

反復針刺激の犬リンパ球に及ぼす影響

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The Effect of Repeated Acupuncture Stimulation on Canine Lymphocyte Response

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ABSTRACT. Twenty normal healthy mongrel dogs were divided equally into four groups. Acupoints were stimulated bilaterally by insertion of sterilized stainless needles at ST-36. The needles were inserted and left in situ for 30 minutes/day. The single acupuncture group was needled for a day without an electric stimulator, and the repeated acupuncture group was needled once a day for five days. The single electroacupuncture group was stimulated for a day with for electric stimulator (1 volt, 2 Hz, 1 msec, positive square wave), and the repeated electroacupuncture group were stimulated once a day for five days. Cellular immunological responses and hematological findings were determined after repeated acupuncture stimulations. ANAE positive cells stained by alpha naphthyl acetate esterase, the stimulation index of lymphocytes blastformed by incubation with phytohemagglutinin and the γ -globulin of serum protein all decreased within several days after repeated electroacupuncture stimulations, representing a decrease in basal immunological response. After the repeated electroacupuncture stimulation on the ST-36, it was suggested that in healthy dogs the depression of the immunological responses was due chiefly to the T-cells.—**KEY WORDS:** acupuncture, ANAE positive cell, cellular immunology, electroacupuncture, phytohemagglutinin.

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Acupuncture stimulation is known to be effective on a number of disease [1, 14, 28]. Several possible mechanisms of its effectiveness have been suggested by many workers of neurophysiology, pharmacology, biochemistry and endocrinology. Lymphocytes are involved in cellular immune responses and play an important role in immunoregulation. Lymphocytes are classified into subpopulations and subsets [16]. *In vitro* response of lymphocytes to phytohemagglutinin (PHA) was related to various clinical features [8, 15]. It has also been reported that lymphocyte response to PHA is enhanced within 1 and 2 days after acupuncture stimulation [11, 12]. However, the period of observation was too short for evaluation of cellular immunological activity. Recent reports on the immunological effects of acupuncture have received a great deal of attention in veterinary medicine

[13]. In veterinary acupuncture, lymphocyte response to PHA has rarely been observed within 2 weeks after repeated acupuncture. The present study was undertaken to determine the differences in cellular immunological responses and hematological findings after repeated acupuncture stimulations.

MATERIALS AND METHODS

Twenty normal healthy mongrel dogs (1.0-2.0 years of age, 5.0-11.5 kg body weight) were divided equally into four groups. Acupoints were stimulated bilaterally by insertion of sterilized stainless needles (0.26 mm×56 mm), at ST-36 [14]. This particular point, below and lateral to the patella in the upper anterior tibial muscle, was chosen because previous reports suggested that the site was useful point for stimulating the immune system [1, 4].

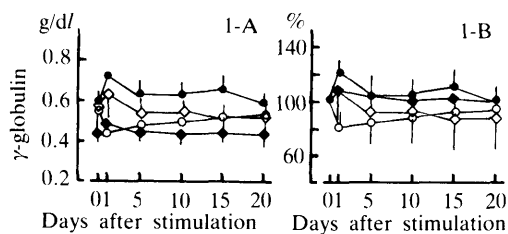


Fig. 1. γ -globulin values of serum protein after single acupuncture (—◆—), single electroacupuncture (—◇—), 5 repeated acupuncture (—●—) and 5 repeated electroacupuncture (—○—) stimulations. Each point represents the mean and standard error. Data are shown in the left panel (1-A), and relative values to the pre-stimulation control values are shown in the right panel (1-B).

The inserted needles were left in situ for 30 minutes/day. The single acupuncture (S-A) group was needed for a day without electric stimulation, and the repeated acupuncture (R-A) group was needed once a day for five days. The single electroacupuncture (S-E) group was treated for a day with an electric stimulator (MSE-3R, Nihon Koden Co.), and the repeated electroacupuncture (R-E) group was stimulated once a day for five days. In the electroacupuncture, each point was stimulated continuously with 1 volt, 2 Hz, 1 msec duration, positive square wave. Blood samples were obtained by venipuncture, using heparinized syringes, immediately before and 1, 5, 10, 15 and 20 days after the acupuncture stimulations.

