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Distribution of Histamine-Positive Mast Cells in the Vicinity of the Needle Tract Following Acupuncture in "Zusanli" (ST_{36}) Acupoint in Rats

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"Zusanli"(ST_{36}) acupoint is commonly used in acupuncture. The mast cells are important object in experimental acupuncture. Histamine is stored in the granules of rat mast cells. The aim of the present study is to examine the distribution of histamine-positive mast cells in the vicinity of needle tract following acupuncture in ST_{36} acupoint in rats. Acupuncture in ST_{36} disrupted the integrity of the epithelium, derma, subcutis, fascia, epimysium and striated muscle tissue. In our research we observed thickening and displacement of the connective tissue in the direction of the needle tract and folding of the fascia. We noticed histamine-positive mast cells in the proximity of needle tract in the tissues of ST_{36} acupoint in rats. We could not establish considerable differences in the number and distribution of histamine-positive mast cells in the needle tract vicinity. Few histamine-positive mast cells in the proximity of the needle tract showed signs of degranulation.

Key words: mast cells, histamine, acupuncture, needle tract, Zusanli (ST_{36}).

Introduction

During the 20th century Traditional Chinese Medicine (TCM) which has been used for thousands of years in China and other countries of the Far East, is being extensively applied in modern medicine both as a modality for treatment or general conditioning in chronic disease as well as supplementary physical therapy, in support of the main therapeutic methods. The acupuncture is one of the main methods of TCM.

"Zusanli"(ST_{36}) acupoint is one of the most commonly used in acupuncture. Acupuncture points, described in the classical texts, are usually detected with the method of standard proportion of anatomical structures under the control of the apparatus measuring skin resistance [17, 4]. The acupuncture channels and acupuncture points of human and animals (including rats) share certain similarities [3], which allows researchers to

use animals as model for experimental acupuncture. Many studies have been devoted to research the local mechanism of acupuncture but there is relatively little research for changes in the vicinity of the acupuncture needle tract, although the regional anatomy has been previously described to include nerve fibres, small vessels and muscle spindles in the proximity of the acupuncture needle tract in the tissue of the acupoints [11].

Histamine is a neurotransmitter involved in local immune responses as well as in regulation of physiological functions [13]. Histamine is stored in the granules of rat mast cells [1, 8, 16]. The mast cells are heavily involved in local acupuncture reaction and are thus important object for experimental acupuncture in rats [18, 14, 20]. Histamine is also the subject of many experimental researches [7, 9, 10, 15].

The normal morphological structures in ST_{36} acupoint are: epidermis, dermis, subcutis, fascia, epimysium and muscle with blood vessels and nerves. There are many hair follicles with piloerector muscles and sebaceous glands in dermis. There are clusters of mast cells in certain areas close to the blood vessels [6]. Our previous results show that the impact of the acupuncture needle in the ST_{36} acupoint induces morphological changes in the tissues and mast cells. As a result of acupuncture of ST_{36} acupoint the integrity of the epithelium, dermis, subcutis, fascia, epimysium and striated muscles are disrupted in the direction of the needle tract [5]. However, the distribution of mast cells in the tissues of the acupoint and their reaction to acupuncture have not been elucidated.

Materials and Methods

The aim of this research is to study histamine-positive cells in the vicinity of the needle tract following acupuncture in ST_{36} acupoint in rats. The studies were carried out on six adult Wistar normotensive rats, weighing between 220 and 350 g. All experiment were carried out in accordance with the standards for ethical treatment of animals in research, set forth by the Ethics Committee of Trakia University. The area around the acupoint was epilated, defined and marked using the method of standard proportion of anatomical structures under the control of the apparatus KWD-808 measuring skin resistance. We used steel acupuncture needle with size 0.25 × 13 mm. The animals were deeply anaesthetized and transcardially perfused with 4% paraformaldehyde solution. Tissue sample with size 5 × 5 × 5 mm from ST_{36} acupoint was taken together with the acupuncture needle, postfixed, embedded in paraffin and cut into 5 μm thick sections. The samples were then deparaffinized with xylene and ethanol, and processed for avidin-biotin-horseradish peroxidase complex (ABC) immunohistochemistry. Briefly, the sections were treated with hydrogen peroxide to inactivate endogenous peroxidase and the background staining was blocked with 5% normal goat serum (NGS) in PBS for 1 hour. Between the steps, the sections were rinsed with PBS/Triton X-100. After that they were incubated with the primary antibody, rabbit anti-histamine (diluted 1:2000, Sigma, Saint Louis, Missouri, USA) overnight at 4 °C in a humid chamber, followed by biotinylated goat anti-rabbit IgG (Sigma, 1:250) for 2 h at room temperature, and finally the ABC complex (Vector Labs, Burlingame, CA, USA) was applied for 2 hours at room temperature. Finally, the peroxidase activity was visualized using diaminobenzidine as a chromogen. After the immunoreaction, the sections were dehydrated in ethanols, cleared in xylene and coverslipped with Entellan.

