

Role of Mast Cells in Acupuncture Analgesia: A Pilot Study

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ABSTRACT

Acupuncture has been practiced throughout Asia for more than 4000 years, however, acupuncture analgesia for surgical procedures was only developed and first used in 1958. The basic mechanism reason for better analgesia effects by stimulation of acupoints than of non-acupoints still remains inconclusive. In this work, the role of mast cells in acupuncture analgesia was investigated in Sprague Dawley (SD) rats. Acupuncture delivered to Zusanli (ST36) significantly increased mast cell degranulation in the acupoint and increased pain threshold (as indicated by increased latency of tail flick response), while injection of disodium chromoglycate (DSCG) to Zusanli (ST36) significantly inhibit mast cell degranulation and attenuated the acupuncture effect. We suggest that mast cell degranulation plays a role in acupuncture-induced analgesia.

INTRODUCTION

Acupuncture is a traditional Chinese therapeutic technique and used widely for pain management¹⁻⁵. However, the mechanisms underlying the acupuncture-induced analgesia are not well understood. One of the major problems remains unsolved is what the physical basis of meridian is if they exist. More recent researches have focused on the correlation between acupuncture points, meridians and connective tissues⁶. During acupuncture, needles pull and distort the surrounding tissue and thus deliver mechanical signals to the cellular level^{7,8,9}, leading to "downstream effects" that could activate certain cellular pathways and facilitate healing. Based on magnetic resonance imaging (MRI), anatomical and X-ray Computed Tomography (XCT) of morphological location¹⁰, all point to the probability that the physical basis of meridians and acupoints was in a complex system mainly of connective tissue.

Mast cells, as a resident of connective tissue, have aroused scientist's attention since early 80's. It was observed that acupoints have a higher concentration of mast cells than that of non-point location in control areas^{11,12}. The same was true for acupoints along the low impedance meridian lines¹³. Mechanically, by causing local microtrauma, acupuncture stimulated the mast cells in-situ to degranulate and release their mediators^{11,14,15}. These active mediators¹⁶, including histamines, caused many local changes such as phagocytosis. The released mediators also cause vasodilation, increased capillary permeability, and triggered a cascade reaction¹⁷.

In the present work, we asked whether mast cell degranulation plays a role in acupuncture-induced analgesia. This work focused on Zusanli (ST36) because Zusanli (ST36) has a high concentration of mast cells^{11,12} and stimulation of this acupoint could induce significant analgesia¹⁸⁻²⁰. Tail flick latency was used as the measurement of pain threshold^{21,22}, and the change rate of it reflected the effects of acupuncture analgesia of rats. Besides, numbers and degranulation rate of mast cells in specimens (skin and tissue) from ST36 were statistically calculated within initialized group of rats. (put in the methods).

MATERIALS AND METHODS

Animals and Groups. A total of forty male and female laboratory-born Sprague-Dawley rats bought from Shanghai Experimental Animal Centre of Chinese Academy of Science were used in this experiment, each with normal tail flick

latency and weighed 200 ± 20 g. Animals were housed in cages at controlled temperature ($20 \pm 2^\circ\text{C}$) with a 14/10 hour light/dark cycle. Food and water were made available optionally. All animals were handled with care to prevent infection and minimize stress. Rats were randomly divided into the following groups: six of normal pain threshold served as the control (A); others were divided into Acupuncture ST36(B1) and sham point Nearby-ST36(B2), Injection of DSCG(C1) and NaCl(C2), Acupuncture after injection of DSCG(D1) and NaCl(D2), and Acupuncture opposite side of injection of DSCG(D3).

Nociceptive Testing Model. Flick latencies to a radiant heat stimulus²³ were assessed for each rat using a Model 33T Tail Stimulator Analgesia Meter (IITC Life Sciences, Woodland Hills, CA). The room temperature was kept at $22^\circ\text{C} \pm 1^\circ\text{C}$. A light beam (40% intensity) was focused on a 2×2 mm region of skin. Flick latencies were obtained by shining the heat light source on the tip of the tail until it flicked. Temperature Change in the tip of the tail was recorded as the flick latency. A 20-s cut-off maximum was programmed into the timer to prevent tissue damage. Rats were habituated to the testing apparatus for 10 min prior to testing on each test day. After the habituation period, the average of 3 times of basic flick latencies was measured for each animal. Then, the changed latencies tested every 5min divided by basic ones are taken as the changed ratio.

