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Regulatory effects of electro-acupuncture at Zusanli on ir-SP content in rat pituitary gland and peripheral blood and their immunity

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INTRODUCTION

It has been reported in many studies that electro-acupuncture (EA) can positive ly regulate erythrocytic immunity and T-lymphocytic subgroups[1-8]. Nevertheless, its mechanism remains to be explored. In the present study, a multi -group, multi-stepped and multi-indexed observation was conducted on the effe cts of EA on erythyrocytic immunity and T-lymphocytic subgroups. A simultaneous assay of the changes in immunoreactive substance-P (ir-SP) content in the pit uitary gland and peripheral blood was also carried out. The objective of the study was to investigate the regulatory effects of the immune system and their poss ible mechanism in the treatment of relevant diseases with EA.

MATERIALS AND METHODS

Animals and groups

Forty healthy Shanghai SD white rats weighing 150 g-180 g, aged 12 wk-14 wk, p rovided by the Center for Laboratory Animals, 4th Military Medical University, Xi'an, were divided into 5 groups with 8 rats in each group: the normal control group, the Zusanli group, the immunosuppressive model group, the non-acu point group, and the Zusanli + immunosuppressive group.

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Instruments and agents

G6805-A EA Instrument was purchased from Shanghai Medical Instrument Factory, Shanghai, China. Radioimmunoassay kit was provided by Beijing Haikerui Biotech Co . Ltd., Beijing, China. Epics-profile II Flow Cytometer was product of Coulter, USA. Anti-rat CD4 FITC/CD8PE was provided by SEROTEC, Britain.

Methods

Rat model of immuno-suppression Rat model of immuno-suppression was est ablished by injecting 100 mg/kg of newly- prepared cytoxan (CY) solution into the rats' abdominal cavity on the 1st and the 4th day. Same amounts of normal saline was injected into the abdominal cavity of the rats of the other 4 groups.

Electro-acupuncture All the rats were raised in a quiet place. The acupo int chosen for EA was *Zusanli* (He-Sea Point, St 36)[9]; the non-a cupoint for EA was 0.5 cm off Zusanli. EA parameters were intermittent waves at 2 Hz and 3 V. The visual param eter was the slight shivering of the hind lim bs of the rats. After they were fixed on a wooden board, the rats of the Zusanli-group were electro-acupunctured at *Zusanli* continually for 30 min at a fixed time for 7 d on a daily basis. The immunosuppressive group and the *Zusanli* + immunosuppressive group were also established according to the above method. EA treatment for the *Zusanli* + immunosuppressive group was the same as that for the *Zusanli* group. Rats of the control and the immunosuppre ssive group were tied by the same method for 30 min at a fixed time on a daily basis but did not undertake EA stimulation.

Sampling and assay Thirty mg/kg of 1% pentobarbital sodium was injected into the abdominal cavity of all the rats on the 7th day of EA. Blood samples were taken by decapitation after anesthesia with 15 U/ml of heparin as, an anticoagulant. Ir-SP content in the pituitary gland and peripheral blood was assayed by radioimmunoassay (RIA) accord ing to the manufacturer's instructions. By micro-whole-blood direct immunofl uorescence staining and flow cytometry, T-lymphocytic subgroups were assayed to reflect the cellular immunity. The rats' RBC-C_3bRR and RBC-ICR were assayed by immune adherence rosette[10].

Statistical analysis SPSS 8.0 (a statistical analysis system) was applied for the data analysis. The methods included variance analysis and linear correlation.

RESULTS

In the Zusanli group, the values of CD4+, RBC-C_3bRR and RBC-ICR an d the conte nt of SP in pituitary gland and peripheral blood were all markedly highe r than that of the control ($P < 0.01$). The values of CD8+ did not change significantly ($P > 0.05$), and the rate of CD4+ positively correlated with RBC-C_3bRR ($r = 0.719$, $P < 0.05$), and so did the content of SP. Th e values of CD4+ and RBC-C_3bRR and the content of SP in rats'pituitary gla nd and peripheral blood of the immunosuppressive group were significantly lower than that of the control ($P < 0.01$, $P < 0.05$). The values of CD8+ did not change significantly ($P < 0.05$). In contrast, after EA at *Zusanli*, there was significant improvement in the indexes of the immuno-suppressive group ($P < 0.01$) whereas there was no statistical difference between the non-point and the control group (Tables 1 and 2).

Table 1 Changes in the values of T-lymphocytic subgroups and erythrocytic immu nity ($n = 8$, $\bar{x} \pm s$)

Group	CD4+	CD8+	RBC-C-3bRR	RBC-ICR
Control	43.1 ± 3.1	23.6 ± 2.6	8.6 ± 2.1	6.8 ± 2.3
Non-point	44.7 ± 4.6	21.0 ± 4.3	11.1 ± 1.2	8.9 ± 2.9
Immuno-suppressed	34.5 ± 2.5[a]	26.2 ± 8.4	5.2 ± 1.2[b]	9.8 ± 4.4
Zusanli	65.6 ± 8.4[a]	29.5 ± 8.2	15.9 ± 3.0[a]	12.1 ± 1.2[a]
Zusanli +Immuno suppressive	48.8 ± 6.0[c]	22.6 ± 7.0	16.4 ± 4.1[c]	15.0 ± 5.2[c]

[a]$P < 0.01$, [b]$P < 0.05$ *vs* control group; [c]$P < 0.01$ *vs* immuno-suppressed group. EA: electro-acupuncture

Table 2 The contents of substance P in pituitary gland and peripheral blood in rats ($n = 8$, $\bar{x} \pm s$)