The specimens were photographed with a Nikon research microscope equipped with a DXM 1200c digital camera. Statistical analysis was performed using SigmaStat®11.0 software package (Systat Software Inc). Experimental data were evaluated by Student's t-test. Differences were considered statistically significant if p-values were < 0.05.

Transmission electron microscopy was also used for visualization of mast cells.

The material from skin in the vicinity of the needle tract, with size 1 × 1 × 1 mm, was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M PBS. The sample was then dehydrated through ethanol series, passed through a propylene oxide and then infiltrated and embedded in a liquid resin such as epoxy. After embedding the resin block was cut into ultrathin sections by a diamond knife in an ultramicrotome. Each section, only 50-70 nm thick, is collected on metal mesh 'grids' and stained with electron dense stains before observation in the TEM.

Results

After acupuncture in ST_{36} acupoint the main alterations happen in the cutis, subcutis and the underlying muscle fibers and fascia. As a result of acupuncture the integrity of the epithelium is disrupted and it folds in the direction of the needle tract. Acupuncture of ST_{36} disrupted the integrity of derma, subcutis, deep fascia, epimysium and striated muscles. We observed thickening and displacement of the connective tissue and formation of folds in the direction of the needle tract and also histamine-positive mast cells

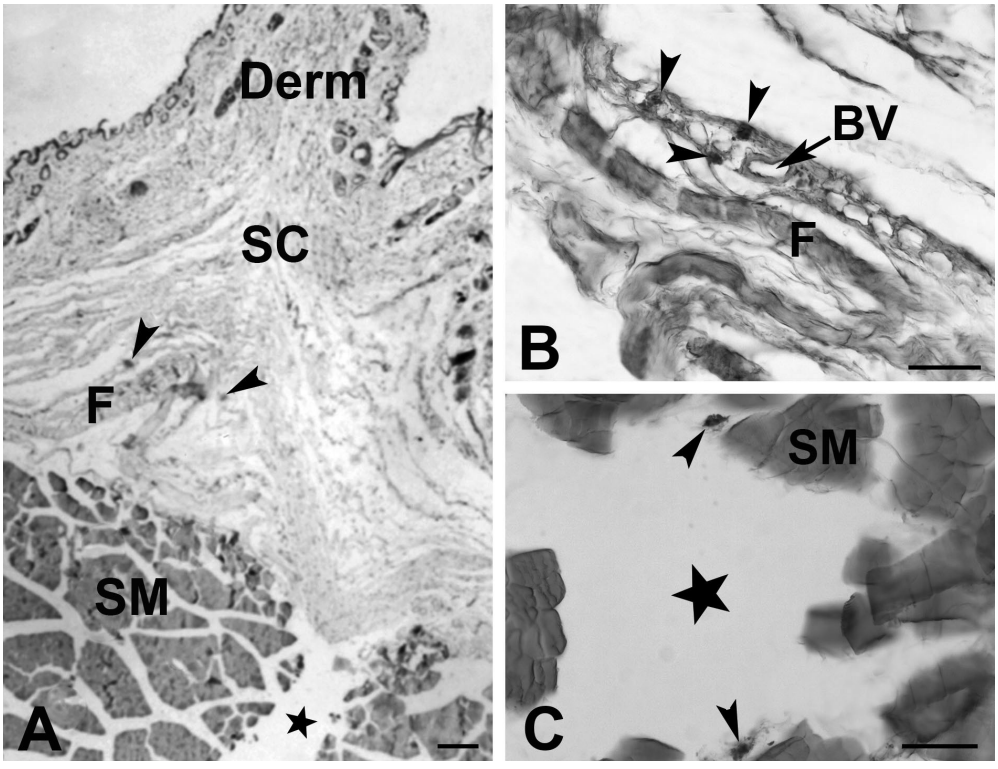

Fig. 1. (A, B, C) Histamine-positive mast cells in the needle tract vicinity of acupuncture point ST_{36} in rats: **A**) Distribution of histamine-positive mast cells (arrow) below the epidermis, dermis (Derm), fascia (F), subcutis (SC) and striated muscle (SM) in the needle tract (star) vicinity; **B**) Demonstrating the localization of histamine-positive mast cells (arrow) next to folded fascia (F) and blood vessel (BV); **C**). Histamine-positive mast cells (arrow) in the connective tissue of striated muscle (SM) in the needle tract (star) vicinity whith signs of partial degranulation. Scale bars = 50 μm

in the vicinity of needle tract in the tissues of ST_{36} acupoint in rats (**Fig. 1A, B, C**). Striated muscle fibers are partially destroyed and we noticed clear evident needle tract (**Fig. 1A, C**). Larger blood vessels are not affected, but rather displaced, by the needle.

The distribution of histamine-positive mast cells in tissue (dermis, subcutis and striated muscle) in the vicinity of the needle tract in ST_{36} acupoint in rats was uneven. The histamine-positive mast cells tended to form clusters in close proximity to the blood vessels (**Fig. 1B**). In our study we were not certain of the presence of histamine-positive mast cells in the epidermis. In the dermis histamine-positive mast cells were located mainly around the blood vessels and close to the hair follicle and sebaceous glands. The number of histamine-positive mast cells in the proximity of needle tract was larger in the border zones epidermis–dermis, dermis–subcutis, subcutis–fascia.

