

ROLE OF MAST CELLS IN ACUPUNCTURE EFFECT: A PILOT STUDY

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To better understand the therapeutic effectiveness of acupuncture, questions about the underlying mechanisms need to be addressed. Here we describe the impact of manual stimulation by an acupuncture needle of *zusanli* (stomach 36 [ST36]) on analgesia in rats. The analgesic effect was more pronounced after stimulation of ST36 than after stimulation of a sham point near the acupuncture point. At the same time, we determined in tissue slices the density of mast cells in the acupuncture points and nearby points, as well as the degree of degranulation before and after stimulation. We found that the density of mast cells from the ST36 of rats was higher than that from a nearby sham

point. In addition, acupuncture resulted in a remarkable increase in degranulation of the mast cells. Pretreatment of the acupuncture point with disodium chromoglycate not only counteracted the phenomenon of degranulation but also reduced analgesic effect of acupuncture. Our experiments on inhibition of degranulation of mast cells in tissue from acupuncture points demonstrates the possible role of mast cells in acupuncture effects.

Key words: Acupoint, mast cells, DSCG, analgesia, degranulation ratio

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INTRODUCTION

Several thousand years of medical practice have revealed that acupuncture, one of the elements in Traditional Chinese Medicine, is an effective therapeutic technique to maintain good health and to treat various diseases.^{1,2} A growing body of scientific literature provides strong evidence for the efficacy of acupuncture in analgesia.^{3,4} Analgesic effects of acupuncture are systemic, whereas acupuncture manipulations themselves are local.⁵⁻⁷ However, the physiological mechanisms underlying the effects following stimulation of acupuncture points (acupoints) are still unclear; this is one of the problems which needs to be solved to establish a scientific basis of acupuncture and is also one of the important problems in meridian research. Meridians indicate pathways along which the Qi (energy) is spread through the human body. These pathways form the basis for the spread of the signals elicited by acupuncture. Meridian research investigates the physiological and physical mechanisms underlying the signal distribution.⁸⁻¹¹ It is believed that during the needling

manipulation process, the application of lift and thrust and twist manipulation pulls the surrounding tissue around the needle body, delivering the cellular signal conducted along the pathway of channels (meridians) and leading to downstream effects that activate certain cellular pathways and facilitate healing.⁹ Examination of tissue morphology using techniques including magnetic resonance imaging and computed tomography have also supported the possibility that the physical basis of meridians and acupoints is a complex system based on connective tissue.¹²

As one of the resident cells in the loose connective tissue from the human body, mast cells have attracted the interest of researchers since the 1980s. One working hypothesis has been that the so-called *De Qi* sensation in response to acupuncture is related to the response of local mast cells.¹³ *De Qi* sensation is a unique needle sensation (grasp), including tingling, numbness, heaviness, and other feelings that occur after an acupuncture needle has properly been placed in the acupoint.^{4,9} Through mechanical stimulation, acupuncture can cause degranulation of the local mast cells in the acupoint and promote release of mediators, including arachidonic acid products, biogenic amines, chemoattractants, cytokines, growth factors, neuropeptides, proteoglycans, and proteolytic enzymes.¹⁴ These mediators act in several biological ways.¹⁵ For example, histamine can dilate capillary vessels and venules, which act on the vascular endothelium to expose the basal membrane and exude the tissue liquid, resulting in a change of electric potential gradient in the tissue along meridians.¹³ Another example of how the release of these mediators can effect systemic change is the way in which the neuropeptide serotonin, involved in analgesia, body temperature regulation, and nerve activity, can affect endocrine function in the body.¹⁶

In the present study, our aim was to investigate the relationship between degranulation of mast cells in acupoints and the analgesic effect of acupuncture. The change of pain threshold (PT) in Sprague-Dawley rat tails was adopted as a measure for the analgesic effect.^{17,18} The acupoint commonly used for analgesia,

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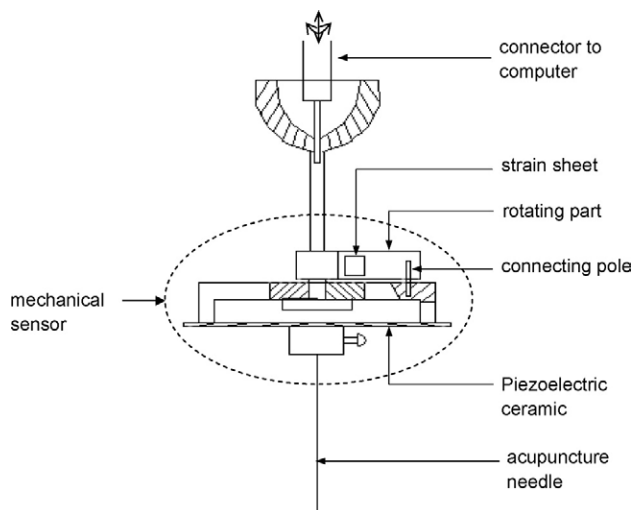


