Visualization of Acupuncture Meridians in the Hypodermis of Rat Using Trypan Blue

Byung-Cheon Lee¹,²*, Kwang-Sup Soh¹

¹Biomedical Physics Laboratory, Department of Physics and Astronomy, Seoul National University, Seoul, Korea
²Pharmacopuncture Medical Research Center, Korean Pharmacopuncture Institute, Seoul, Korea

Abstract
Bonghan Kim claimed that the primo-vessel (Bonghan duct) and the primo-node (Bonghan corpuscle) are anatomical structures corresponding to acupuncture meridians and acupoints, respectively, but this claim has not been confirmed. Recently we found that Trypan blue preferentially stained primo-vessels and primo-nodes on the surfaces of internal organs, not staining other structures like blood or lymph vessels or nerves. In this work, we applied Trypan blue to visualize the putative acupuncture meridian along skin skeletal muscles in the hypodermal layer of a rat. The Trypan blue stained-structures are morphologically similar to the meridian primo-vessels, as claimed by Bonghan Kim. Further study is needed to investigate the network of these Trypan blue-stained structures in order to establish them as acupuncture meridians.

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acupuncture; meridian; primo-node; primo-vessel; Trypan blue

1. Introduction
In the 1960s, Bonghan Kim claimed that acupuncture meridians were anatomical structures named meridian primo-vessels (superficial Bonghan ducts) [1]. Over several years we have conducted investigations to rediscover primo-vessels (Bonghan ducts) and primo-nodes (Bonghan corpuscles) in various tissues of mouse, rat, and rabbit [2–10]. In addition to these anatomical observations we found catecholamines in the primo-fluid (Bonghan liquid) extracted from primo-nodes, and we measured the flow speed of the primo-fluid [11,12]. Additionally, we performed proteomics analysis on the primo-fluid extracted from the primo-vessels of rabbit [13]. We have very recently found an effective dye, Trypan blue, which strongly and preferentially stained the primo-vessels on internal organs and other tissues [14,15]. In this work, using Trypan blue, we were able to visualize novel muscles containing sinuses that lay in parallel with skin skeletal muscles in the hypodermal layer of a rat. We observed several features of the Trypan blue stained structures (TBS) that were similar to the characteristics of the meridian primo-vessels described by Bonghan Kim [1].

2. Materials and Methods
Rats (Wistar/ST males, ≈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. Under urethane (1.5g/kg) anesthesia, we dissected the medial alba of a rat to expose the subcutaneous layer. The exposed subcutaneous area was stained with 0.4% Trypan blue (Sigma-Aldrich, St-Louis, MO, USA) and washed with saline two or three times. The TBS along the skin skeletal muscle was observed and pictures were taken under a stereomicroscope using a CCD camera (SZX 12 with DP 70; Olympus, Tokyo, Japan).

We isolated the TBS for light microscopic examination. For the histological study, the TBS-containing skin was fixed with 10% neutral buffered formalin. After fixation, we made paraffin blocks of the TBS-containing skin and sliced 5-μm sections from these blocks for hematoxylin and eosin staining.

3. Results

Figure 1 shows images of the meridian primo-vessel (superficial Bonghan duct) in the hypodermal layer, which were taken by Bonghan Kim [1]. Figure 1B, a magnified view of a part of Figure 1A, shows the presence of slits which are indicated by arrows. Figure 2A shows an image of the hypodermal layer before staining with Trypan blue. After staining with Trypan blue, a straight blue line, which is indicated by arrows, emerges as shown in Figure 2B.

Figure 1  (A) Light microscopic image of a meridian primo-vessel (superficial Bonghan duct) reported by Bonghan Kim [1]. (B) Magnified view of (A). The slits indicated by arrows are a noticeable feature. A primo-vessel is composed of many channels like a vascular bundle. This original picture had no information on the scale bar and staining name.

Figure 2  Stereoscopic images of skin that is (A) without staining, (B) with Trypan blue (TB)-staining and (C) magnified view of (B). TB-stained subcutaneous skin (B) reveals a blue-colored threadlike structure, part of which is magnified in (C). Notice that the skin skeletal muscle (SSM) just near the blue line was not stained by TB. H=hypodermis; D=dermis.
A magnified view of this line in which the putative primo-vessel is seen as a distinctive blue line (arrows) just beside the skin skeletal muscle (SSM), can be seen in Figure 2C.

Figure 3A presents a light microscopic image of the TBS which had some slits (arrows) similar to those in the image in Figure 1B. Figure 3B shows a magnified view of the dotted rectangle in Figure 3A, which shows the difference between the TBS and the SSM. The SSM has distinctive striated lines, which are indicated by arrows; however, the TBS has no striated lines. After the TBS had been cut, its tip was a little shrunken and round in shape (see the solid circle in Figure 3B), but the tip of SSM tip was sharply cut (see the dotted circle in Figure 3B). Figure 4A presents cross-sectioned images of the TBS and the SSM. The TBS is magnified in Figure 4B, which shows small or big-sized sinuses, which are indicated by arrows and dotted arrows, respectively.

4. Discussion

The TBSs we found in the hypodermis of rat have features similar to those of primo-vessels (superficial Bonghan ducts). These include the preferential
staining by Trypan blue [14], the bundle of muscle-like structures and their thickness (about 150 μm) [2], and the presence of sinuses of various sizes in the muscle-like structures [4,14]. The sinuses are strong evidence that the TBSs are channels where fluid flows, much like the primo-vessels [12]. Finally, we note that there are slits in the TBS (Figure 3A) as in the meridian primo-vessel of Kim (Figure 1B). This kind of slit was also noticed in the primo-vessel observed in the brain ventricles of a rabbit [3]. The presence of slits suggests that the TBS may have a contractile property to transport fluid in the channels depicted by the sinuses.

Even though we found the TBS along the skeletal muscle in the hypodermis of the abdominal skin of a rat, the success rate was low because the staining technique was difficult. The putative acupuncture meridian is thought to form a network that has not yet been found by using the current method. According to Bonghan theory [1], an acupuncture point is a rather thick and bulged body connected to a meridian which has not yet been observed in our work. Further work and improvement of technique is needed to establish the whole primo-vessels and primo-nodes as anatomical structures corresponding to acupuncture meridians and acupuncture points.

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References