Acupuncture Stimulation. Manual acupuncture stimulation was performed for 30 minutes with twirling and lift-thrust manipulation every 5min. Sterilized stainless steel acupuncture needles of 0.3 mm diameter were inserted into the loci of Zusanli (ST36) on left feet, located²⁴ at 5 mm lateral and distal to the anterior tubercle of the tibia. Sham point Nearby-ST36²⁵, which is 0.3mm away from ST36 (in direction to the fibula), was adopted as control point. To quantify the manual acupuncture stimulation^{26,27,28,29}, a self-made acupuncture needle real-time forced monitored was used to detect the wave shape of needle manipulation at ST36 on rats. During the acupuncture stimulation, animals were kept in plastic holders with their tails and hind legs protruding out. Animals in the control and injection groups were similarly kept in holders without acupuncture stimulation in order to rule out variations due to stress caused by the restraint.

Inhibition of Mast Cells. An inhibitor of degranulation function has been applied to mast cells in Zusanli (ST36), in order to determine the role of mast cells in acupuncture analgesia. Twenty μl of 5% DSCG was injected into ST36 by microliter syringe. The amount and concentration were decided referring to the method of conversion between experiment

animals and human²⁴. The same amount of 9% NaCl was used as control solution.

Specimen Preparations and Microscopic Examination. Specimens at Zusanli (ST36) with a volume of 0.33 cubic millimeters were collected after decapitation under narcotism of the experimental and control animals. Continuous paraffin slices of 4 μ m in thickness were made from corresponding groups. Some specimens with the same volume were collected from the non-acupoints, which was 0.3mm away from ST36. The slides were then stained with two methods³⁰: Toluidine Blue³¹ and Neutral Red³². Photomicrographs of the slides were taken at 400 with a 40 objective and investigated using light microscopy. The population of mast cells per microscopic view ($\times 200$) were taken count of. Only cells with visible nuclei were counted. And the ratios of degranulation and normal mast cells were calculated. To observe the degranulation inside mast cell more clearly, transmission electron microscope (TEM) slice was made for acupuncture and control group. The collection of specimen was the same as paraffin slice. Photomicrographs of the TEM slides were taken.

Statistical Analysis. Statistical analyses were performed to compare the tail flick latencies of experimental and control animals. Influence of disodium chromoglycate on the role of mast cells in acupuncture analgesia was estimated as changes in pain threshold $R_{pt} = \Delta P/P_0$, Where P_0 represented the average baseline flick latencies average of 3 times and ΔP represented the increase or decrease of tail flick latencies. The quantities and degranulation ratios of mast cells per unit area in specimens from all groups were calculated. Differences between groups were considered significant if $p < 0.05$. All data are represented as mean \pm SD. Tests performed to assess significance of differences in the estimates were two-tailed with no adjustments for multiple comparisons. The test statistic used to determine statistical significance of differences between two percents was $Z = |X_a + X_b| / \sqrt{S_a^2 + S_b^2}$, where X_a and X_b are the two percents being compared, and S_a and S_b are the standard errors of those percents. The critical value used for two-sided tests at 0.05 level of significance was 1.96.

RESULTS

The Criterion of De-qi Sensation. (Fig. 1, Fig.2). Based on previous studies^{27,28}, a self-made acupuncture needle real-time forced monitor was used to detect the wave shape of needle manipulation at ST36 on rats. The mean force of 240-280mN in lifting and thrusting and moment of 10-15mN·mm in twirling may be regarded as a regular range and

parameter of De-qi. According to this method, each manual needle insertion in the following experiment could be basically identified to achieve de-qi sensation.