We observed thickening and displacement of the connective tissue in the vicinity of the needle tract (**Fig. 1A**). We also found histamine-positive mast cells adjacent to the folded fascia and blood vessels (**Fig. 1B**). More histamine-positive mast cells were visualized in subcutis and dermis than in striated muscles. Histamine-positive mast cells were also identified in the connective tissue of striated muscle in the vicinity of needle tract (**Fig. 1B**). We investigated the distribution of histamine-positive mast cells in the tissues in the vicinity of needle tract after acupuncture in Zone I (0-50 μm from the center of the needle tract) and Zone II (50-100 μm from it) (**Fig. 4**). In our results we could not establish considerable differences in the number and distribution of histamine-positive mast cells between Zone I and Zone II in ST_{36} acupoint. There were not statistically significant differences between the two areas ($p > 0.05$) (**Fig. 3**). We observed that mast cells show signs of partial degranulation in the proximity of the needle tract (**Fig. 1C, Fig. 2**).

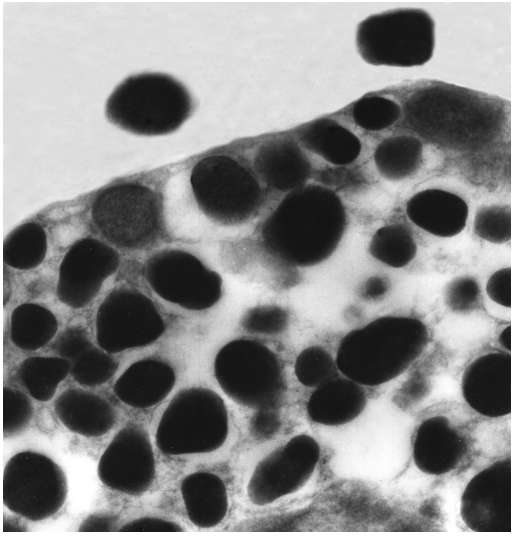

Fig. 2. Electron micrograph with signs of degranulation of mast cells in the needle tract vicinity (12700×)

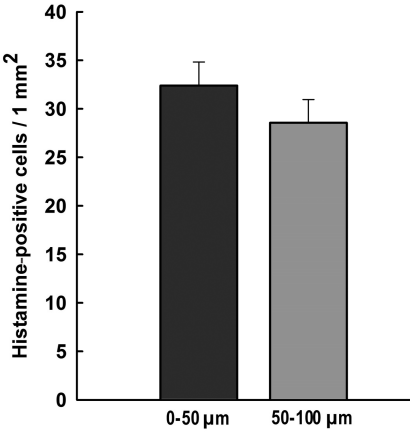

Fig. 3. Graphical representation of the distribution of histamine-positive mast cells in the tissues in the vicinity of the needle tract after acupuncture in acupoint ST_{36} in rats. Distribution of histamine-positive mast cells in Zone I (0-50 μm from the needle tract) and Zone II (50-100 μm from the needle tract). There are not statistically significant differences between the two areas ($p > 0.05$). Experimental data were evaluated by Student's t-test to parametric data. The data represent the average of the distribution of histamine-positive mast cells in an area of 1 mm^2 + SEM

Discussion

The results of the present study show thickening and displacement of the connective tissue in the proximity of needle tract. Our previous results also reported alterations in the connective tissue after acupuncture [7]. In the present study we observed single histamine-positive mast cells with signs of degranulation in the vicinity of the needle tract. The observed single histamine-positive mast cells with signs of degranulation were consistent with our research in rats, since we were able to show that degranulation of some mast cells takes place after acupuncture [5]. The influence of acupuncture on mast cell degranulation has been confirmed by other authors [2, 12]. Researchers have found that the acupuncture causes degranulation of closely located histamine-positive mast cells [19]. Other researchers reported that there were no significant differences in the distribution of mast cells between ST_{36} acupoint area and the nonacupoint areas, and most of the mast cells were mainly arranged along the small blood vessels. These researchers have also found that the influence of acupuncture on the cell number and degranulation of mast cells was unremarkable [21]. In our study we also could not establish considerable differences in the number and distribution of histamine-positive mast cells between center of acupoint with the needle tract and neighboring tissues in ST_{36} acupoint in rats. We suppose that the observed thickening and shifting of the connective tissue (together with the histamine-positive mast cells contained in it) by the needle could lead to small increase of the number of histamine-positive mast cells found in the connective tissue in the vicinity of the needle tract. We also confirm the presence of a large amount of histamine-positive mast cells in the proximity of blood vessels.

Conclusions

We could not establish considerable differences in the number and distribution of histamine-positive mast cells in the needle tract vicinity. Few histamine-positive mast cells in the proximity of needle tract are with signs of degranulation.

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