10 ml of phosphate-buffered saline (PBS, pH 7.4) and was filtered by using a 0.45- μ m membrane filter (Merck Millipore, Darmstadt, Germany) with a syringe (BD, Franklin Lakes, NJ, USA). After the sides of the rabbits' abdomens had been incised, Alcian blue solution, preheated to 37°C in a water bath, was injected into a lymph vessel or a primo vessel.

Acridine orange and 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) nuclear staining were carried out as follows: To discriminate primo vessels from lymphatic vessels, we fixed the

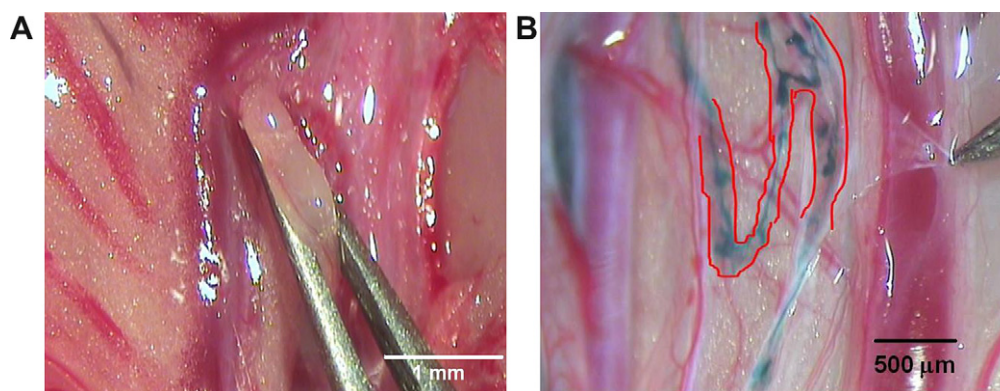


Figure 1 (A) Rabbit lymph vessels before Alcian blue injection; (B) lymphatic primo vessels stained by Alcian blue and floating in lymph vessels (red solid lines).

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