The Effect of Acupoint Specificity (Fig.3). During 30min acupuncture at acupoint ST36, pain threshold increased significantly during the first 20min of acupuncture. In contrast, acupuncture in the groups of sham point Nearby-ST36 and blank control did not induce analgesia (How about Nearby-ST36? See Fig 3 Legend).

Influence of DSCG injection on the pain thresholds (Fig.4). Same dose of DSCG (20%) and NaCl (0.9%) were merely injected to ST36 acupoint as well as the blank control point. After injection, there was a slight increase in pain thresholds at first. Twenty minutes later, there were no significant differences between the injections with two kinds of solutions.

Influence of DSCG on the role of mast cells in acupuncture analgesia. (Fig.5, Table.1) Since the injection temperately increased pain thresholds (Fig. 4), acupuncture was made 20min after the injection. Figure 5 summarized the results of acupuncture effect on various groups. When DSCG was injected to Zusanli (ST36) to inhibit the mast cells, acupuncture analgesia was attenuated. However, injection of NaCl solution at the opposite side did not significantly affect the acupuncture effect (Table 1).

Light photomicrographs of stained mast cells in specimens at Zusanli (ST36) and non-acupoint under different statuses (Fig.6a-h). Paired continuous paraffin slices in 4 μ m thickness were stained with TB and NR respectively. Mast cells were identified by their metachromatic granules. Before acupuncture, mast cells at ST36 did not degranulate both in skin and muscle. After acupuncture, most of the mast cell have degranulated obviously. Large amount of their granules scattered around the cells. After DSCG inhibited, most of the mast cells were stabilized even after acupuncture, and the edge of mast cells remained smooth. In contrast, mast cells in non-acupoints, which were not so dense as those in acupoints.

TEM photomicrographs of mast cells in specimens at Zusanli (ST36) in acupuncture and control group (Fig.7a,b). Most of the skin mast cells in acupuncture group were in the degranulation status. Its cell membrane became thin and was broken out. Compare to the acupuncture group, the cells were in a stabilized status and the cell membrane kept rounded.

Comparison of Numbers of MCs in acupoints and non-acupoints and Degranulation Ratio of MCs in acupoints.

(Table.2, Table.3) There are significant difference ($P < 0.001$) between the number of mast cells per microscopic view ($\times 200$) from 20 slices randomly chosen in acupoints and non-acupoints respectively. Here we define mast cell without smooth round as degranulation. According to the degranulation ratio of mast cells among each group, both in skin and muscle, acupuncture enhanced the degranulation phenomena ($P < 0.05$); whereas DSCG inhibited this phenomena in skin ($P < 0.001$ compare to acupuncture group; $P > 0.05$ compare to control group); we take NaCl as contrast, it showed no significant difference compare to acupuncture group in skin ($P < 0.05$).

DISCUSSION

Interplay of Acupuncture and Mast Cell in acupoints. With the wideuse of acupuncture clinically as alternative or complimentary therapies for treating chronic pain³³ and acupuncture analgesia for surgical procedures³⁴, increasing concerns regarding the analgesic mechanism of acupuncture and physical basis of acupoints along meridians were aroused. The present study is an important initiative toward investigating the interplay of acupuncture and mast cells located in acupoints. Our fiest data suggest that acupuncture analgesia was attenuated when the mast cells in ST36 were inhibited by DSGG.

Manual Acupuncture Control and Point Specificity. As for acupoint specificity, in adult rats' skin and muscle samples, we found that the local mast cells were increased in number at the acupoint when compared with that beside the acupoint ($p < 0.05$), which is in accordance with the previous reports^{12-15,35,36}.

