FURTHER STUDY ON THE ANATOMICAL, HISTOLOGICAL AND BIOCHEMICAL BASES UNDERLYING CLINICAL ACUPUNCTURE EFFECTIVENESS

Ifrim-Chen Feng and Mircea Ifrim

Faculty of Medicine & Pharmacy, University of Oradea Oradea, Romania (Received 6th January 2004, accepted 29th January 2004)

The study was undertaken in order further to elucidate the relationship between structural specificities of acupoints and meridians and their clinical effectiveness. 356 biopsis samples were processed, 287 of which were taken from 48 adult and 2 newborn cadavers and the rest 69, from living patients, at three different levels beneath the acupoints, between meridians and at some distances away. Six types of staining methods were applied to the materials obtained from diversified areas in relation to acupoints and meridians, so as to manifest the distribution of collagen fibers, reticular fibers, mucopolysaccharides (MPS), connective tissues, nerve threads, blood vessels etc. The findings revealed that structural and biochemical specificities associated with acupoints and meridians included (1) amount of mucopolysaccharides, in particular acid MPS, the concentration of collagen fibers and, richness in nerve endings etc. with the intensities being highest around the acupoints, higher at the levels of meridians and low in areas at some distances away from meridians (2) constitution of the adjacent connective tissue and the afferent nerve formations and (3) functional structures constituting an energetic complex system.

Key words: Structures, Acupuncture, Meridians, Acupoints, Connective tissues, Mucopolysaccharides, Nerve fibers, Blood vessels, Staining methods.

INTRUDUCTION

Typical Traditional Chinese Medicine comprises herbal medicine, acupuncture, moxibustion, therapeutic massage, prevention and surgery. Acupuncture, moxibustion and therapeutic massage depend on application to meridians, also called channels as well as their collaterals and acupoints^{1,2}.

Correspondence to: Frim-Chen Feng, Faculty of Medicine & Pharmacy, University of Oradea, Fax: 40-259-418266, E-mail: cfeng@uoradea.ro

For thousands of years and in particular in the last half century, meridians and acupoints have been one of the most intriguing areas attracting high research interest. Main findings from modern research include the following: (1) the general recognition of the functions of meridians as well as collaterals in connection, transportation, induction, transmission and regulation of essential physiological functions, qi (understood as energy), blood and body fluid³⁻⁵; (2) the nature of the meridians being expounded on the bases of neural segments, central nerve system, blood vessels, neuro-humural system, bio-electricity and cybernetics³⁻¹¹ and; (3) the association of acupoints with specificities in nerve concentration, richness of blood vessels, lymph plexus, difference in Ca⁺⁺ and other minerals, etc.¹¹⁻¹⁷

However, the rich findings do not signal the end but rather call for further research.

OBJECTIVES OF THE STUDY

The objectives of the study are two-fold: (1) Is there a connection between structures of acupoints and connective tissues? (2) Are structures of acupoints functionally important?

The first hypothesis is based on the facts that both connective tissues and acupoints are ubiquitously distributed from extremities to internal organs; and secondly connective tissues consist of cellular components and ground substance – mainly acid and neutral mucopolysaccharides, and fibrillar components such as collagen, reticulin, elastic and oxytalamic fibers. Therefore, an association between the structures of acupoints and richness in the components of connective tissue would suggest a correlation.

Based on Darwin's well known principle – "struggle for life", Dr. W. Roux' conclusion that "connective tissue is a functional structure" (1885), Prof. Benighoff's "functional arrangement of morphological structures" in the early 20 century, and later reports as "bio-physico-chemical mechanism contributes to the formation of structures", "the embryonic mezenchim is rich in mucopolysaccharides", etc., we accordingly hypothesized that correlation between the structures of acupoints and connective tissue would suggest a functional correlation.

MATERIALS AND METHODS

356 biopsis samples were processed, 287 of which taken from 48 adult and 2 newborn cadavers and, the rest 69, from living patients during operations. The samples were fixed either in 10 % formalin or Carnoy's fixative. The embedding in paraffin was achieved by the rapid method, followed by sectioning at 5 microns (frozen sections permitted better preservation, especially Toluidin-Blue to acid mucopolysaccharides), and stained by Hematoxylin-eosin, Van Giesson, Mallory, Masson, Gomori, PAS and Toluidin-Blue.