Figure 1. The structure and principle of force detection. The mechanical sensor was used to detect the real-time force on the needle during acupuncture manipulation.

zusanli (stomach 36, [ST36]), was selected to apply the lifting and thrusting and twisting stimulation¹⁹⁻²¹ under the control of a clinic needle real-time force monitor.^{22,23} To investigate the role of mast cell degranulation in the process of generation of pain analgesia in response to acupuncture, we treated the acupoint with the mast cell stabilizer disodium chromoglycate (DSCG)²⁴ and compared the effects of acupuncture in the treated points with those not treated with DSCG. In addition, from in vitro skin and tissue specimens, we estimated the density of mast cells in acupoints and nearby sham points, and also the change of degree of degranulation of mast cells from the acupoints in response to acupuncture.

METHODS

Animals and Groups

We used 52 male and female laboratory-born Sprague-Dawley rats provided by the Shanghai Experimental Animal Centre of the Chinese Academy of Science. They all exhibited normal tail flick latency and weighed 200 ± 20 g (at age of six weeks). The animals were housed in cages at controlled temperature (20 ± 2 °C) with a 14/10 hour light/dark cycle. Food and water were made available ad lib. All animals were handled with care to prevent infection and to minimize stress. Two rats each were used, for control and acupuncture, and for transmission electron microscopy. The remaining rats (48) were randomly divided into eight groups using a random-number table, six rats each for control group (A); acupuncture to ST36 (B1); acupuncture to sham point nearby ST36 (B2); injection of DSCG to ST36 (C1); injection of NaCl to ST36 (C2); acupuncture after pretreatment of DSCG to ST36 (D1); acupuncture after pretreatment with NaCl to ST36 (D2); and acupuncture after DSCG pretreatment in the sham point (D3). All the experiments were performed in accordance with the principles and procedures outlined in the *Guide for the Care and Use of Laboratory Animals* issued by the U.S. National Academy of Sciences.

Nociceptive Testing Model

The rat tail was exposed to radiation heat, and the tail flick latency was measured to detect the heat sensitivity. This model has been extensively applied in previous research on acupuncture analgesia.²⁵ The method is characterized by simplicity, easy use, and reproducibility of results. In our experiments, a model 33T tail stimulator analgesia meter (IITC Life Sciences, Woodland Hills, Calif) was used to apply the heat stimulation. Each time, the skin of the rat tail, with an area of 4 mm^2 and 2 cm from the tail tip, was stimulated with 40% of maximum light strength. The room temperature was controlled within 22 ± 1 °C. A 20-second cutoff maximum was programmed into the timer to prevent tissue damage. Rats were habituated to the testing apparatus for 10 minutes prior to testing. Then, we successively determined the temperature for tail flick three times to obtain the basic PT as average (five-minute intervals were allowed between each test). Ten minutes after testing the basic PT, the experiment was started.

Acupuncture Stimulation

Since *zusanli* is a popular acupoint for analgesia studies in animal experiments^{20,21} as well as for clinical treatment,^{3,4} it was selected as the acupoint in our experiments. Sterilized stainless steel acupuncture needles (0.25 mm, 1 inch, Suzhou Kangnian Medical Devices Co, Lt, Suzhou, China) were inserted into ST36 of the right hind leg, located 5-mm lateral and distal to the anterior tubercle of the tibia.²⁶ (The acupuncture point dosage was as follows: from Table 6-1 of Li (2003),²⁶ the equivalent dosage was calculated according to the surface area ratio between experimental animal and the human being, which in our case is 0.018, according to conversion formula: $DB = (DA \times WA) \times \text{RATE}/WB$. [Note: DB is the dosage for each kilogram weight of the rat. DA is the dosage for each kilogram weight of the human being. WA and WB are the weight of the human being and the rat. RATE is the ratio coefficients of the surface area from rat and human being, $\text{RATE} = 0.018$]. $DA \times WA = 1 \text{ mL}$, $WB = 200 \text{ g}$; thus, $DB = 0.09 \text{ mL/kg}$. It is about $20 \mu\text{L}$ of DSCG solution for each injection with a concentration of DSCG of 0.02 g/mL). The