Mechanism of Signal Pathway in Acupuncture Modulation. According to the classification³⁷ of rodent mast cell subtypes, mast cells in ST36 belonged to connective tissue mast cells (CTMC), particularly of the skin. CTMC showed characteristics of inhibition by DSCG³⁸. In correlation with the morphological study, more than 80% of acupuncture points and 50% of meridian intersections of the human arm appeared to coincide with inter- or intramuscular connective tissue planes^{10,7}. Our results are consistent with the findings reported by other investigators that the mast cells in the adult rat connective tissue were distributed rich in acupoints along the meridians. That could result in histamine flare or meridian phenomenon³⁵. As we known, a variety of biologically active substances reserved in mast-cell granules, such as histamine, 5-HT, substance-P, heparin, leukotriene C4, etc³⁸. A previous study has reported that the number of the mast cell could serve

as an indicator of metabolism activity of local connective tissue⁴⁰. Collectively, these data lead us to posit that mast cell plays a key role in the channel through which acupuncture makes clinical phenomena & curative effect. When acupuncture, the needle inserts into the acupoint, rotating and lifting and thrusting techniques will cause the winding of subcutaneous collagen fibers⁸. All of the reactions from mechanical signal, mechanotransduction and cellular response, combine together and result in clinical phenomena and curative effect of acupuncture (relation as Fig.8).

Our experiments demonstrated that the number of mast cells in ST36 of rats showed an increase compared with that of beside the acupoint. Manual acupuncture promoted the mast cell to degranulate. The inhibition of mast cell secretion function weakened the effect of acupuncture analgesia. The difference in the activated condition of mast cells between the groups led us to the conclusion that mast cells participate in some way or another in mechanism of signal pathway in acupuncture modulation. This para is better.

In our observation, when needles stimulate these acupoints, mast cells in connective tissue was activated and released granules. The released substance may alter excitability of nerve endings together with the permeability of blood vessel walls⁴¹. Acupuncture can induce the degranulation of local mast cells and then hasten the downstream effects of mast cells along meridians by means of directional flow of tissue fluid³⁹. As a positive feedback, this procedure improves the vascular permeability and brings a series of biological effects in bodies, including the needling sensation of 'de-qi' and the transmission phenomenon along meridians.

This work presents a pilot research aiming at manifesting the role of mast cells play in acupuncture. In a word, there are close relationships between connecting mast cell and acupoints in respect of structure and function. A deeper explore of it should be followed.

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LEGENDS

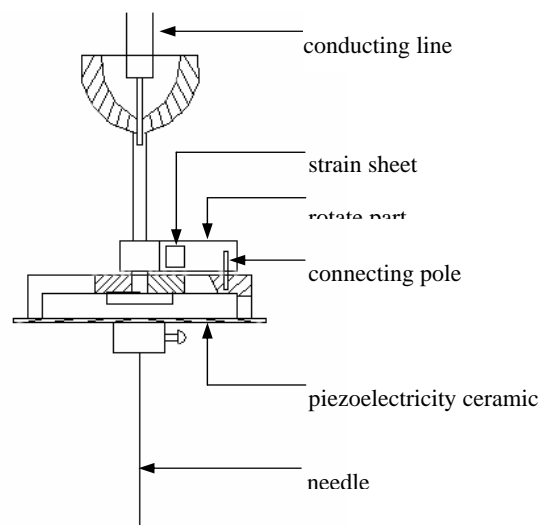


Fig.1. The structure and principle of the detection needle. The mechanical sensor was used to detect the real-time force of the needle as acupuncture manipulation.

