Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 25 (3-4)
Sofia ● 2018

Experimental Acupuncture of the Human ST_{36} Acupoint

Nikolay Dimitrov[1], Dimitrinka Atanasova[1,2], Nikola Tomov[1], Sevinch Hamza[1], Dimitar Sivrev[1]*

[1] *Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

[2] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

* Corresponding author e-mail: nikolaydd@abv.bg

The aim of the present study is to examine the structures that interact with the acupuncture needle. For this purpose, we examined the vicinity of the needle tract formed after experimental acupuncture in ST_{36} acupoint in humans. We used the method for needle tract visualization, developed by us, to demonstrate the tissues in a condition, maximally close to the condition during the needling. As a result of acupuncture the integrity of the epithelium, dermis, subcutis and striated muscles is disrupted and folds are formed in the direction of the needle tract. Elastic and collagen fibers in dermis and subcutis are partially destroyed. We observed hair follicles, nerve fibers, blood vessels, and muscle spindles in the vicinity of the needle tract. Some of them are partially destroyed by the acupuncture needle. Larger blood vessels and nerves are not affected by it. Needling of the acupoint generally causes displacement and destruction of the soft tissue.

Key words: acupoint, Zusanli (ST_{36}), muscle spindles, needle tract

Introduction

Traditional Chinese Medicine (TCM) and acupuncture in particular continue to gain more and more popularity in Bulgaria, Europe, and all over the world in the last years, which is one of the reasons for our struggle to clarify the morphological basis of this ancient healing method. Acupuncture points (acupoint) are important for treatment in TCM and they can be found by applying the method of standard proportions of anatomical structures under the control of an apparatus measuring skin resistance [12]. Acupuncture point ST_{36} is one of the most important acupoints used for treatment in TCM. In human, ST_{36} acupoint is located on the stomach meridian – from ST_{35} ('lateral eye of the knee', at the level of the knee joint space) 3 cun (1 handbreadth) down and 1 fingerbreadth lateral to the anterior crest of the tibia, on the tibialis anterior muscle [8]. In TCM for therapeutic intention, acupuncture needles are inserted into the acupoint. The depth of the needling ST_{36} acupoint in the human is 1–1.5 cun [8]. Researchers investigate the mechanism of acupuncture and the nature of acupuncture points [1, 13], but there is a lot of uncertainty and controversy. Some physicians use the electrical conductance of the skin at the acupuncture points, for diagnostic purposes [2].

The aim of the present study is to examine the structures that interact with the acupuncture needle. For this purpose, we examined the vicinity of the needle tract formed after experimental acupuncture in ST_{36} acupoint in humans.

Materials and Methods

We used the method for needle tract visualization, developed by us in rats and explained in detail in previous publications [3, 5, 6] to demonstrate the tissues in a condition, maximally close to the condition during needling. This method enables the investigator to demonstrate the tissues in a condition, maximally close to the condition during the needling process, after retracting the needle. This method was applied on human cadavers to obtain samples from ST_{36} acupoint. The experiments were carried out on six human cadavers of different ages available at the Department of Anatomy in the Faculty of Medicine of the Trakia University. ST_{36} acupoint was located, defined and marked using the method of standard proportion of anatomical structures – 3 cun down from ST_{35} and 1 fingerbreadth lateral to the anterior crest of the tibia, on the tibialis anterior muscle [8]. We used standard steel acupuncture needles with size 0.30 × 40 mm. Acupuncture needles were inserted 25 mm deep into ST_{36} acupoint. Samples from ST_{36} acupoint with different size 10 × 10-15× 25 mm was excised together with the needle and fixed in formalin for 48h – 96h. Tissue was paraffin-embedded and sectioned on a conventional microtome in 5 μm sections. The acupuncture needle remained in the paraffin block and was taken out immediately before its plane was reached by the microtome blade. We used classical histological techniques of staining: H&E, Orcein and Van Gieson for visualization of morphological structures in the vicinity of the needle tract.

Results

The method used successfully visualizes needle tract formed after experimental acupuncture in ST_{36} acupoint in humans (**Fig. 1, 2**). We demonstrate the needle tract and tissues in the vicinity in a condition, maximally close to the condition during the needling. The needle tract defect seen is with a minimum size and is observed across all tissues through which the needle has passed. The needle tract is visible from the surface of the ST_{36} acupoint point to its depth. The acupuncture needle destroys and shifts the tissues through which it passes.