Group	Pituitary gland	Peripheral blood
Control	569 ± 8	20 ± 7
Non-point	547 ± 22[b]	20 ± 8[b]
Immuno-suppressed	273 ± 106[a]	12 ± 4
Zusanli	592 ± 142[ab]	92 ± 22[a]
Zusanli +immuno-suppressive	554 ± 32[b]	30 ± 9[b]

[a]$P < 0.01$ *vs* control group; [b]$P < 0.01$ *vs* immuno-suppressed group. EA: electro-acupuncture

DISCUSSION

Regulatory effects of EA at Zusanli on the neuroendocrine system

Zhao *et al*[11] discovered the cubic structure of the microangium of Zusanli and Lin *et al*[12] reported that there might be a nerve network in the colloid of the spine and brain stem that corresponds with the stomach channel of Foot Yangming, St, runs through the spine, and reaches the nucleus of spinal tract of trigeminal nerve. These studies have proved the regulatory effect of EA at *Zusanli* on neuroendocrines. With the development of studies on the mechanism of acupuncture, the relation between acupuncture and endocrine hormone has become a focus of interest for many researchers. Many studies have indicated that acupuncture can regulate thyroid hormones, sex hormones, adrenocortical hormones, insulin, brain-gut peptide, etc[13]. Substance P, an important cerebral and brain-gut peptide is widespread in the central nervous system and gastrointestinal tract. It exists not only in the endocrine and paracrine cells, playing the roles of hormones and the local transmitter, but also in the endogenous and exogenous neurons, playing the role of a neural transmitter. The present study has proved that EA at *Zusanli* can significantly increase the synthesis of substance P in the pituitary gland, and its content in the peripheral blood, thus bringing into play its unique physiological and pathophysiological regulatory functions.

The general regulatory effects of Zusanli EA and the theory of neuroendocrine-immune network

It has been reported that many hormones can modify the body's immunity by their intervention at macrophages, T-lymphocytes and B-lymphocytes[14-20] receptor sites whereas immune cells can also secrete various neuropeptides and hormones as immune transmitters. What is more, some neuroendocrine cells can secrete cellular factors. These cells can influence both the neuroendocrine system and the immune system. The three systems of nerve, endocrine and immunity depend mainly on the peptidergic factors they generate and the receptors of the latter to communicate with each other. Therefore, immune cells can also be regarded as a kind of receptors in the body that receive stimulation from antigens, secrete immune transmitter, transmit information to the central nervous system before it regulates, as a feedback, the body's immunity by means of transmitting nerves and hormones. This is the so-called "theory of neuroendocrine-immune regulatory network". The regulatory effect of EA at *Zusanli* on the nervous, endocrine and immune systems cannot be separated from its internal association with the neuroendocrine-immune regulatory network. In addition to its direct regulation of the above systems, acupuncture can also indirectly act upon the systems through their internal association with each other. This is probably the theo retical basis of Traditional Chinese Medicine's general regulatory principle. It is also a point of collision between Traditional Chinese Medicine and modern medicine, namely the holistic medical mode[21].

Role of SP in the mechanism of acupuncture's effects on the immunoregulation

SP is an important biologically active substance of information channel transmission[22]. Modern immunological research has proved that cells of the immune system can release SP and contain SP conjugative sites of

high affinity[23]. SP can affect the immune system in the following ways. It can 1) stimulate and proliferate lymphocytes *in vivo* and *in vitro*; 2) improve the proliferative reaction of the spleen, mesenteric lymph nodes and intestinal aggregated lymphatic follicles[24]; 3) at milli-Molar level, it can induce interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factors (TNF) from monocytes[25]; 4) increase lipopolysacchraide (LPS)-induced secreti on of IL-10 from monocytes in human umbilical cord blood and reverse the inhibitory effect of INF-¦Ã on LPS-induced secretion of IL-10[26]; 5) stimulate the synthesis of PGE2, which in turn stimulates the generation of cellular factors[27]. The present study indicates that after electro-acupuncture at Zusanli, ir-SP in the rats' pituitary gland and peripheral blood increased significantly in comparison with that of the control and were positively correlated with the change in CD4+. This attests that electro-acupuncture can regulate cellular immunity by exciting organism synthesis and releasing SP and affect the neuroendocrine-immunoregulatory network through the indirect ef fect of SP on the change of various cellular factors. However, the change in SP after electro-acupuncture at Zusanli had nothing to do with the immune indexes of RBC, which indicates that electro-acupuncture probably does not depend on SP to influence RBC immune indexes.

The effects of EA at Zusanli on the mucosal immunity of gastrointestinal tract

Modern immunological studies have confirmed the significant regulatory effects of gastrointestinal hormone on the intestinal mucosal immunocytes[28-30]. The intestinal mucosal immuno-tissues are controlled by peptidergic nerve fibres, which were located adjacent to lymphatic systems in the epithelial layer, lamina propria and immunocytes such as macrophages, mastocytes, etc. can directly regulate the intestinal immune system. In the intestineassociated lymphatic tissues, the existence of T-lymphocytes and SP receptors specific to B cells in dense SP teleneurons and aggregated lymphatic follicles indicates that SP might be acting as a nutritious factor for the intestinal immunocytes. It is by stimulating its organic synthesis and release that EA at *Zusanli* regulates the intestinal immunity, thus adjusting gastrointestinal diseases and functions. Furthermore, EA at *Zusanli* can also positively regulate the general immune system by the exchange between GALT and the general lymphatic tissues. According to Traditional Chinese Medicine, pathogenesis boils down to three factors, i.e., spiritual, exopathic and constitutional factors. The three factors constitute the gist of the theory of neuroendocrine-immunoregulation network.

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