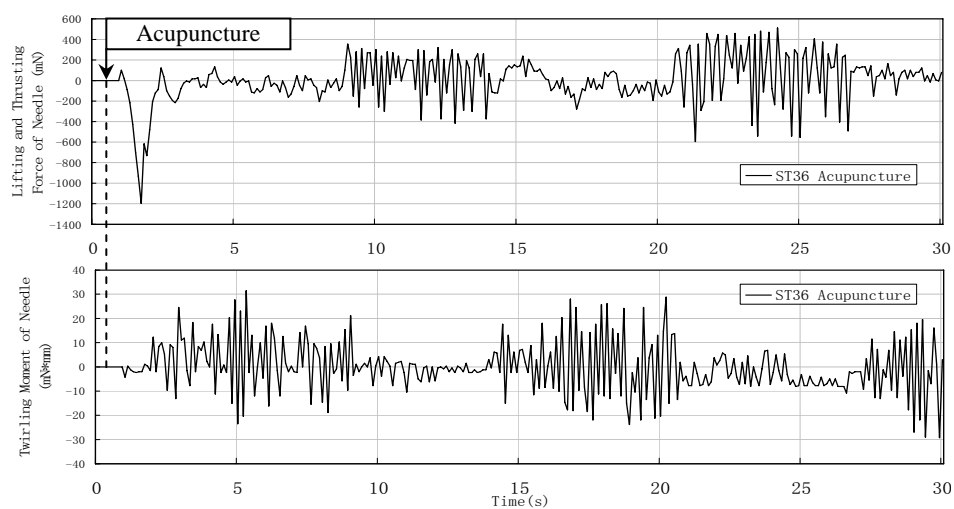


Fig.2. Wave shape of needle manipulation at ST36 detected by a self- made acupuncture needle real-time forced monitored.

Upper: Lifting and Thrusting Force of Needle (mN); Under: Twirling Moment of Needle (mN*mm). The mean force of 240-280mN in lifting and thrusting and moment of 10-15mN*mm in twirling may be regarded as a regular range and parameter of De-qi.

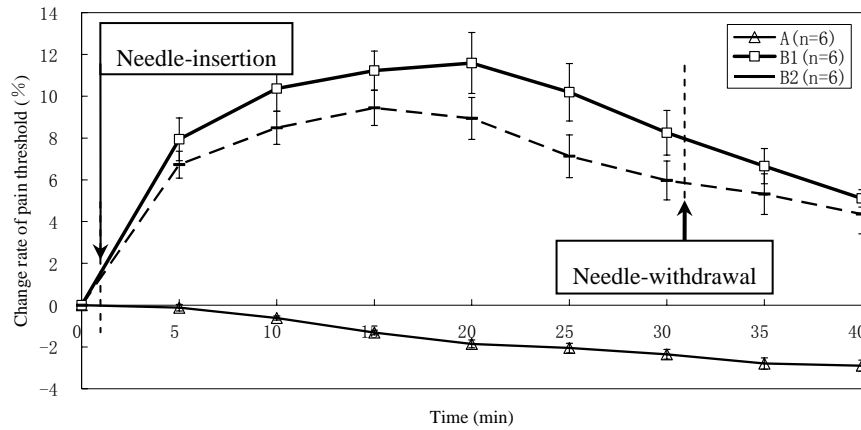


Fig.3. **Effect of acupuncture on pain threshold.** B1, acupoint ST36. B2, sham point Nearby-ST36. A, blank control.

Manual acupuncture stimulation was performed for 30 minutes with twirling and lift-thrust manipulation every 5min. Tail flick latency was used as the measurement of pain threshold, and the change rate of it reflected the effects of acupuncture analgesia of rats. Note that real acupuncture induced analgesia effect by increasing the pain threshold change rate from -1.85 ± 0.46 to 11.59 ± 3.57 (at 20 min), which had much better effect than sham acupuncture 8.94 ± 2.46 (at 20 min)