As a result of acupuncture the integrity of the epithelium, dermis, subcutis and striated muscles is disrupted and folds are formed in the direction of the needle tract. We observed indentation of the epidermis and compression and displacement of the connective tissue from acupuncture needle (**Fig. 1A**). The needle tract formed as a result of acupuncture is clearly visible in the depth of investigated tissues (**Fig. 1, 2**) Changes in the structure are most clearly differentiated in the vicinity of the needle tract formed by the acupuncture needle. Particles of loose connective tissue and collagen and elastic fibers fall inside the needle tract into the plane of disrupted striated muscle. We observed destroyed elastic and collagen fibers in dermis, subcutis and striated muscles in the needle tract (**Fig. 2A-D**). We also observed hair follicles, nerve fibers, blood vessels, sebaceous glands, sweat glands, lymph vessels, striated muscle fibers (**Fig. 1**) and muscle spindles in the vicinity of needle tract (**Fig. 2C**). Some of them are also destroyed from the acupuncture needle (**Fig. 1B-D; 2B**). Very interesting observation was the presence of blood vessels (**Fig 1B; 2B**), hair follicles (**Fig. 1B**) particles of striated muscles (**Fig. 2C**) and muscle spindles (**Fig. 1D**) inside in the needle tract.

The destroyed blood vessels in the needle tract were cut into two parts by the needle. The muscle spindle observed in the needle tract was removed from its normal position between striated muscles by the needle and moved along the needle tract (**Fig. 1D**). Blood vessels with reduced cross section and in various degrees of compression were found in the vicinity of the needle tract (**Fig. 1B**). The larger blood vessels and larger nerves deeper in tissue were not affected by the needle.

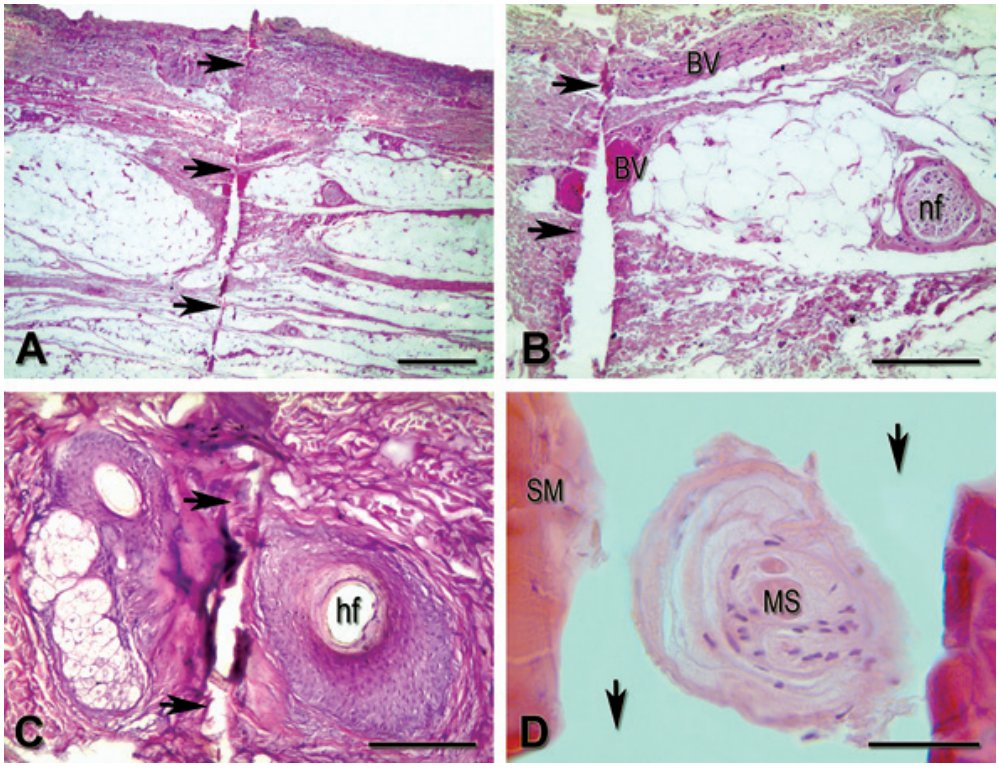

Fig. 1. The representative Hematoxylin & Eosin (H&E)-stained structures in the vicinity of the needle tract formed after acupuncture in ST_{36} acupoint in humans. (**A)** The needle tract (arrow) formed after acupuncture and structures in the vicinity of the needle tract. **(B)** Needle tract formed after acupuncture (arrow) and nerve fibers (nf), and blood vessels (BV) in the vicinity of the needle tract (arrow). **(C)** Hair follicles (hf) in the vicinity of the needle tract (arrow) destroyed by the acupuncture needle. (**D)** The muscle spindle (ms) in the needle tract (arrow) destroyed by the acupuncture needle between striated muscles (SM). Scale bars: 500 µm (A), 200 *µm* (B), 100 *µm* (C), 50 *µm* (D).

