Original Contribution

MAST CELL REACTION TO ACUPUNCTURE ON TONGUE

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ABSTRACT

PURPOSE: The aim is to find out the mast cells (MCs) reaction in tongue after experimental acupuncture. **METHODS:** For experiments were carried 10 adults rats (28 months age). The needles used for the acupuncture is 0.22x13mm, and were placed for 10 minutes into standard acupuncture point Ex-HN-10 (Juquan) corresponding to that of humans. This point is located on the upper surface in the sagittal plane of the tongue and is close to the center of the tongue body. As normal consequence of every acupuncture is the forming of a needle tract and also here in the tissues of the rats tongue we could demonstrate this. This was done with a visualization method for the needle tract that we developed for tongue. The proximity of the needle tract was examined for MCs. Two stains were used for proper visualization: Toluidine blue and Bismarck brown staining. **RESULTS:** In close proximity of the needle tract we observed degranulation of MCs that was massive and few destroyed MCs in the needle tract itself. At a considerable distance from the MCs some discharged granules from them was found. **CONCLUSIONS:** There is a MCs reaction on the acupuncture of tongue that includes a degranulation of the MCs that was massive in proximity of the acupuncture needle tract. Some of the effects of acupuncture could be due to the demonstrated MCs degranulation.

Key words: rat, tongue, mast cells, acupuncture, Juquan, needle tract

INTRODUCTION

The rat tongue is an appropriate structure to access and examine different anatomical structures as blood vessels, muscles, nerves, glands and immune competent cells (1). The normal rats tongue structure is observed by researcher and typical structures as mast cells, nerve bundles, blood vessels, and skeletal muscle are described (2). Mast cells are of particular interest because they have a fast responsiveness to stimuli of different type. Mast cells (MCs) have diverse properties that include tissue repair, immunomodulation, defense mechanisms against parasite infections. (3). Many researches look at the MCs as an important subject in regarding the changes in the tissue after acupuncture (4-7).

The changes in MCs in rats ST₃₆ acupoint after experimental acupuncture have been investigated in our previous researchers (8-10). Previously published research of ours found out that in rats of different ages the distribution of MCs in rat tongue was different (1). The researchers use classical histological staining techniques: Toluidine blue and Bismarck brown for visualization of MCs in rats' tissue (11, 12). Bismarck brown was more selective stain for mast cells (13). Toluidine blue histochemistry and tryptase immunohistochemistry are comparable for the demonstration of MCs in the skin (14).

Acupuncture of the tongue is not as popular as corporal but nevertheless very interesting and promising. Specific acupoints in the tongue corresponding to various organs, and meridians and acupuncturing the surface or base of the tongue in specific acupoints in improving various functional modalities in patients with

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chronic neurological disorders such as ASD, cerebral palsy, stroke, and drooling problems (15). We used Ex-HN-10 (Juquan) acupuncture point (acupoint) for the present study, because it is commonly used in Traditional Chinese Medicine (TCM) and easily accessible in tongue acupuncture. The acupuncture point Ex-HN-10 (Juquan) in humans is located with maximal extension of the tongue, in the centre of the tongue body (16).

MATERIALS AND METHODS

The experimental animals were 10 Wistar rats of age 2 years and 4 months. The experimental procedure was approved by the Research Ethics Committee, Faculty of Medicine, Plovdiv University (project number: HII 09/20 21/12/16, License 193). The animals were handled with care and the intention to minimize the number of animals used and the suffering. Ketamine-Xylazine with the usual dosage was used for deep anaesthesia before the histological preparations. The acupuncture needles were 0.22×13 mm in size, made of steel. Acupuncture needle were placed for 10 minutes deeply into standard acupuncture point Ex-HN-10 (Juquan) located on the upper surface in the sagittal plane of the tongue in the center of the tongue body corresponding to that of humans. This was done with a visualization method for the needle tract that we developed for tongue so that it could be demonstrated in a circumstance as close as possible to the condition during the needling process. The proximity of the needle tract formed after experimental acupuncture in the rats tongue was examined. Needle was left in place while the animals were perfused first with 0.05 M PBS followed by 4% PFA in 0.1 M phosphate buffer, pH 7.36. A tongue sample was excised together with the acupuncture needle. The needle was included in the paraffin blocks together with the samples of tongue and removed immediately before the sectioning on a conventional microtome in 5 and 7 µm sections. The animals were prepared; tongues were dissected, and postfixed overnight in the same fixative at 4 °C. Tissue was paraffin-embedded and sectioned. Two histological techniques of staining were applied: Toluidine blue and Bismarck brown. Cells were counted per view field at x200 (0,163 m²) using LAS v.4.12 (Leica, Germany). Mast cell reaction was documented on a high magnification pictures.

