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RESEARCH ARTICLE

Number Density of Mast Cells in the Primo Nodes of Rats



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Abstract

Mast cells (MCs) play a major role in allergic reactions. Surprisingly, the acupuncture points have a higher density of MCs compared with nonacupoints in the skin, which is consistent with the augmentation of the immune function by acupuncture treatment. We hypothesized that the primo vascular system (PVS), which was proposed as the anatomical structure of the acupuncture points and meridians, should have a high density of MCs. In order to test that hypothesis, we investigated the primo nodes isolated from the surfaces of internal organs, such as the liver, the small and the large intestines, and the bladder. The harvested primo nodes were stained with toluidine blue, and the MCs were easily recognized by their red–purple stains and their characteristic granules. The results showed a high density of MCs in the primo nodes and confirmed the hypothesis. The MCs were uniformly distributed in the nodes. The relative concentration of the MCs with respect to other cells was ~15%. We divided the sizes of the primo nodes into three classes: large, medium, and small. The number density and the

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relative concentration of MCs did not show a size-dependence. The current work suggests that the PVS may participate in the immune response to allergic inflammation, which closely involves MCs.

1. Introduction

Allergic diseases are increasing in prevalence and among the commonest causes of chronic ill-health worldwide. In order to reduce the burden of health care costs alternative medicines such as acupuncture are increasingly tried besides conventional Western medicine. Augmentation of the immune function is one of the widely-acknowledged effects of acupuncture treatment [1]. The active carriers of the defense mechanism are immune cells such as mast cells (MCs), neutrophils, eosinophils, basophils, macrophages, and lymphocytes. Among these, MCs are closely related to acupuncture treatments: There are more MCs at acupoints and acupuncture meridians than at nearby nonacupoints [2]; electro-acupuncture induces more MCs at other acupoints [3,4]. In addition, stimulation of the acupoints results in a significant increase in the degranulation of the mast cells [5].

Bong-Han Kim (BH Kim) [6], the first discoverer of the new circulatory system, the so-called primo vascular system (PVS) claimed that the anatomical structure corresponding to the classical acupuncture meridians was a subsystem of the PVS distributed in the skin of animals and humans. However, there has been no confirmation of his claim, and only one paper has presented a negative result [7]. Even though that is not direct proof of BH Kim's claim, the high concentration of MCs in the PVS could be thought of as supportive data for his claim because MCs are more highly concentrated at acupoints than at nonacupoints.

The presence of immune cells, such as MCs, macrophages, neutrophils, and others, were observed in the PVS on the surfaces of internal organs of rats [8] and in the primo nodes (PNs) obtained above the epicardia of rat hearts [9]. The MCs amounted to up to 20% of residential cells in the PNs harvested from the surfaces of internal organs and from the insides of lymphatic vessels of rats [10]. However, another count of the MCs reported only ~4% in the PNs on the surfaces of internal organs of rats [11]. The reason the relative number density shows such relatively large differences is still not clearly understood. Therefore, investigations of the density of MCs by independent teams are worth doing.

In the current work, we report on the number density and the relative concentration of the MCs in the PNs on the surfaces of internal organs of rats. With toluidine-blue staining, the MCs were easily distinguishable from other types of cells, and the areas of the PNs could be determined by counting the number of pixels in charge coupled device (CCD) images (Microscope Digital Camera, (DP70, Olympus, Tokyo Japan)). We classified the PNs into three groups, large (L), medium (M), and small (S), according to the areal-sizes of the middle sections of the PNs. We hypothesized that either the absolute number or the relative density of the MCs depended upon the sizes of the PNs.

MCs are bone marrow-derived and particularly depend on the stem-cell factor for their survival. They are found to reside in tissues near blood vessels and nerves and in proximity to surfaces that interface with the external environment [12]. MCs, vascular vessels, and nervous fibers form a composite structure. MCs were found to be migrated and recruited in the acupoints and meridians by acupuncture stimuli [13]. The biological functions of MCs appear to include roles in host defense mechanisms against pathogens, in tissue repair, and in angiogenesis. MCs may be activated by various stimuli, and, after activation, MCs may extrude granules that contain histamine and induce allergic inflammation [12]. Some of the MCs were observed to degranulate in the PNs [8] and in the pericardial space [9]. Substantial numbers of granules from MCs were found in primo subvessels [10], suggesting that primo vessels are conduits for the granules.

MCs have emerged as a connection between Western physiology and Eastern acupuncture via the new circulatory system, the PVS. In terms of Western physiology, the positive effects of acupuncture on the immune function can be explained through a combination of MCs and the PVS.

2. Materials and methods

2.1. Animal preparation and harvesting of the Organ Surface-Primo Node (OS-PNs)

Ten male Sprague-Dawley (SD) rats (7–9 weeks of age) were obtained from DooYeol Biotech (Seoul, Korea). The animals were housed in constant temperature and humidity conditions (23°C, relative humidity 60%) with a 12-hour/12-hour light/dark cycles and were provided water and commercial rat chow *ad libitum*. The procedures involving the animals and their care were in full compliance with current international laws and policies and were approved by the Institutional Ethics Committee of the Advanced Institute of Convergence Technology, Seoul National University (Approval Number: WJIACUC20140807-03-07). The rats were anesthetized by using an intramuscular injection of a regimen consisting of 1.5 g/kg of urethane and 20 mg/kg of xylazine. The rats were sacrificed by using an intracardiac injection of 1 mL of urethane after the experiments.

An incision of the subcutaneous layer of the abdominal skin along the midline, but slightly off the linea alba, was performed with surgical scissors. We avoided cutting the linea alba in order to maintain the abdominal wall fat band located at the midline of the ventral peritoneal wall because some PNs are often found in the abdominal wall fat band. All procedures of observations and operations were performed under a stereomicroscope (SZX12, Olympus, Tokyo, Japan). We searched for the Organ Surface-PVS (OS-

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