The tissues processed were divided into three groups according to the areas from which they were obtained, with the tissues from groups 1 and 2 obtained from 18 different acupoints on 10 diverse meridians. The areas from which sample tissues were taken, from both cadavers and living patients, were as follows:

In Group 1: at the level of and beneath the acupoints.

In Group 2: along the meridians between the acupoints.

In Group 3: out of points and meridians.

The examined parameters included 1) general status of connective tissues; 2) biochemical statuses of mucopolysaccharides, collagen, reticulin, etc.; 3) statuses of nerve fibers and; 4) muscle fibers.

RESULTS

In Masson staining, connective tissues were stained in blue, muscles, reddish; mucus, pink and amyloid, light violet.



Fig. 1. Point 7, Small Intestine Meridian of Hand–*Taiyan* (Masson × 400) – Cadaver



Fig. 3. Triple Warmer Meridian of Hand –Shaoyang $(Masson \times 100) - Living \ Subject$



Fig. 2. Point 8, Liver Meridian of Foot–Jueyin (Masson \times 100) – Living Subject



Fig. 4. Skin (Masson \times 100) – Cadaver



Fig. 5. Point 13, Governing Meridian (VG \times 100)–Cadaver



Fig. 7. Skin (VG × 100) – Cadaver



Fig. 9. Spleen Meridian (PAS \times 100) – Living Subject



Fig. 6. Governing Meridian (VG \times 100) – Living Subject



Fig. 8. Point 36, Stomach Meridian of Foot- Yangming (PAS \times 100) – Cadaver



Fig. 10. Skin (PAS \times 100) – Living Subject

Group 1 demonstrated a very high density of connective tissues in both the cadavers and the living subjects (Figs. 1-2); while Group 2 exhibited a higher but less marked density than Group 1 (Fig. 3); Group 3 had low density of connective tissues (Fig. 4).

Van Giesson staining collagen fibers in red-yellow yielded similar results i.e. Group 1 had the very high density (Fig. 5); Group 2, high density (Fig. 6) and Group 3, low density (Fig. 7).

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Fig. 11. Point 57, Urinary Bladder Meridian of Foot-*Taiyang* (TBM × 100) – Living Subject



Fig. 13. Skin (TBM \times 100)



Fig. 15. Stomach Meridian (Gomori × 100)



Fig. 12. Large Intestine Meridian of Hand Yangming (TBM \times 100) – Living Subject



Fig. 14. Point 8 (Quqan), Liver Meridian of Foot–*Jueyin* (Gomori × 100)



Fig. 16. Stomach Meridian (Gomori × 100)

PAS staining acid and neutral mucopolysaccharides in red-purple demonstrated the same results with Group 1 exhibiting the maximum density (Fig. 8); Group 2, lesser density than Group 1 (Fig. 9) and Group 3, a low density (Fig. 10).

In order to discriminate acid and neutral mucopolysaccharides, the staining method of Toluidin Blue was applied, acid mucopolysaccharides were stained in pink-purple with this technique. The results shown in the following Figs. 11-13, are



Fig. 17. Point 10, Small Intestine Meridian of Hand–Taiyang (HE \times 100)

the same as in the previous.

Reticular fibers blackened by Gomori staining did not display any differences between the three groups seen in Figs. 14-16.

Hematoxilin Eosin stained nerves threads in dark-blue and collagens in pink, demonstrating the same outcomes in three groups above. However, due to poor micrograph effect, one image only from the group 1 is here presented (Fig. 17).

DISCUSSION

Microscopic examinations revealed no differences between the biopsis samples processed from either cadavers or living subjects and from either adult or newborn cadavers, but there existed in the following significant discrepancies between three groups.

- I. Mucopolysaccharides are clearly concentrated around the areas of various acupoints. The concentration of MPS diminishes at the level of the meridians and decreases even more in the areas between the meridians. PAS staining permitted estimation of the general level of MPS while Toluidin Blue permitted the identification of the acid mucopolysaccarides, which were more concentrated in the regions of acupoints but declined according to the order of energy levels of and proximities to the meridians.
- II. Special staining methods demonstrated increased concentration of collagen as well as nerve fibers in areas of the acupoints, with lower concentration in meridian levels between loci and even much lower concentrations in areas outside of both acupoints and meridians.
- III. The concentration patterns, namely, highest around the acupoints, high but less marked at meridians and much lower concentration in neutral areas without acupuncture loci and channels were also seen in total MPS and in particular acid MPS. Such findings appeared not to be artifacts due to the fixatives used (10 %)

formalin or Carnoy's fixative) nor origins of the biopsies samples (cadavers or living tissues) as histochemical processes demonstrated the same results.