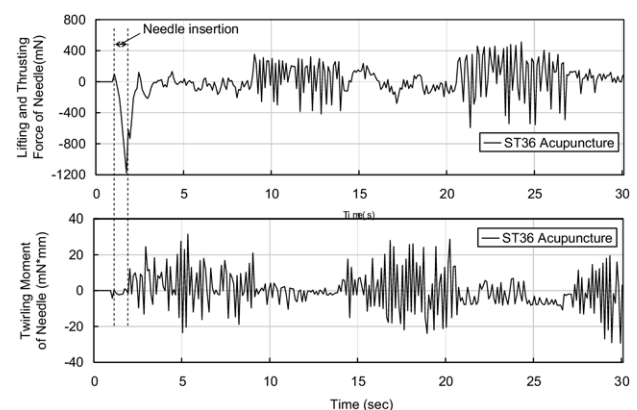


Figure 2. Wave form of needle manipulation at ST36 detected by acupuncture-needle real-time force monitor. Upper graph: lifting and thrusting force of needle (mN). Lower graph: twirling moment of needle (mN*mm).

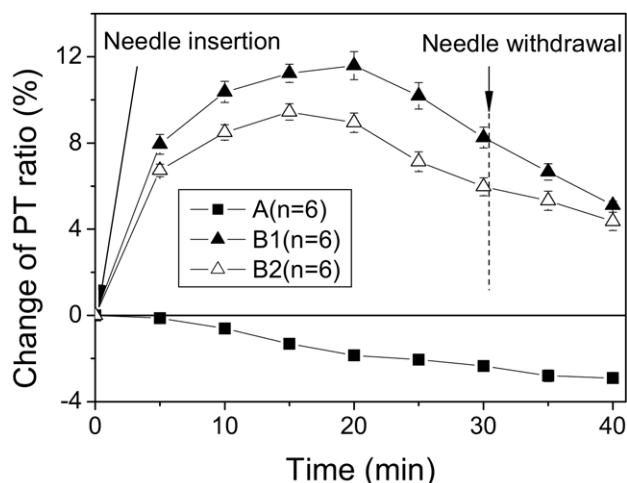


Figure 3. Effect of acupuncture on PT ratio. Manual acupuncture stimulation was performed for 30 minutes. Tail flick response was used as the measurement of PT and tested every five minutes, and the increased PT ratio reflected the effects of acupuncture analgesia of rats. The negative change of PT ratio in the control group could be pain hypersensitivity resulting from the repeated thermal stimulation at the same point. If error bars are not seen, they are within the size of the symbols. *PT*, pain threshold; *A*, control; *B1*, acupoint ST36 stimulation; *B2*, sham point nearby ST36 stimulation.

site 3 mm nearby ST36 (in the direction of the fibula) was taken as the sham point. The locations of acupoint and nearby sham point were clearly separated as shown previously by adding gen-

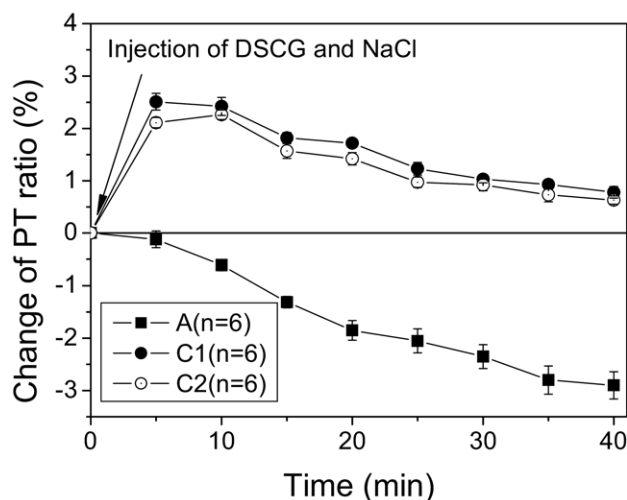


Figure 4. Influence of injection of DSCG or NaCl on the PT ratio. For C1, 20 μ L of DSCG (20%) or C2, 20 μ L of NaCl (0.9%), was injected. Pain threshold was measured every five minutes after injection. The injection slightly increased the PT ratio during the first 10 minutes, but became reduced again thereafter, and there was no significant difference between the two solutions. If error bars are not seen, they are within the size of the symbols. *PT*, pain threshold; *DSCG*, disodium chromoglycate; *A*, control; *C1*, injection of DSCG to ST36; *C2*, injection of NaCl to ST36.

