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RESEARCH ARTICLE

Primo Vascular System in the Subarachnoid Space of the Spinal Cord of a Pig

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Abstract
The primo vascular system was recently observed in the central nervous systems of rabbits and rats, but no investigations in large animals have been reported. In the present work we found a putative primo vascular system in the spinal cord of a pig. We obtained spines from four healthy pigs and fixed them with paraformaldehyde. The primo vessels were expected to lie in the subarachnoid space between the pia mater and the arachnoid mater. The composite of three membranes (the pia, the arachnoid, and the dura maters) wrapping the spinal cord was peeled off, isolated from the spine, and put on a slide glass. This composite was stained with 4′,6′-Diamidino-2-phenylindole (DAPI) and phalloidin to show the nuclei and the f-actin, respectively, in the cells of the primo vessels. We observed eleven pieces of the putative primo vessels in the subarachnoid space of the spines at the thoracic spinal nerve area. They had the typical rod-shaped nuclei distributed in a broken line, and f-actin signals around nuclei. The lengths of the nuclei were 12–15 μm, and the thicknesses of the primo vessels were 8∼20 μm, which were consistent with other primo vessels that had been observed in the various organs of rabbits, rats, and mice. In addition, we observed branching of the primo vessels, which is again an expected result from previous works. In conclusion, a primo vessel was observed in the subarachnoid space of the spinal cord of a pig. This was the first observation of a primo vessel in a large animal, and the staining method used to observe the primo vessel in a fixed sample was newly developed in this work.

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1. Introduction

The spinal cord is a long, thin, tubular bundle of nervous tissue and support cells that extends from the medulla of the brain. The brain and the spinal cord together make up the central nervous system. The spinal cord extends down to the space between the first and the second lumbar vertebrae; it does not extend the entire length of the vertebral column. The spinal cord is the main information pathway connecting the brain and the peripheral nervous system [1].

The three meninges that cover the spinal cord are continuous with that in the brainstem and cerebral hemispheres. The cord is stabilized within the dura mater by the connecting denticulate ligament, which extends from the enveloping pia mater laterally between the dorsal and the ventral roots. The spinal cord is protected by the three layers of tissue, called spinal meninges, which surround the cord. The dura mater is the outermost layer, and it forms a tough protective coating. The arachnoid mater is the middle protective layer. The space between the arachnoid and the underlying pia mater is called the subarachnoid space. The pia mater is the innermost protective layer [1,2].

The primo vascular system (PVS) in the central nervous system was first reported by Bong-Han Kim in North Korea in the 1960s. He stated, "The nerve PVS is soft and semi-transparent and its color looks milky white. It has two or four subvessels and their nuclei are separated from each other farther than those of other primo vessels. And their cells have elliptical nuclei from 10 to 20 μm long" [3,4].

The research on the PVS was newly started in 2002 by the Seoul National University group [5,6]. They found the PVS inside blood vessel and analyzed it with histological methods such as hematoxyline & eosin (H&E), Verhoeff [7]. Since 2007, visualization of the PVS has been performed by using Alcian blue [8], and a technique with trypan blue spreading on adipose tissue or on organ surfaces with subsequent washing with phosphate buffer solution was developed to reveal hardly visible primo vascular systems [1,9,10]. The trypan-blue-stained structures were examined with 4′,6′-Diamidino-2-phenylindole (DAPI) staining. DAPI staining showed the characteristic shapes of nuclei and the distribution of the PVS, which were different from blood vessels or lymphatic vessels [1,11—13]. In addition, phalloidin staining of f-actin of some cells was helpful in differentiating the PVS from blood or lymph vessels and from nerves.

As Bong-Han Kim stated [3,4] and has been confirmed by recent work on the PVS in the brains of rabbits [11] and rats [14] examining the shape and the distribution of the nuclei of the isolated threadlike tissue specimens have so far been the most effective way to detect and identify the PVS, even though more histological analyses are necessary to prove its genuineness. Thus, we used this feature, which is effective in distinguishing the PVS from blood vessels, nerve bundles, and torn pieces of membranes, to find the PVS in the spinal cord of a pig. One of the most difficult problems in identifying the PVS is the similarity between primo vessels and lymph vessels. Fortunately, in the present case, no lymphatic system exists in the central nervous system [13], so no such problem occurs.

The significance of the current work is the first finding of the PVS in the subarachnoid space of the spinal cord of a pig. Even though a trypan blue injection method was used to observe the PVS in the rat spinal cord [1], this is the first time a large animal was examined in this respect. The PVS in a pig was previously studied for the case of organ surfaces [15], but observation of the PVS in the spinal cord of a pig opens a new frontier of spine anatomy that may provide hitherto unknown physiological functions in connection with various spine-related diseases like amyotrophic lateral sclerosis.

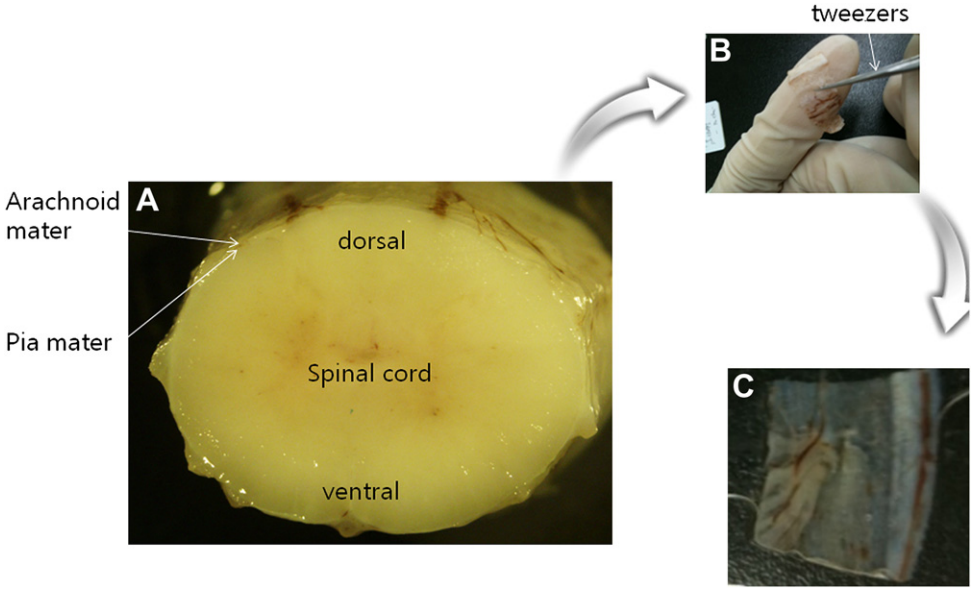

Figure 1 (A) Cross section of the spinal cord of a pig after the dura mater was peeled away; (B) composite of the arachnoid and the pia maters (arrow) was stripped from the spinal cord and put on the finger with tweezers; (C) composite (arrow) with the pia mater was put on a slide glass for 4′,6′-Diamidino-2-phenylindole (DAPI) and phalloidin staining.