Local Changes in Microcirculation and the Analgesic Effects of Acupuncture: A Laser Doppler Perfusion Imaging Study

Seorim Min, KMD, MS,^{1,2} Hyangsook Lee, KMD, PhD,¹ Song-Yi Kim, KMD, PhD,^{1,2} Ji-Yeun Park, KMD, PhD,^{1,2}
Younbyoung Chae, KMD, PhD,^{1,2} Hyejung Lee, KMD, PhD,^{1,2} and Hi-Joon Park, KMD, PhD^{1,2}

Abstract

Objectives: Although the local response induced by acupuncture manipulation has been considered to be among the important factors that induce the effects of acupuncture, this connection has not yet been properly studied with standardized tools. The aims of this study are to examine the local changes in microcirculation that occur at different manipulation intensities and explore any associations of these changes with the analgesic effects of acupuncture.

Participants/Interventions: Twelve healthy volunteers received three acupuncture interventions (insertion only, a single manipulation, and repeated manipulations) at the right LI4 (Hegu or Hapgok) in random order.

Outcome measures: Skin blood perfusion was measured in a 100-mm² area ellipse centered on LI4 by using laser Doppler perfusion imaging (LDPI) before, during, and after acupuncture stimulation. Pressure pain thresholds (PPTs) were measured at ipsilateral areas, including acupoints ST25 (abdomen), LI5 (hand), LI10 (arm), and SP9 (leg).

Results: Repeated acupuncture manipulations enhanced microcirculatory perfusion compared with the insertion-only ($p < 0.01$) and the single-manipulation ($p < 0.05$) conditions. The repeated acupuncture manipulations significantly decreased the pressure pain at ST25 compared with the other groups (each $p < 0.05$). Of note, Spearman correlation analysis revealed significant correlation between changes of local perfusion and PPTs ($r = 0.393$; $p = 0.018$).

Conclusions: These results suggested that repeated manipulation induced higher local microcirculatory changes that were correlated with the analgesic effects at the relevant sites. The findings suggest that a proper dose of acupuncture stimulation might be essential to elicit the acupuncture effects.

Introduction

ACUPUNCTURE IS KNOWN TO BE a therapeutic approach that is especially beneficial for pain management.^{1–3} Acupuncture is a complex intervention that includes inserting needles into the skin and/or muscle at the acupuncture points and stimulating them to ultimately result in therapeutic effects. It has been suggested that acupuncture affects autonomic functions and the cardiovascular system.⁴ Microcirculation, primarily regulated by the autonomic nervous system, is a physiologic factor important in acupuncture's effects.⁵ Acupuncture stimulation induces alterations in microcirculation (i.e., increases in blood flow in the skin and muscle)⁶ and releases biological factors, such as nitric

oxide^{7,8} and calcitonin gene-related factor⁹, which play important roles in the modulation of vasodilation.¹⁰

Proper acupuncture manipulation is an important component of the analgesic effects of acupuncture. Recent studies have reported that changes in the stimulation parameters or stimulating techniques, such as the depth of needle penetration, result in different clinical outcomes.¹¹ Other studies have demonstrated that manipulation combined with electroacupuncture produces more potent antinociception compared with electroacupuncture alone in rats.¹² However, the following two factors are prerequisites for the development of clinically meaningful acupuncture manipulations: First, a standardized and reproducible manipulation method that is based on the observation of a

¹Studies of Translational Acupuncture Research, Acupuncture and Meridian Science Research Center, Kyung Hee University, Seoul, Republic of Korea.

²Department of Korean Medical Science, Graduate School of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea.

measurable biological parameter, such as microcirculation, must be defined; second, the changes in this biological parameter must be evaluated in terms of its relation to acupuncture's effects.

The recent development of noninvasive and remote sensing methods, such as laser Doppler perfusion imager (LDPI) technology, permitted in-depth analysis of changes in skin blood perfusion.¹³ This type of biomedical equipment makes it possible to easily quantify changes in microcirculation according to different acupuncture stimulation parameters.⁵ Thus, the current study proposes that LDPI is a useful tool to standardize the methods of acupuncture manipulation.