Discussion

In our previous investigation of morphology of ST_{36} acupoint in the human we demonstrated normal anatomical structures in this acupoint: skin, subcutaneous adipose tissue, blood vessels, nerves, sebaceous and sweat glands, and mast cells. In some areas of the skin indentations and differences in the thickness of the epidermis and the loose connective tissue layers were found, but these differences were not pronounced [7]. In this study we observed normal anatomical structures in this acupoint in the human but

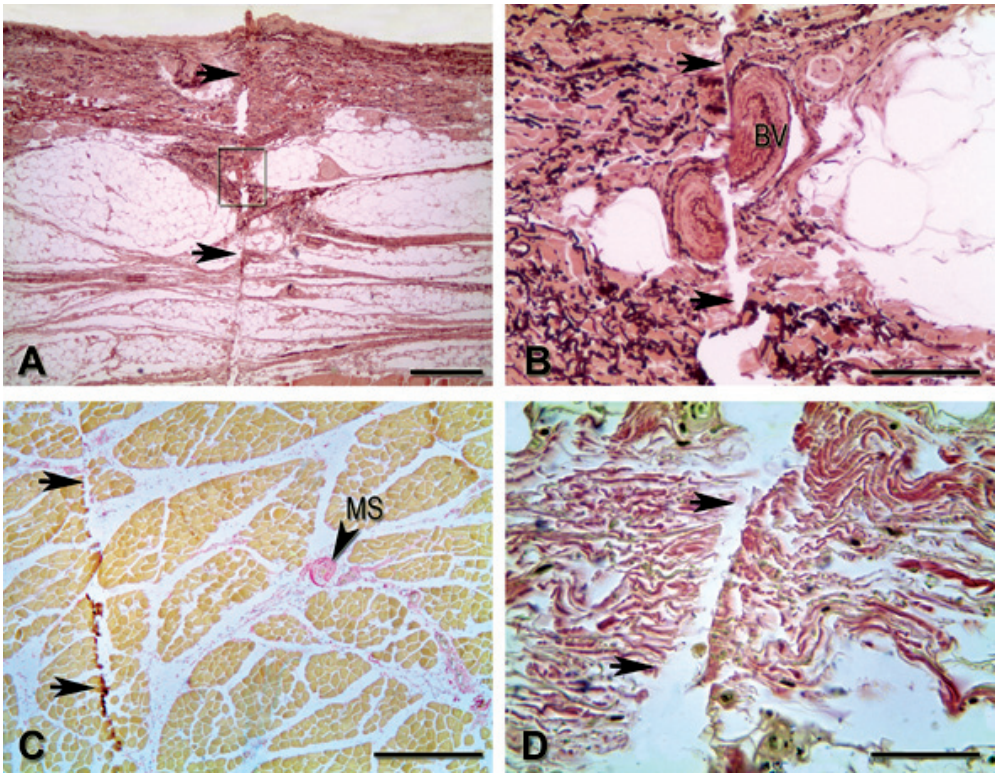

Fig. 2. The structures in the vicinity of the needle tract formed after acupuncture in ST_{36} acupoint in humans. **(A)** Needle tract (arrow) formed after acupuncture and elastic fibers in dermis and subcutis in the vicinity of the needle tract, partially destroyed by the acupuncture needle (Orcein). **(B)** Elastic fibers and blood vessels (BV) in the vicinity of the needle tract (arrow) destroyed by the acupuncture needle (Orcein). **(C)** The needle tract (arrow) destroyed striated muscles. Muscle spindles (MS) in the vicinity of needle tract (arrow) (Van Gieson). **(D)** Collagen fibers in the vicinity of the needle tract, partially destroyed by the acupuncture needle (arrow) (van Gieson). Scale bars: 500 μm (A, C), 100 μm (B), 50 μm (D).

also alterations of the structure of elastic and collagen fibers in the vicinity of needle tract. Our previous results in rats ST_{36} acupoint show normal anatomical structures and the presence of a thicker layer of loose connective tissue in some areas of the skin, indentations and differences in the thickness of the epidermis and folding of the deep fascia [4]. Following acupuncture in ST_{36} in rats, a needle tract is formed in the tissues, affected by the needle. The influence of the acupuncture needle in ST_{36} in rats induces morphological changes in the examined tissues – compression and displacement of the connective tissue in the vicinity of the needle tract. The integrity of derma, subcutis, deep fascia, epimysium and striated muscle was disrupted by the acupuncture needle [3]. Changes in the structure of elastic and collagen fibers in ST_{36} in rats are most clearly defined in the vicinity of the needle tract formed by the acupuncture needle. The defect is seen with a minimum size, mainly because tissue integrity recovers fast after the removal of the needle [5]. In this study we successfully visualized needle tract formed after experimental acupuncture in ST_{36} acupoint in humans and tissues in the vicinity of this tract. We observed analogous to those in experimental animals changes in the structure of elastic and collagen fibers in the vicinity of the needle tract. In this