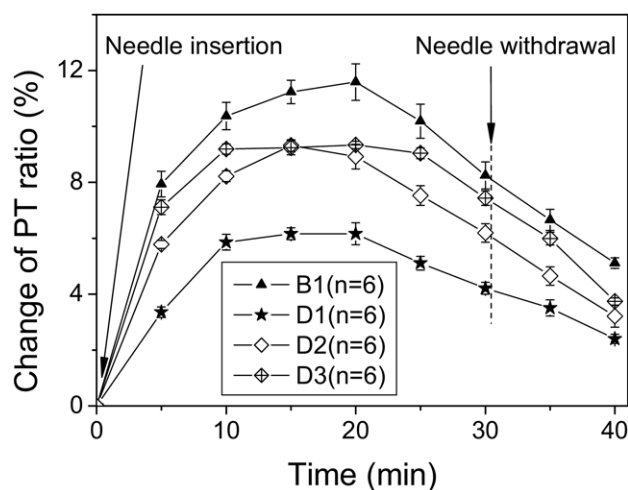


Figure 5. Influence of disodium chromoglycate (DSCG) on the role of mast cells in the effect of acupuncture analgesia. B1-PT ratio in acupuncture group was compared with that of D1, acupuncture after DSCG pretreatment, and D2, acupuncture after NaCl pretreatment, and D3, acupuncture after DSCG pretreatment in the opposite leg. If error bars are not seen, they are within the size of the symbols. *PT*, pain threshold.

tian violet solution to the tip of needle.²⁷ The perpendicular needling depth was approximately 7 mm, and we applied the lift and thrust and twist manipulation every five minutes. The needling manipulation was performed for 30 minutes.

Quantification of Needling Manipulation. For the quantification of the manual acupuncture stimulation, we used a clinical

Table 1. Change of Pain Threshold Ratio at 20 minutes

ID	Group Name	No.	Average $\pm$ SEM, %
A	Control	6	-1.9 ± 0.1
B1	Acupuncture at ST36	6	11.6 ± 0.6^a
B2	Acupuncture at nearby ST36	6	$8.9 \pm 0.3^{a,b}$
C1	Injection of DSCG at ST36	6	$1.7 \pm 0.1^{a,c}$
C2	Injection of NaCl at ST36	6	$1.4 \pm 0.1^{a,b}$
D1	Acupuncture after DSCG at ST36	6	$6.2 \pm 0.4^{a,b}$
D2	Acupuncture after NaCl at ST36	6	$8.9 \pm 0.4^{a,d}$
D3	Acupuncture opposite side after DSCG	6	$9.3 \pm 0.5^{a,e}$

ST36, stomach 36; DSCG, disodium chromoglycate.

