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RESEARCH ARTICLE

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Novel Anatomic Structures in the Brain and Spinal Cord of Rabbit That May Belong to the Bonghan System of Potential Acupuncture Meridians

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Abstract
Novel threadlike structures of 20 to 40 μm in diameter were observed running afloat in the cerebrospinal fluid of the brain ventricles and the spinal central canal of a rabbit. We developed an effective *in situ* staining technique using hematoxylin to visualize the threadlike structure. The presence of the rod-shaped nuclei in the threadlike structure was confirmed by various nucleus specific staining dyes such as 4′,6′-diamidino-2-phenylindole, propidium iodide and yoyo-1. The threadlike structure was surrounded by a cellular membrane, whose presence was visualized by using 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate staining. The location, diameter, optical transparency and the presence of rod-shaped nuclei in and the surrounding membranes of the threadlike structure were consistent with a nerve Bonghan duct. The Bonghan duct was claimed to be the extension of the physical substance of acupuncture meridians and to be a distinct type of circulatory system present in mammals. Although Bonghan theory has not been reproduced for a long time, recently, some portions of the Bonghan duct network were confirmed in various organs in mammals including blood vessels, lymphatic vessels and enteric organs. The novel threadlike structure in the central nervous system, more specifically in brain ventricles, is one in a series of findings in an attempt to rediscover the Bonghan duct network.

1. Introduction

Acupuncture-meridian-like structures, also referred to as Bonghan ducts (BHDs), that form a novel circulatory system throughout an animal's body were first discovered by Bonghan Kim [1], but have been neglected for a long time. Only recently, several reports on observations of BHDs on the surface of the internal organs of rats and rabbits [2–4] revived the interest in the long forgotten work. The novel circulatory system is thought to be the anatomic basis of classical acupuncture meridians and consisted of several subsystems: superficial BHDs in the skin, intravascular BHDs inside large blood and lymphatic

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vessels, organ-surface BHDs on the surfaces of various internal organs and brain BHDs in brain ventricles.

The intravascular BHDs were confirmed by using an acridine-orange staining method [5,6] and those inside lymphatic vessels were observed by applying three different staining techniques [7–9]. Extensive histologic studies have been performed for organ-surface BHDs by using conventional staining methods [10], various electron microscopes [11] and immunohistochemistry [12].

In this article, we report on novel threadlike structures which are thought to be the brain BHDs in brain ventricles of rabbits. In neuroanatomy, brain ventricles are known to be sinuses containing cerebrospinal fluid (CSF) which has important roles in brain physiology [13–15]. There are also choroid plexuses [14,16,17] and Reissner fibers [18] in the ventricles. A choroid plexus is a cellular structure comprised of many blood capillaries. It generates cerebrospinal fluid and is embedded in the ependymal walls. Reissner fibers [19] are partially suspended in the cerebrospinal fluid and are made of material secreted from the subcommusura organ located in the circumventricular zone of the brain. A Reissner fiber is a fibrous structure inside the brain ventricle. It is not a cellular structure, but is comprised of accumulations of glycoprotein [18–20]. Because brain BHDs and Reissner fibers are both similar looking threadlike structures, we needed to clearly distinguish between them.

We detected brain BHDs in the third and the fourth ventricles that extended into and through the central canal of the spinal cord of a rabbit. The BHDs were afloat in CSF and did not adhere to the walls of the ventricles. After presenting the anatomic observations, we examined the novel threadlike structures to differentiate them from Reissner fibers and to identify them as BHDs by showing the presence of rod-shaped nuclei and outer membranes.

2. Materials and Methods

Ten New Zealand white rabbits (female, 12 weeks old) were obtained for this study from the Jung Ang Laboratory Animal Company of Korea. The animals were housed in a constant, temperature controlled environment (23ºC) with 60% relative humidity under a 12 hour light/dark cycle. All animals had *ad libitum* access to food and water. The procedures involving the animals and their care were in full compliance with institutional regulations and current international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996). The rabbits were anesthetized by using an intra-peritoneal injection with urethane (1.5 g/kg). Under deep anesthesia, the animals were decapitated without any perfusion. After a 1 hour freezing of the heads at −70ºC, the brains were isolated from the skulls as quickly as possible. In order to maintain the original shape of the brain, we put an ice pack beneath the isolated brain during dissection. The fourth ventricle was exposed and mildly cooled on an aluminum foil-covered ice pack, followed by careful dissection which was performed under a stereomicroscope. Hematoxylin filtered through a 0.2 μm pore-sized filter paper was poured into the exposed fourth ventricle of the brain drop by drop and kept there for 1 to 2 minutes. After hematoxylin *in situ* staining of the exposed fourth ventricle, 0.1 M phosphate buffered saline, pH 7.4, was applied drop by drop into the fourth ventricle. The pink colored choroid plexus in the fourth ventricle, which was readily identified by its many capillaries, was carefully removed. After staining and washing, a thin threadlike structure emerged along the midline of the fourth ventricle. The threadlike structure led to the third ventricle through the aqueduct. The *in situ* features were recorded using a CCD camera (Olympus DP70) coupled to a stereomicroscope (Olympus SZX12).

We cut the threadlike structure in the middle of the third ventricle and fixed it in neutral phosphate buffered formalin, pH 7.4, for study under a light-microscope. The threadlike structure specimen was stained by using a DNA specific dye, 4′,6′-diamidino-2-phenylindole (DAPI) and was observed by using phase-contrast microscopy. Confocal laser scanning microscopy (CLSM) was applied to optically examine sections of the threadlike structure. For a cross-sectioned image of the threadlike structure, we cut a 20 μm section of the specimen and stained the specimen with yoyo-1, a DNA-specific dye. After characterization of the nuclei of the threadlike structure, 10 μM of a phospholipid staining dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI), was applied in order to visualize the outermost membrane surrounding the threadlike structure.

3. Results

In order to demonstrate the effectiveness of hematoxylin for making the novel threadlike structure visible, we carried out a stereoscopic observation with and without hematoxylin application. Figure 1 illustrates the pictures of the fourth ventricle of the same rabbit before and after hematoxylin staining. The picture before hematoxylin staining on the fourth ventricle, shown in Figure 1A, hardly shows any floating structure in the sulcus of the fourth ventricle. Hematoxylin staining and washing made