RESULTS

The experimental acupuncture in rats tongue was successful and the method used by us visualized the needle tract formed after it. We demonstrate the needle tract in a condition, similar to the condition during the needling as well as the tissues in proximity. The needle tract is visible as a defect seen from the surface of the tongue to its depth and with a minimum size. The tissues are partial destroyed and shifted from acupuncture needle.

MCs were observed in the tissues of rats tongue also in the acupoint. As a result of the needle incertion, a needle tract is formed in the tongue tissues at the place of the penetration. Interesting distribution of mast cells in the different tissue of the rat tongue could be seen on the pictures. We observed MCs in proximity of this tract (**Figure 1 C, D; 2 B, D**). Because of the acupuncture needling, the integrity of tissues is disrupted. We observed compression and displacement of the tissue in the proximity of the needle tract, altogether with MCs within it (**Figure 1 A, B; 2 A, C**). Folds in the direction of the needle tract from the disrupted epithelium were formed (**Figure 1 A, B; Figure 2 C**).

In all stainings, the needles tract was visualized well. The MCs reaction was present as massive degranulation of the cells and group of cells in proximity of the needle tract (**Figure 1 C, D; 2 B, D**). Few single MCs were destroyed by the acupuncture needle and were found inside the needle tract.

Grouping of mast cells around the needle tract was visible on some of the pictures similar to the one on (**Figure 1 D; Figure 2 B, D**). The mast cells not in contact with the needle tract were not degranulation. Significant blood vessels near the needle tract were pressed, deformed and flattened by the acupuncture, but not damaged.

DISCUSSION

Normal rats tongue morphology has mast cells scattered throughout the submucosal region or adjacent to nerve bundles and blood vessels, and also between the fibers of the skeletal muscle, which was observed by numerous researchers (2). This is in line with the results of this study. The observed mast cells are in the proximity of nerve bundles and blood vessels. A numerous mast cells in rats tongue were scattered throughout the submucosal region and in the deep muscular layer as solitary cells or in groups of several cells, also in the vicinity of needle tract.

Figure 1. Needle tract (arrow), is well visualized on picture A and part of it is magnified on picture B. Mast cells (MC) are visible in groups in lamina propria of rat tongues and the muscle layer. Prominent mast cells degranulation in near vicinity of the needle tract (arrow) is presented on bigger resolution on picture C and D. All pictures are from the same area. Stained with Bismarck Brown. Ep - epithelium, MC - mast cells, MCd- mast cells degranulation, NT- needle tract. Scale bars = 500 µm on A; 200 µm on B; 100 µm on C&D.

Figure 2. Picture A and C are form the needle tract (NT). Massive degranulation is visible on higher resolution on picture B and D. On pic. A and C the needle tract (arrow) is visible and on B and D the focus is on a group of mast cells with degranulation is the lamina propria of the rat tongue. Stained with Toluidine Blue. Ep - epithelium, MC - mast cells, MCd- mast cells degranulation, NT- needle tract. Scale bars = 500 µm on A&C; 100 µm on B&D.

Some investigators have also observed the needle tract in the tissue of acupoints, but they explore canine acupoints (17), and not tongue acupoints. A relationship between MCs degranulation and some acupuncture effects has been proposed from many researchers (6, 7). Our previous research in rats also reveals that acupuncture causes degranulation of MCs (8- 10).

The present study provides clear morphological evidence for MCs degranulation in the vicinity of needles tract, following acupuncture. The acupuncture on the tongue was carried out with no complications in rats. This is in concordance with results of (15), where the method was applied to people with no complications, even though that we kept the needles in place for 10 minutes compared to 15 sec. It is possible that the prolonged time that the needle remained in the tissue could have stimulated the mast cell to migrate closer to the needle tract. The MCs reaction could explain some of the effects of the tongue acupuncture.

CONCLUSION

There is a MCs reaction on the acupuncture of tongue. It is a massive degranulation of the MCs in the vicinity of the acupuncture needle tract. The demonstrated MCs degranulation may be involved in the effect of acupuncture.

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