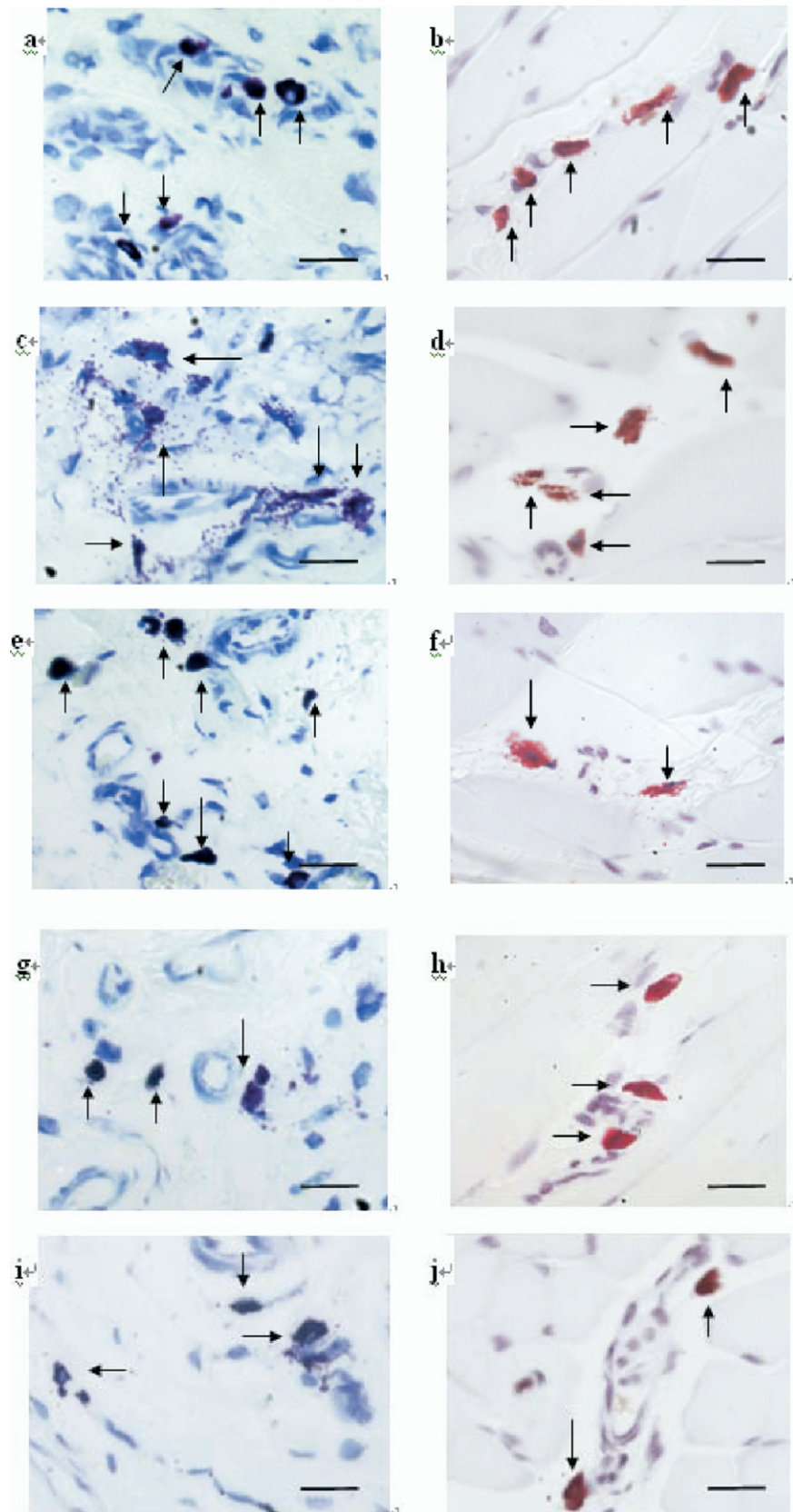
^a $P < .05$ vs group A.

^b $P < .05$ vs group B1.

^c $P < .01$.

^d $P < .01$.

^e $P < .05$ vs group D1.



monitor system, developed in our laboratory, for recording needle force in real time (Figure 1).^{22,23} This allowed the synchronous observation of the force with the needling process and gave a quantification index of the needling strength and frequency. During the needling and recording process, the rats were fixed within the testing apparatus; the two hind legs extended from the openings of apparatus and were fixed, leaving the tail exposed naturally. The same conditions were also used to fix the rats for the nonacupuncture group and the medicine injection group to rule out intergroup variations due to stress caused by the fixation procedure.

Medicine Pretreatment at the Acupoints. As a mast cell stabilizer,²⁴ 20 μ L of 0.02g/mL DSCG was injected into ST36 by microliter syringe to inhibit degranulation in the acupoint. The dosage and concentration were selected according to the accepted method of conversion between experimental animals and humans.²⁶ The same amount of physiological saline (9% NaCl) was used as the control solution.

Specimen Preparations and Microscopic Examination

Tissue samples from acupoints and nearby sham points were collected after decapitation under narcosis of the animals (1% sodium pentobarbital 1 mL/100 g). Based on the assumption that the effects of needling have uniform spherical spreading in the acupoint with the needle tip as center, we took the upper part of tissue from ST36. After cutting, the final size of skin was $3 \times 3 \text{ mm}^2$ and of muscle was $3 \times 3 \times 3 \text{ mm}^3$. Sequential paraffin slices of 4- μ m thickness were made after 48 hours of fixation at 4°C in fixing solution (10% formalin). The direction of section was longitudinal to the skin and muscle tissue. The skin sample was stained with 0.5% toluidine blue²⁸ and the muscle sample with 0.5% neutral red.²⁹ We took 20 slices uniformly distributed along the depth of the tissue sample. The numbers of mast cells per microscopic view (0.16 mm^2 at $\times 200$ magnification) were counted from four areas per slice and then averaged. Mast cells with more than three granules outside of the cell shape or with empty cavities in the cytoplasm were considered to be degranulated. The ratios of degranulated to total mast cells were calculated. Representative photomicrographs were taken at magnification of $\times 400$ for morphological evaluation. To more closely observe the features of degranulation of mast cells, transmission electron microscopy slices from acupuncture and control groups were made with the same collecting methods of specimens as described above.