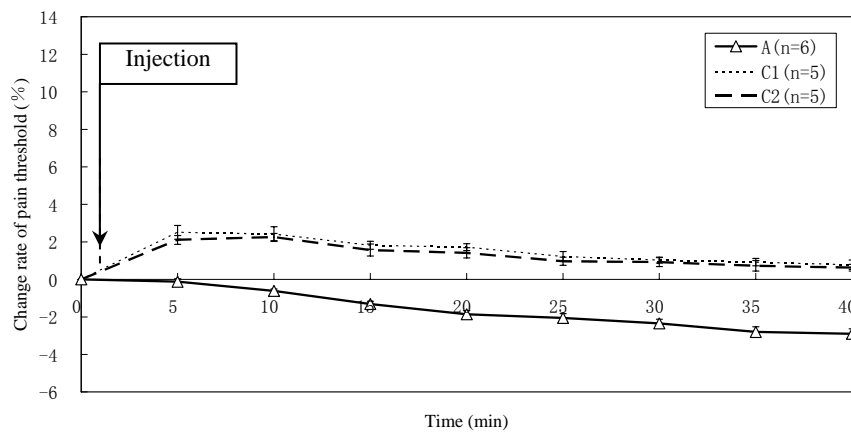


Fig.4. Influence of injection to the pain thresholds. C1, $20 \mu\text{l}$ of DSCG (20%) and C2, $20 \mu\text{l}$ of NaCl (0.9%) were merely injected to compare with control group, A, blank control. Pain threshold was measured every 5min immediately after injection. The injection temperately increased pain thresholds. Significance of differences between C1 and C2 data were assessed (result are not showed completely). Twenty minutes later, there were no significant differences between the injections influences with two kinds of solutions.

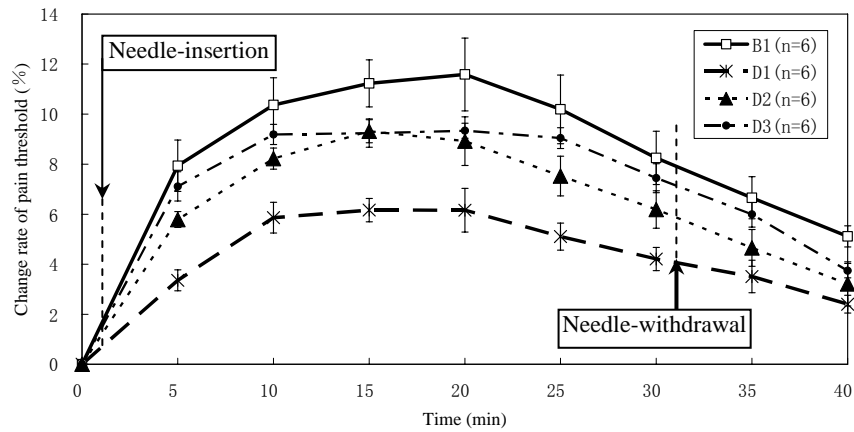


Fig.5. Influence of DSCG on the role of mast cells in the effect of acupuncture analgesia of rats. B1, acupuncture group was compared with D1, acupuncture after DSCG, D2, acupuncture after NaCl, and D3, acupuncture opposite side after DSCG. Methods of measurement are the same as in Fig.3. DSCG attenuated acupuncture analgesia by reducing the pain threshold change rate to 6.16 ± 2.15 at 20min ($P < 0.05$ vs Group Acupuncture). However, injection of NaCl solution at the opposite side did not significantly affect the acupuncture effect ($P > 0.05$ vs Group Acupuncture)