study we observed destroyed muscle spindles in the needle tract. This indicates that the acupuncture needle is able to destroy at least some of muscle spindles. We also observed large nerve fibers and large blood vessels in ST_{36} acupoint in the humans. However, they were not affected. This confirms our previous investigation in rats, which shows that larger neurovascular structures remain relatively intact following needling of the acupoint [3]. All this leads to the conclusion that morphological alterations following experimental acupuncture in ST_{36} acupoint in humans and rats are similar. Other researchers have observed the needle tract in the tissue of acupoints. In its vicinity, they observed nerve fibers, small vessels and muscle spindles in canine acupoints [9]. This confirms our investigation in humans. Observed changes after acupuncture that occur in the connective tissue are important because they are probably related to the effect of acupuncture. Some investigators have suggested that the mechanism of the acupuncture effects is the reaction of the connective tissue near the needle tract [10, 11, 14]. The investigators suggest that collagen fibers play an important role in the degranulation of mast cells and acupuncture analgesia [14]. This shows the important role of the changes of connective tissue observed after insertion of acupuncture needle, which we confirm on a morphological level.

Conclusion

The method for needle tract visualization, developed by us successfully visualizes needle tract formed after experimental acupuncture in ST_{36} acupoint in humans. Needling of the acupoint generally causes displacement and destruction soft tissue. The alterations are mainly in the connective tissue of the acupoint. However, smaller blood vessels and nerve structures, such as muscle spindles, are also affected. The observed morphological effects of acupuncture are probably related to at least some of the mechanisms of post-acupuncture reactions.

References

1. **Bowsher, D. Mechanisms of acupuncture**. – In: *Medical Acupuncture: A Western Scientific Approach*, Edinburg, Churchill Livingstone, 1998, 69-82.
2. **Comunetti, A., S. Laage, N. Schiessl, A. Kistler.** Characterization of human skin conductance at acupuncture points. – *Experientia,* **51**, 1995, 328-331.
3. **Dimitrov, N**. Morphological changes in biologically active point /BAP/ ST36 after acupuncture in rat. – *Acta morphol. anthropol.,* **19**(1), 2012, 30-33.
4. **Dimitrov, N.** Normal morphology of biologically active point BAP/ST36 rat. – *Acta morphol. anthropol.*, **19**(1)*,* 2012, 34-37.
5. **Dimitrov, N., D. Atanasova, J. Staykova, N. Pirovski, D. Sivrev**. Changes in collagen and elastic fibers in biological active point ST_{36} of rats after experimental acupuncture. – *Acta morphol. anthropol.,* **21**, 2015, 115-118.
6. **Dimitrov, N., D. Atanasova, N. Tomov, D. Sivrev, N**. **Lazarov.** Acupuncture causes serotonin release by mast cells. – *Rom. J. Morphol. Embryol.*, **58**(3), 2017, 961-968.
7. **Dimitrov, N., D. Sivrev, D. Atanasova.** Histological structure of the human biologically active point (BAP) ST36. – *TJS,* **13** (2), 2015, 67-69.
8. **Focks, C.** Atlas of acupuncture. China, Churchill Livingstone Elsevier, 2008, 168.
9. **Kim, M., T. Nam, M. Kim, J. Kim, D. Kim, K. Lee, C. Song.** Histological observation of canine acupoints. – *J. Vet. Clin*., **23** (2), 2006, 102-104.
10. **Langevin, H., N. Bouffard, G. Badger, D. Churchill, A. Howe.** Subcutaneous tissue fibroblast cytoskeletal remodeling induced by acupuncture: evidence for a mechanotransduction-based mechanism. – *J. Cell Physiol.*, **207**(3), 2006, 767-774.

11. **Langevin, H., N. Bouffard, D. Churchill, G. Badger**. Connective tissue fibroblast response to acupuncture: dose-dependent effect of bidirectional needle rotation. – *J. Altern. Complement. Med., **13***(3), 2007*,* 355–360.
12. **White, A., M. Cummings, J. Fishie. How to locate acupuncture points.** – In: *An introduction to western medical acupuncture*. Churchill Livingstone Elsevier, 2008, 185-189.
13. **Wick, F., M. Wick, MC. Wick.** Morphological analysis of human acupuncture points through immunohistochemistry. – *Am. J. Phys. Med. Rehabil*., **86**(1), 2007, 7-11.
14. **Yu, X., G. Ding, W. Yao, R. Zhan, M. Huang.** The role of collagen fiber in "Zusanli" (ST 36) in acupuncture analgesia in the rat. – *Chinese Acupuncture & Moxibustion*, **28**, 2008, 207-213.