Statistical Analysis

Statistical analysis was performed to compare the tail flick latencies of experimental and control animals. The influence of DSCG on the effect of acupuncture analgesia of rats was estimated from the change of PT ratio $R_{pt} = \Delta P/P_0$, where P_0 represented the average baseline flick latencies averaged from three measurements and ΔP represented the increase or decrease of tail flick latencies. The density and degree of degranulation of mast cells in specimens from all groups were calculated. Differences between groups were considered significant if $P < .05$. All data are represented as mean $\pm$ SEM.

RESULTS

Quantification of Manual Acupuncture Stimulation

We used the clinical monitor system (Figure 1) for recording in real-time acupuncture needle force. Figure 2 shows the strength and frequencies of manual stimulation. During the acupuncture on rats, the mean force of lifting and inserting the needle was kept in the range of 240 to 280 mN, the torque in the range of 10 to 15 mN $\times$ mm, and the frequency at approximately 3 Hz.

Specificity of the Acupoints

The influence of acupuncture on ST36 for 30 minutes on rat PT was observed (Figure 3). We found that PT increased with time of stimulation reaching a maximum after about 20 minutes (curve B1). The increase in PT ratio reached 13% above the control value (curve B1-curve A), which means that the tail flick occurred after 20 to 25 minutes at about 3.5°C higher temperature. During the period of 25 to 30 minutes, the PT ratio declined gradually. After withdrawal of the needle at 30 minutes, the PT ratio continued to decline but did not return to the basic PT ratio within the following 10 minutes. Acupuncture with the same intensity was given to the sham point (Figure 3, curve B2). During the entire needling process at the sham point, the PT ratio also increased but not as much as after stimulation of ST36. From this, we conclude that acupuncture at ST36 has a significantly superior effect of analgesia than acupuncture at the sham point, which shows the specificity of the acupoints.

Influence of DSCG and Physiological Saline on PT

We selected DSCG pretreatment to inhibit degranulation of the mast cells from the acupoint. Since injection itself may interfere with the acupuncture and analgesic effects, we first investigated the effect of injecting DSCG and physiological saline to ST36

Figure 6. Representative photomicrographs of stained mast cells (arrows) in specimens at *zusanli* (ST36) and nonacupoint. Left panels are from skin stained with toluidine blue and right panels are from muscle stained with neutral red. (A and B) ST36 before acupuncture; large rounded or elliptical mast cells are observed in skin and muscle. (C and D) ST36 after acupuncture; most of the mast cells show evidence of degranulation, as judged by extensive changes in appearance with intragranular matrix material released to the pericellular space. (E and F) ST36 after acupuncture with disodium chromoglycate (DSCG) pretreatment; majority of mast cells remain non-degranulated after acupuncture, demonstrating that the mast cell stabilizer treatment is partially effective. (G and H) Nonacupoints before acupuncture; mast cells have a similar appearance but a lower density compared with those in acupoints. (I and J) Nonacupoints after acupuncture; portion of mast cells degranulated in the skin. Scale bar is 10 μ m.

Table 2. Comparison of Mast Cells in Acupoints and Nonacupoints

Group Name	No. of Mast Cells ^a	
	Skin	Muscle
Acupoint (n = 20)	34.2 ± 2.5	12.4 ± 1.4
Nonacupoint (n = 20)	22.1 ± 1.7 ^b	8.5 ± 1.0 ^b

^aAverage ± SEM; 0.16 mm²/microscopic view (×200).^bP < .01 vs acupoint group.

without acupuncture (Figure 4). It is shown that injection of these two solutions had similar effects. After a slight increase of PT ratio within the first 10 minutes, a slow gradual decrease could be observed that may be attributed to sensitization as also seen in the control group. Hence, the influence from injection itself became very weak after 20 minutes.

Influence of DSCG and Physiological Saline on Acupuncture Effect

Based on the above result on the influence of injection, we applied the needling 20 minutes after injection. The PT value before injection was taken as the basal PT and subtracted. Figure 5 and Table 1 demonstrate that pretreatment of DSCG (curve D1) attenuated the analgesic effects of acupuncture. After 20 minutes of acupuncture, when the maximum PT ratio was obtained, the sites with DSCG pretreatment demonstrated a reduction of the ratio by 5.4 (D1-B1), which was twice the decrease (2.7) seen in the sites pretreated with physiological saline 2.7 (D2-B1). A similarly small effect (2.3) on the PT ratio was obtained after 20 minutes of acupuncture at the opposite leg (D3-B1). We concluded from these findings that the three kinds of pretreatment all weakened the effects of acupuncture on PT, but to different degrees. Stabilizing the mast cells in acupoint with DSCG clearly made the most pronounced attenuating difference.