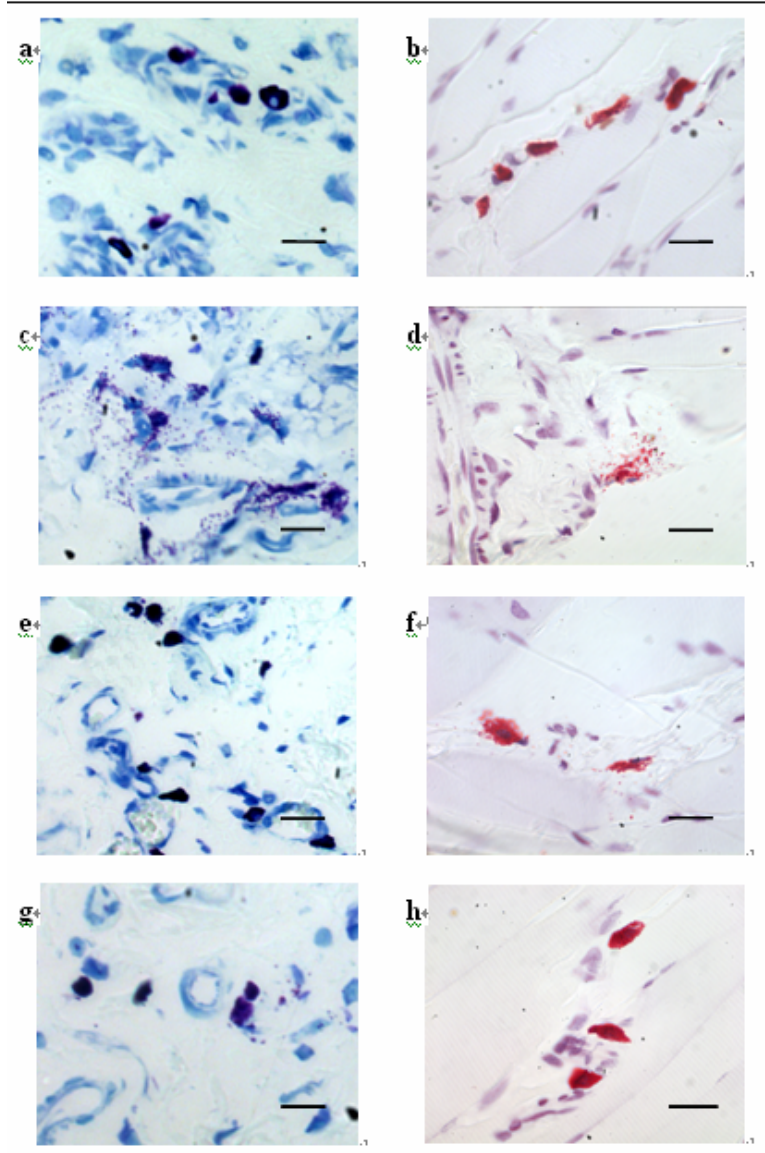


Fig.6. Photomicrographs of stained mast cells in specimens at Zusanli (ST36) and non-acupoint under different statuses.

a and b showed specimens before acupuncture, mast cells at Zusanli did not degranulate both in skin and muscle. **c and d**, showed specimens after acupuncture, most of the mast cell have degranulated obviously. Large amount of their granules scattered around the cells. **e and f** showed specimens after DSCG inhibited, most of the mast cells were stabilized even after acupuncture. The edge of mast cells remained smooth. **g and h** showed specimens of mast cells in non-acupoints, which were not so dense as those in acupoints. Scale bar is 10 μ m

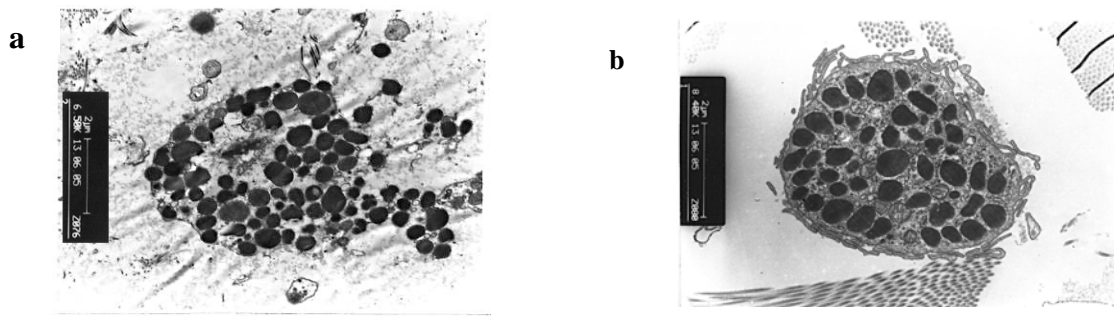


Fig.7 TEM photomicrographs of mast cells in specimens at Zusanli (ST36) in acupuncture and control group. **a** showed the skin mast cell in acupuncture group, which was in the degranulation status. Its cell membrane became thin and was broken out. Compare to the cell in acupuncture group, **b** showed the skin mast cell in blank control group, which was in a stabilized status and the cell membrane kept rounded.