Measurements of erythrocytes, packed cell volume, hemoglobin values, leukocytes and differential leukocyte count (percentage), serum protein and fractions were obtained using routine techniques [7]. Acid alpha naphthyl acetate esterase (ANAE) positive cells were counted after ANAE stain of lymphocytes [18], and the ANAE positive cell/lymphocyte ratio was calculated. Stimulation index (SI) of lymphocyte blastformation was measured using macroculture methods with phytohemagglutinin (PHA) [24].

The data obtained were analyzed statisti-

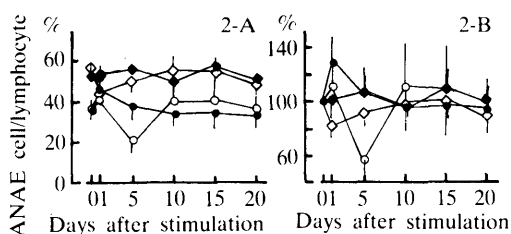


Fig. 2. ANAE positive cell/lymphocyte ratio after single acupuncture (—◆—), single electroacupuncture (—◇—), 5 repeated acupuncture (—●—) and 5 repeated electroacupuncture (—○—) stimulations. Each point represents the mean and standard error. Data are shown in the left panel (2-A), and relative values to the pre-stimulation control values are shown in the right panel (2-B).

cally by Student's *t*-test. Relative values to the pre-stimulation values were analyzed for statistical significance between the groups.

RESULTS

The experimental data and relative values to the pre-stimulation values of erythrocytes, packed cell volume, hemoglobin values, leukocytes, lymphocytes, serum protein, albumin, and albumin/globulin ratio displayed no significant differences among the 4 groups ($p > 0.05$). Experimental values of γ -globulin showed no significant differences among the 4 groups within 20 days after stimulations ($p > 0.05$) (Fig. 1-A), but the relative values to the pre-stimulation control values of the R-E group were lower than in the R-A group on the 1st day after stimulation ($p < 0.05$) (Fig. 1-B). The ANAE positive cell/lymphocyte ratio of the R-E group was lower than both the S-A and S-E groups on the 5th day after stimulation ($p < 0.05$) (Fig. 2-A), and the relative values to the pre-stimulation control values of the R-E group were lower than in S-A group on the 5th day after stimulation ($p < 0.05$) (Fig. 2-B). The number of ANAE positive cells of the R-A group were fewer than in either the S-A or S-E groups on the 10- and 15th day after stimulation ($p < 0.05$) (Fig. 3-A). The

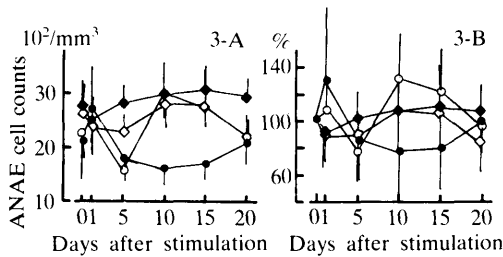


Fig. 3. ANAE positive cell counts of lymphocytes after single acupuncture (—◆—), single electroacupuncture (—◇—), 5 repeated acupuncture (—●—) and 5 repeated electroacupuncture (—○—) stimulations. Each point represents the mean and standard error. Data are shown in the left panel (3-A), and relative values to the pre-stimulation control values are shown in the right panel (3-B).

stimulation index of lymphocytes (SI) of the R-A group was lower than in the S-E and S-A groups on the 1st and 5th day after stimulation ($p < 0.01$), and that of the R-E group was lower than in the S-A group on the 10-, 15- and 20th day after stimulation ($p < 0.05$) (Fig. 4-A). The relative values to the pre-stimulation control values of the SI in the R-A group were lower than in the S-E group on the 1st and 5th day after stimulation ($p < 0.05$) (Fig. 4-B).