Number of Mast Cells and the Degranulation Phenomenon

Mast cell density and degranulation was investigated by light and electron microscopy. In the stained tissue slices, we could observe the basophilic granules of the metachromia in mast cells by light microscopy (Figure 6A-J). The density of mast cells in

Table 3. Degranulation Ratio of Mast Cells in Acupoints

Group Name	MC Degranulation Ratio ^a	
	Skin, %	Muscle, %
Control (n = 6)	29.2 ± 1.2	26.3 ± 1.8
Acupuncture (n = 6)	48.9 ± 3.9 ^b	32.2 ± 3.1 ^c
Acupuncture after DSCG (n = 6)	33.7 ± 2.8 ^d	28.5 ± 2.3
Acupuncture after NaCl (n = 6)	50.4 ± 2.5 ^b	29.7 ± 2.3

MC, mast cell; DSCG, disodium chromoglycate.

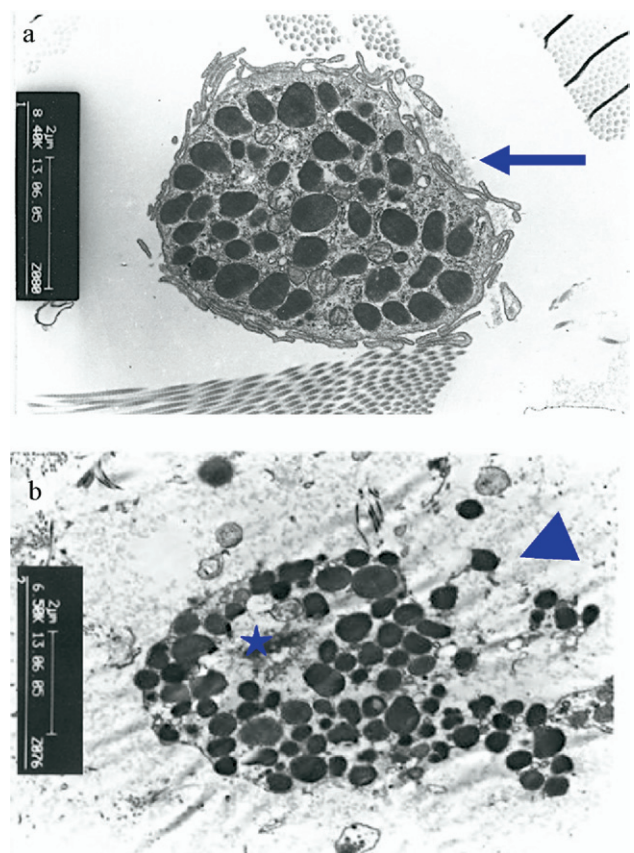
^aAverage ± SEM.^bP < .01.^cP < .05 vs control group.^dP < .01 vs acupuncture group.

Figure 7. Transmission electron microscopy photomicrographs of featured mast cells at *zusanli* (ST36) in control and acupuncture group. (A) Skin mast cell without acupuncture, rounded in shape with a complete intact cytomembrane densely packing the intragranules (arrow). (B) Skin mast cell after acupuncture, which has a thin and broken cytomembrane, with signs of degranulation, granules outside of the cell shape (arrowhead) and empty cavity in the cytoplasm (asterisk).

acupoint skin and muscle tissues was significantly higher than that from the sham point (Table 2). The acupuncture raised the degree of degranulation of the mast cells in the acupoint area of both skin and muscle (Table 3). After DSCG pretreatment, the acupuncture could not significantly facilitate degranulation of mast cells in skin and muscle of the acupoints. In electron microscopy (Figure 7), we observed more closely the degranulation feature in tissue slices after acupuncture; the cell membrane thinned and extensive intragranular matrix material appeared to be released to the pericellular space with empty cavities in the cytoplasm.

DISCUSSION

This article presents results that suggest a role of mast cells from the acupoints in the analgesic effects of acupuncture. The measurements of the analgesia and the observations of mast cell density and degranulation in tissue slices indicate a correlation between them.

First, with the same stimulation intensity during acupuncture, better effects of analgesia were obtained by stimulation at acupoints than at the nearby sham points. In addition, the densities of mast cells in acupoints in skin was 54.9% higher than in nearby points, and in muscle was 45.3% higher.