As known, mucopolysaccharides make up a complex group of substances, containing in their molecule an amino carbohydrate. They are presented both in the cells and in connective vascular interstices, more or less bound to collagenous proteins. Although they only represent 0.02 % - 5 % of the total weight of the constituent tissue substances they play very significant physiological roles. Studies on the MPS contained in the connective – vascular interstices have yielded the following findings: 1) binding of water, ions, acids and bases in the tissues; 2) good permeability of the basement membrane; 3) association between fibrillogenesis and ageing; 4) involvement in inflammation, regeneration, cicatrization and tumoral proliferation; 5) differences in appearance, growth, differentiation and maintenance among the various connective tissue types and; 6) modulation of metabolism of MPS possibly underlying the therapeutic actions of hormones and substances acting on the connective – vascular interstices.

While connective tissues, their components as well as their complex physiological roles are common to many tissues and organs, the singular concentrations of certain elements around the functional acupoints and meridians, which are central to actions in acupuncture, moxibustion and therapeutic massages would suggest functional involvements in those actions. Proteic synthesis occurring at the level of the ground substance of the connective tissue and the collagen fibers, as proven by the histogenesis itself of the connective tissue, and as a corollary the tissue electric potential changes support such a viewpoint. The present work furnishes arguments regarding involvement of the connective tissues energy changes physiologically, pathologically and therapeutically. Further work is warranted for further elucidation of this complex system.

Differences in therapeutic effectiveness were observed when the same meridians were used in the treatment of different diseases^{18,19}, co-stimulation of the same meridians was applied to treat the same or different disorders²⁰⁻²³, the use of certain specific points relieved certain suffering^{24,25} or changing loci at the same meridians in certain treatments was practiced in clinical medicine^{25,26}. Findings from the present study should help to shed light on the mechanisms underlying these phenomena.

CONCLUSIONS

In the light of the up mentioned demonstrations, the conclusions can be drawn as follows.

- 1. In the areas at and beneath acupoints, a significant increase in amount of mucopolysaccharides (MPS), in particular the acid MPS was observed. Intensive or increased concentrations of collagen fibers were presented.
- 2. Such concentrations are less marked in the meridians but still significantly higher than those in tissues some

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distances away from points and meridians.

- 3. No changes in distribution of the reticular fibers and elastic network were observed among the different examined groups.
- 4. The presence of a multitude of nerve endings at the level of acopoints confirmed the previous findings reported in the literature and suggest the involvement of the central vegetative nervous system in the action of acupuncture.
- 5. Acupuncture points and meridians are associated with structural specificities with regards to the constitution of the adjacent connective tissues and the afferent nerve formations.
- 6. The acupuncture points and the meridians are functional structures, constituting an energetic complex system of the human organism
- 7. These findings should help inspire further research in understanding the holistic concept of human physiology, the etiopathogenesis of diseases and effective therapeutic measures.

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針灸臨床效益與解剖、組織與生化 相關性之深入研究

Ifrim-Chen Feng and Mircea Ifrim

Oradea 大學 醫學與藥劑學院

Oradea 羅馬尼亞

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為分析針灸點、經脈結構與臨床針灸效果之關係,本研究共分析了 356 個樣本,其中 287 個 取自 50 具屍體,69 個取自活病人。樣本取自針灸點下三層次組織即位於針灸點,經脈及離開經 脈較遠之部位。共採用了六種染色方法以顯示採自上述不同部位和層次組織中膠原纖維、網狀纖 維、粘多糖(MPS)、結締組織、神經纖維、血管等之分佈。結果顯示與針灸點及經脈有關之特 殊結構,包括(1)粘多糖,特别是酸性粘多糖之量,膠原纖維與神經之密度等顯示針灸點濃/密度 最高,經脈次之,離開經穴較遠之中性組織則相對稀少;(2)鄰近之結締組織、輸入神經組織與其 功能之結構,合成一複雜之能量系統。

關鍵詞:結構,針灸,經脈,針灸點,結締組織,粘多糖,神經纖維,血管,染色法。

聯絡人: Frim-Chen Feng, Faculty of Medicine & Pharmacy, University of Oradea, 傳真: 40-259-418266, E-mail: cfeng@uoradea.ro。