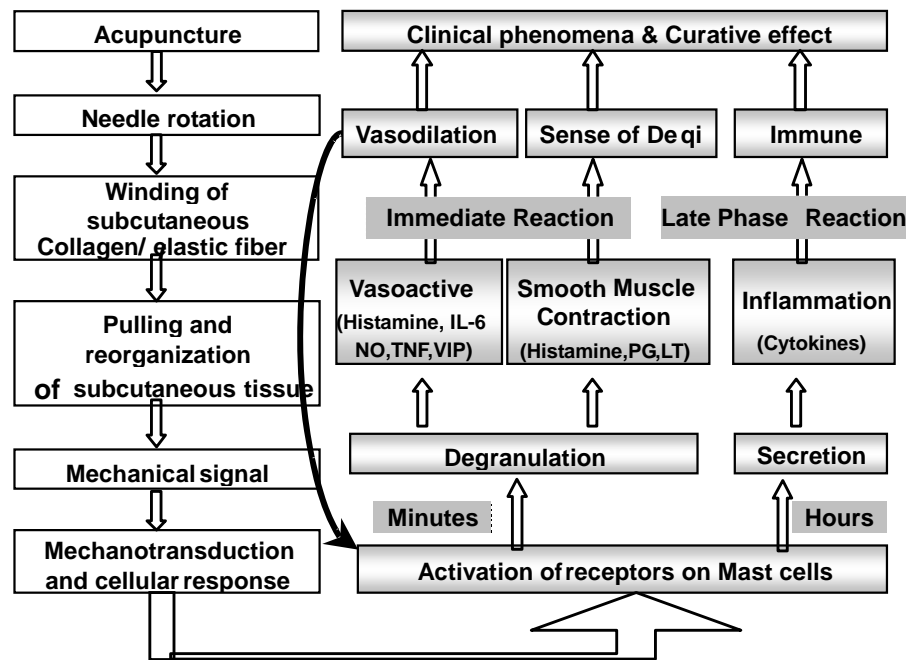


Figure.8. A hypothesis of the channel through which acupuncture makes clinical phenomena & curative effect. When acupuncture, the needle inserts into the acupoint, rotating and lifting and thrusting techniques will cause the winding of subcutaneous collagen fibers. All of the reactions from mechanical signal, mechanotransduction and cellular response, combine together and result in clinical phenomena and curative effect of acupuncture.

TABLES

Table.1 Change Rate of Pain Threshold at Peak Value (at 20min)

ID	Group Name	n	Average±SD
A	Control	6	- 1.85 ± 0.46
B1	Acupuncture at ST36	6	11.59 ± 3.57*
B2	Acupuncture at Nearby-ST36	6	8.94 ± 2.46*^{△△}
C1	Injection of DSCG	5	1.72 ± 0.45*[△]
C2	Injection of NaCl	5	1.42 ± 0.67*[△]
D1	Acupuncture after DSCG	6	6.16 ± 2.15*^{△△}
D2	Acupuncture after NaCl	6	8.92 ± 2.38*[△]
D3	Acupuncture opposite side after DSCG	6	9.34 ± 0.74*^{△△}

Note: *P<0.01 vs. Group A; [△]P<0.01, ^{△△}P<0.05 vs. Group B1; [▲]P<0.01, ^{▲▲}P<0.05 vs. Group D1;

Table.2 Comparison of Numbers of MCs in acupoints and non-acupoints (per microscopic view (×200))

Group Name	MCs Number	
	Skin	Muscle
Acupoint (n=20)	34.15±6.06	12.35±3.33
Non-acupoint (n=20)	22.05±4.24*	8.50±2.40*

Note: *P<0.001 vs. Acupoint Group

Table.3 Degranulation Ratio of MCs in acupoints

Group Name	MCs Degranulation Ratio	
	Skin	Muscle
Control (n=6)	29.18 ± 2.82	26.31 ± 4.49
Acupuncture (n=6)	48.90 ± 9.45*	32.18 ± 7.46**
Acupuncture after DSCG (n=6)	33.67 ± 6.84[△]	28.48 ± 5.72
Acupuncture after NaCl (n=6)	50.39 ± 6.01*	29.74 ± 5.55

Note: *P<0.001, **P<0.05 vs. Control Group; [△]P<0.01, ^{△△}P<0.05 vs. Acupuncture Group.