Second, along with the increase in PT caused by acupuncture, a facilitation in degranulation of the mast cells was observed in the acupoints. After the inhibition of degranulation of mast cells in acupoints by DSCG, this analgesia was significantly attenuated. This suggests that mast cells from the acupoint area participate in the acupuncture effect, and degranulation of mast cells is an important link in producing this effect.

Third, there was also an increase of PT in response to acupuncture of nonacupoints, and the mast cells in nonacupoints also degranulated, but the effects were significantly less pronounced. We hypothesize that, although acupuncture at nonacupoints, the manipulation of lifting and thrusting, twisting and twirling will be partially transmitted to the neighboring acupoint. Nevertheless, compared with ST36, the nearby sham point uniformly displays weaker analgesia than the real point during the entire acupuncture procedure. Based on the observation that the densities of mast cells in acupoints are significantly higher than in nonacupoints, the influence from degranulation in acupoints can be expected to be stronger than from the degranulation in the nonacupoints.

Our results confirm previous findings that the mast cell densities are about 50% higher in acupoints than in nonacupoints; this was shown previously for several other acupoints, including *sanyinjiao* (SP6), *yanglingquan* (GB34), *hegu* (LI4), *quchi* (LI11), and *neiguan* (PC6).¹³ In this study, we also demonstrate that the phenomenon of mast cell degranulation may be correlated with the analgesic effect of acupuncture. The reason for greater analgesic effectiveness in acupoints compared with nonacupoints may be the result of the higher densities of mast cells in acupoints. As one of the resident cells in loose connective tissue of the human body, mast cells are in contact with surrounding blood vessels, nerves, and lymph channels, forming a complex system of intercellular communications (Figure 8). This network may be one of the major physiologic mechanisms underlying the effectiveness of the acupoints.

After activating the mast cells in the connective tissue, biologically active components are released in a complex cascade of intercellular signaling.^{15,24} Our experimental results show that in acupoints, large quantities of these substances are released after the mast cells were mechanically stimulated (Figure 6C and 6D). The released substances include many biologically active substances, such as histamine, 5-HT, substance P, heparin, and leukotriene C₄.^{14,15} These substances not only affect the excitability of the nerve endings but also affect the functional condition of the blood vessels.³⁰

In conclusion, these data suggest that mast cells participate in the signaling pathway of acupuncture modulation (Figure 8). Acupuncture—the needle insertion into the acupoint, and the rotating and lifting and thrusting techniques—causes the winding of subcutaneous collagen fibers.^{8,9} At the same time, the needle movements may transfer the mechanical signal to the mast cells via the collagen fibers. This triggers the immediate degranulation of the local mast cells. Through the directional

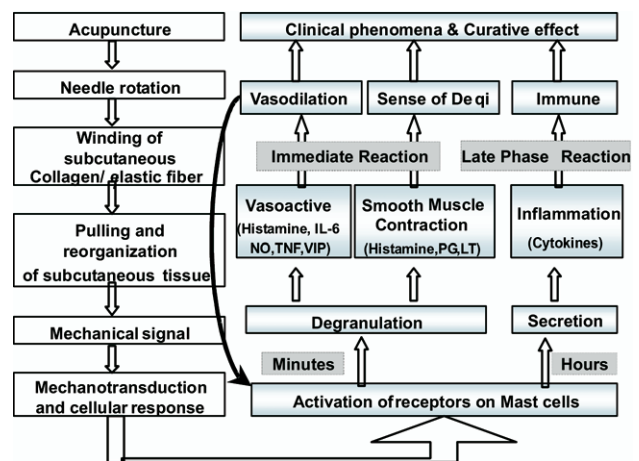


Figure 8. A hypothesis of the channel through which acupuncture makes clinical phenomena and curative effect. On acupuncture, the needle is inserted into the acupoint by applying rotating and lifting and thrusting techniques. This will cause the winding of subcutaneous collagen fibers. All of the reactions from mechanical stimulation, mechanotransduction and cellular responses combine together and result in clinical phenomena and curative effects of acupuncture.

flow of the tissue fluid, the locally released mediators can promote the activation of mast cells along the meridians.³¹ Since the histamine release in response to acupuncture is a positive feedback process leading to further degranulation of mast cells, it can change vascular permeability, resulting in the modulation of different systems, tissues, and organs of human body, causing the occurrence of a series of systemic biological effects and potentially impacting function of the endocrine, immune, and neurological systems. Further research is needed on the role of acupuncture-induced mast cell degranulation on local and systemic effects of acupuncture.

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