DISCUSSION

Acupuncture has been shown to be useful in managing patients with infection and inflammation [8, 17, 25]. Possible theories behind the effectiveness of acupuncture remain to be established. Most of theories are based on physiological view points. Our results demonstrated that the repeated stimulation of St-36 acupoints in dogs resulted in a significant reduction in immunological responses. It is known that the stimulation of St-36 points affects the immunological system of man and animals [2, 6, 19]. Several preliminary experiments showed that electroacupuncture stimulation with 1.0 volts and 2-Hz was necessary to produce a weakness muscle twitch response in animals

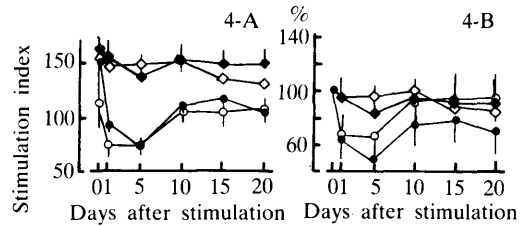


Fig. 4. Stimulation index of lymphocytes blast-formed with phytohemagglutinin after single acupuncture (—◆—), single electroacupuncture (—◇—), 5 repeated acupuncture (—●—) and 5 repeated electroacupuncture (—○—) stimulations. Each point represents the mean and standard error. Data are shown in the left panel (4-A), and relative values to the pre-stimulation control values are shown in the right panel (4-B).

[2, 14, 21, 26]. Changes of γ -globulin values with acupoint stimulation became evident 1 and 2 weeks after other hematological changes occurred [21, 23]. Based on these observations, the dogs in the present experiment were stimulated continuously with 1 volt, 2 Hz, 1 msec duration, positive square wave, and consequently changes in lymphocytes and γ -globulin were observed for 20 days following the acupoint stimulation.

There are limited reports on erythrocytes, packed cell volume and hemoglobin values after acupuncture stimulation. It has been reported that no significant decreases of erythrocytes and packed cell volume were seen in dogs after acupuncture anesthesia, and the changes were even smaller than after anesthesia by pentobarbital sodium [26]. In man, packed cell volume decreased during needling stimulation and/or electric stimulation, but recovered gradually after the stimulation [5]. In our experiments, it was expected that the erythrocytes, packed cell volume and hemoglobin value would decrease after the acupuncture stimulations, but such changes were not seen.

The effect of acupuncture on leukocytes varies widely depending upon the acupuncture locus selected for needling, the method of needling and the functional condition. After needling, the leukocyte count was

reported to increase [9], decrease slightly [3], and decrease slightly followed later by an increase [20]. No definite effects of acupuncture on leukocytes were recorded in this study.

ANAE stains mature T-cells of lymphocytes in peripheral blood [10] and reflects the presence of resting T-cells [27]. Canine T-cell subpopulation is difficult to divide into subsets [18]. In humans, following acupuncture stimulation, increases of T-cell counts, NK-cells and B-cell/lymphocyte ratio and decreases of T γ -cell/T-cell and K-cell/T-cell ratio were reported [11, 12]. The present results were that ANAE positive cell/lymphocyte ratio decreases within 5 days after repeated electroacupuncture stimulations. This suggests that the subsets of the canine ANAE positive cell population changed with stimulation.

In humans, increases of the SI of lymphocytes after incubation with PHA and T-cells immediately after acupuncture stimulation [11, 12] were reported. However, repeated stimulation was not used and the period of observation was only a few days. The present results suggested decreases of the SI with PHA within 5 days after the stimulation in the R-E group, and a depression of ANAE positive cell activities after repeated electroacupuncture stimulation.

In this study, the decreases in ANAE positive cells, SI with PHA and γ -globulin within several days after repeated electroacupuncture stimulations, indicated a decrease in basal immunological activity. However, there was recovery to normal conditions from the 10th day after the stimulations. The findings suggested that acupuncture stimulation of healthy dogs does not cause long-term hematological and immunological changes, but in diseased dogs it affects the immunological mechanisms.

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要 約

反復針刺激の犬リンパ球に及ぼす影響：工藤忠明・加藤由美子・増野裕英¹⁾・本所久男・北澤 馨（岐阜大学農学部家畜外科学教室，¹⁾愛知県刈谷保健所）——犬の左右の足三里に1日1回30分間置針及び通電刺激（1 volt, 2 Hz, 1 msec, 陽性矩形波）し，あるいは1日1回30分5日間置針及び通電刺激して，末梢リンパ球に及ぼす針の影響を観察した。反復刺激後数日間にわたり ANAE 染色陽性細胞は減少，PHA による幼若化リンパ球の刺激指数及び血清 γ -グロブリンは低下し，T-細胞を中心とした免疫能の抑制が示唆された。