Study of Novel Threadlike Structures on the Intestinal Fascia of Dogs

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Abstract

The primo-vascular system was visualized in the mesentery surrounding the small intestine of a dog using Trypan blue. This structure, which was first observed in a rat, formed a network as primo-vessel branches were joined to primo-nodes. Other characteristic features of the primo-vascular system, such as bundles of tubes with fibrous extracellular matrix in a primo-vessel and a broken-line alignment of rod-shaped nuclei along the primo-vessel, were observed. Blood vessels, lymph vessels, and primo-vessels were present in the same mesentery, and they could clearly be distinguished by histological differences.

KEY WORDS:
dog; fascia; mesentery; primo-node; primo-vascular system; primo-vessel (Bonghan duct)

1. Introduction

The primo-vascular system (PVS), a newly described circulatory system that corresponds to acupuncture meridians, was first proposed by Bong-Han Kim [1] in the early 1960s and partially confirmed by Fujiwara and Yu [2]. However, Kim’s claims have been long neglected, because his results could not be reproduced despite many attempts by research groups in China.

Only recently has an intensive reinvestigation [3] of the PVS slowly revealed its presence in various parts of the body, such as inside blood vessels [4], bovine hearts [5], lymph vessels [6–8], brain ventricles, central canal of the spinal cord [9], along the sciatic nerve [10], adipose tissues [11], and on the surfaces of intestinal organs [12] and tumor tissues [13]. The PVS was observed primarily in small animals, such as rabbits, rats, and mice. The only large animal was a cow, in which a PVS network was observed in the bovine heart [5].

In this work, we observed a PVS network in the mesentery surrounding the intestinal organs of dogs. To the best of our knowledge, this is the first observation of the PVS in dogs. Kim, Fujiwara, Soh, and other groups primarily investigated rabbits, rats, and mice, but published no reports on dogs. The PVS network is similar to the network seen on the omentum below the stomach and over the small intestine of rats [14]. The characteristic features of the PVS in small animals, like the distribution of rod-shaped nuclei, were confirmed in the PVS of dogs, and were distinct from those of blood or lymph vessels.
The observation of the PVS in dogs is significant because it suggests that other animals, including humans, have a PVS. In addition, a larger animal will make further analysis more practical, because a physiological analysis usually requires a large amount of PVS tissue. Furthermore, the clinical implications of the PVS can be investigated in dogs before human studies are begun.

2. Materials and Methods

The dogs (male Beagles, 1–2 years old, 2.8–5.0 kg) were obtained from Aijian Dog Company (Harbin, China). Five Beagle dogs were used in this study and examined to ensure that they were in good health. The animals were housed in a constant-temperature environment (26ºC) with 60% relative humidity under a 12-hour light/dark cycle. The dogs were given ad libitum access to food and water. Animal procedures were carried out in accordance with international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 2010, ISBN-10: 0-309-15400-6). The dogs were anesthetized with zolazepam-tiletamine (Virbac, France) administered by intraperitoneal injection (1.5 mL/kg body weight), and all surgical procedures were performed under systemic anesthesia.

Under deep anesthesia, we cut the medial alba of the abdomen carefully to prevent the skin from bleeding and exposed the internal organs. We then used a 0.4% Trypan blue solution (Sigma-Aldrich Co., St-Louis, MO, USA) to stain the primo-vessels on the small intestine, liver, and stomach. After about 15 seconds, we washed the internal organs several times with physiological saline. We were able to observe the threadlike net structure stained by Trypan blue and take pictures with a digital camera (Olympus, Tokyo, Japan).

To visualize the nuclei, we used ethidium bromide to stain the tissue specimens. One or two drops of dye (50 μg/mL) were dropped on the specimen, which was incubated at room temperature in the dark for 1 minute. The tissue was then washed with phosphate buffered saline and observed under the fluorescent microscope.

For further analysis, the tissue of interest was isolated, embedded in paraffin, and stained with hematoxylin and eosin. The PVS network was observed using a phase-contrast microscope (Olympus).

3. Results

A PVS network stained with Trypan blue was observed in the mesentery covering the small intestine of a dog, as shown in Figure 1. The primo-vessels and blood vessels nearly overlapped, but they could clearly be distinguished (Figure 1, inset). The primo-vessels consisted of two or three branches and were joined at a node.

Figure 2 shows a primo-vessel along with a blood vessel in the mesentery, where the two vessel types were packed close together. Ethidium bromide staining clearly revealed the characteristic distribution and shapes of nuclei in the primo-vessel, which are distinct from those in blood vessels.

Figure 3 shows a hematoxylin and eosin-stained paraffin-embedded tissue section (4-μm thick) of a mesentery specimen composed of connective tissue, blood vessels (arrows), and a primo-vessel. The magnified image shows a longitudinal section of the primo-vessel, which has a multiple tubular structure and is filled with fibrous material.

Figure 4 compares a lymph vessel with a blood vessel. The lymph vessel has a valve, and its nuclei are aligned transversally to the vessel wall. The lymph vessel is a single tube.

4. Discussion

The PVS network was first observed in the omentum of a rat [12] and was then visualized in the bovine heart [5] by Trypan blue staining. Using a similar technique, we observed a PVS network in the mesentery of a dog, which is significant because it strongly suggests that such a network also exists in humans. This implies that a drastic change is needed when performing abdominal surgery to take this PVS network in the abdominal cavity into account.

In this study we observed the characteristic features of the primo-vessels, such as a broken-line alignment of rod-shaped nuclei, lack of a basal lamina or accessory cells along the primo-vessel, and...
Figure 2  Ethidium bromide-stained primo-vessel running along the blood vessel in the mesentery. At 400× magnification, the blood vessel (arrows) and primo-vessel (arrowheads) are clearly observed to be packed tightly together. In addition, the primo-vessel can be easily distinguished from the blood vessel by the rod-shaped nuclei arrayed in the longitudinal direction of the primo-vessel.

Figure 3  Hematoxylin and eosin staining of an oblique section of the dog mesentery showing the histological structure of the mesentery. The mesentery is composed of connective tissues, blood vessels (arrows), and a primo-vessel (arrowheads). The primo-vessel is apparent not as a single tube, as is a blood or lymph vessel, but as multiple lumens between rod-shaped nuclei.

Figure 4  Lymph vessel (arrowheads) along with a blood capillary (arrows) in the dog mesentery. The most characteristic feature of a lymph vessel (arrowheads) is the valve (square).

The most characteristic feature of a lymph vessel (arrowheads) is the valve (square). These features are distinct from those of blood and lymph vessels, as shown in Figures 2 and 4. It is remarkable that a primo-vessel
and a blood vessel were tightly packed together, as Bong-Han Kim described in his pioneering work [1]. A brief report on a long threadlike freely moving primo-vessel extending from the small intestine to the bladder in a dog will be presented elsewhere.

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References