J Acupunct Meridian Stud 2015;8(6):281–287

Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Study of Mast Cells and Granules from Primo Nodes Using Scanning Ionic Conductance Microscopy

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Available online 6 November 2015

Received: Jun 1, 2015
Revised: Oct 28, 2015
Accepted: Oct 28, 2015

KEYWORDS
acupuncture;
histamine granule;
mast cell;
primo vascular system

Abstract
Acupuncture points have a notable characteristic in that they have a higher density of mast cells (MCs) compared with nonacupoints in the skin, which is consistent with the augmentation of the immune function by acupuncture treatment. The primo vascular system, which was proposed as the anatomical structure of the acupuncture points and meridians, also has a high density of MCs. We isolated the primo nodes from the surfaces of internal abdominal organs, and the harvested primo nodes were stained with toluidine blue. The MCs were easily recognized by their stained color and their characteristic granules. The MCs were classified into four stages according to the degranulation of histamine

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pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2015.10.003

scanning ion conductance microscope (SICM)

granules in the MCs. Using conventional optical microscopes details of the degranulation state of MCs in each stage were not observable. However, we were able to investigate the distribution of the granules on the surfaces of the MCs in each stage, and to demonstrate the height profiles and three-dimensional structures of the MCs without disturbance of the cell membrane by using the scanning ion conductance microscopy.

1. Introduction

Mast cells (MCs) play a key role in the inflammatory process, and are implicated in the pathology associated with autoimmune disorders such as rheumatoid arthritis. MCs are the active components in acute allergic reaction [1], and the body's antimicrobial reaction [2,3]. The diverse roles of MCs are still being revealed [4,5].

MCs are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the external world and the internal milieu, such as the skin, digestive tracts, and mucosa of the lungs [6].

MCs are more abundantly present in the acupuncture points compared with neighboring nonacupoints in skin [7,8] which could be related to the augmentation of immune function after acupuncture treatment [9]. Acupuncture treatment increased the degranulation of MCs [10]. Recently, MCs were found to be abundant in the primo node (PN) of the primo vascular system (PVS) which is a new anatomical structure corresponding to the acupuncture meridians [11,12]. Interestingly, there was very low concentration of MCs in lymph nodes. However, lymph nodes contain a high concentration of lymphocytes such as T cells and B cells. By contrast, the PNs have scarce lymphocytes but a high concentration of granulocytes such as MCs, basophils, and eosinophils [13—16]. The immune function is orchestrated by the collaboration of lymphocytes and granulocytes, therefore the lymph system and the PVS could work together.

When activated, a MC rapidly releases its characteristic granules into the interstitium. These granules carry a variety of bioactive chemicals, most notably histamine and heparin. MCs can be stimulated to degranulate by direct injury, cross-linking of immunoglobulin E receptors, and acupuncture stimulation [17].

In this article we report the study of MCs isolated from PNs that were harvested from the surfaces of internal organs such as the bladder, and large and small intestines of a rat. We focused our attention on the four stages of degranulation of MCs. In order to examine the detailed states and the changes of the granule distribution in the surface of the cell membranes we utilized the new scanning probe microscope technique, called scanning ion conductance microscopy (SICM) [18].

The SICM was the offspring of scanning probe microscopes and was developed for the purpose of studying the three-dimensional (3-D) topography of a live single cell in high resolution and natural conditions without disturbing the surface structure by chemical or physical probes. In this study, we only studied MCs that were fixed and stained with toluidine blue. We could more clearly investigate the distribution of granules in each stage using SICM than an optical microscope and an atomic force microscope because it could image the surface with much higher resolution and there was no interaction between an imaging probe and sample surfaces [19]. Another benefit of using SICM is a lack of severe sample preparation processes that are essential to electron microscopes [20,21].

2. Materials and methods

2.1. Animal preparation and harvesting of the primo nodes

Ten male Sprague—Dawley rats (7—9 weeks old) were obtained from DooYeol Biotech (Seoul, Korea). The animals were housed under constant temperature and humidity conditions (23°C, relative humidity 60%) with 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 (*Guide for the Care and Use of Laboratory Animals*, National Academy Press, 1996) 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 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 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 using 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 PVS in the abdominal cavity under a stereomicroscope (STZ10; Olympus). During the procedure, avoiding blood flow into the abdominal cavity and keeping the surfaces of internal organs humid by dripping phosphate buffer solution (PBS) onto them frequently were important.

2.2. Isolation and adhesion of MCs

The isolated PNs were fixed with neutral buffered formalin at 23°C for 24 ± 2 hours. The cells were isolated by gently rubbing two slides between which the PN was placed. The cells were placed on a poly-L-lysine coated or gelatin coated Petri dish. Floating cells and other debris were