On the basis of the preceding ideas, the aims of this study were as follows: (1) to measure changes in microcirculation perfusion in response to different numbers of acupuncture stimulations using LDPI and (2) to investigate whether changes in microcirculation after different manipulations are related to the effects of acupuncture.

Materials and Methods

Participants

Healthy participants were recruited from Kyung Hee University, Seoul, Republic of Korea, by using an advertisement. Participants were excluded if they had any history of cardiac, respiratory, neurologic, or hormonal diseases. All participants refrained from consuming alcohol, caffeine, or medication for 12 hours before the experiment. All participants received a detailed explanation of the study and provided written informed consent. The expert committee of Acupuncture & Meridian Science Research Center, Kyung Hee University, reviewed the study protocol and judged it to be in compliance with the Declaration of Helsinki. The study was performed in a quiet, light-conditioned room with an ambient temperature of 22–24°C. Room temperature changes during the experiments were less than 1°C.

Acupoint selection

Acupoint LI4 (Hegu or Hapgok) is located on the dorsum of the hand, between the first and second metacarpal bones, in the middle of the second metacarpal bone on the radial side. It has been well established that acupuncture stimula-

tion at LI4 elicits both microcirculatory^{14,15} and analgesic effects.^{16,17}

Needling details (depth of insertion, rotation, and needle retention time)

Acupuncture stimulation was accomplished with acupuncture needles (0.30 mm in diameter and 30 mm in length; Dong Bang, Gyeonggi-do, Korea) that were inserted at acupoint LI4 to a depth of 15 mm. The manipulation technique was a balanced “tonifying and reducing” technique that involved bidirectional rotation at a rate of 1 Hz. All acupuncture interventions were sustained for 21 minutes.

To ensure that the acupuncture stimulations were standardized, the depths of insertion and the rotational forces of the acupuncture needling were measured by using an Acusensor (acupuncture needling force and motion sensor system; Stromatec, Inc., Burlington, VT) (Fig. 1).

Pilot experiment: choosing proper acupuncture manipulation parameters

Five volunteers (mean age $\pm$ standard deviation, 26.8 $\pm$ 0.4 years; 3 women and 2 men) participated in a pilot trial to determine the experimental manipulation conditions. As a first step in the standardization of the acupuncture manipulations, the number of rotations was chosen to be the primary parameter. Normally, a multiple of nine rotations is considered one unit in the tonifying methods of traditional acupuncture. Thus, to determine whether a dose-response relationship existed, microcirculation perfusion values after 0 (i.e., only insertion), 9, 27, and 54 rotations of the acupuncture needle were compared by using a PIM3 system (Perimed AB, Järfälla, Sweden). Each participant took part in four kinds of manipulating conditions (0, 9, 27, and 54) in a random order, with an interval of several days.

Figure 2A showed that acupuncture rotation produced an increase in microcirculation perfusion that was dose dependent as for rotation number: Twenty-seven rotations of acupuncture stimulation resulted in the maximum perfusion values. In contrast, perfusion values resulting from 54 rotations declined. Therefore, we defined 27 rotations of the needle as one manipulation unit.

FIG. 1. The depths and the rotation rates of acupuncture needling, as measured by Acusensor (A). Acupuncture needles were inserted at LI4 to a depth of 15 mm (B) and rotated at a rate of one spin per second (1 Hz, C). Rev, revolution.

FIG. 2. Determination of acupuncture stimulating parameters using laser Doppler imaging (pilot data). **(A)** Changes in perfusion units according to the numbers of rotations (0, 9, 27, and 54). **(B)** Changes in perfusion units according to the numbers of sessions of manipulation (single versus triple) over 21 minutes. Values are presented as the percentage changes in mean $\pm$ standard error. * $p < 0.05$ compared with the single manipulation condition. AP, acupuncture; INS, insertion-only condition; MAN, single manipulation condition; PU, perfusion unit; RMAN, repeated manipulation condition.

Next, the study investigated whether repeated manipulations elicited greater perfusion compared with a single manipulation unit. The repeated manipulation method involved three sessions of manipulations that were given at 7-minute intervals. Figure 2B shows the results of repeated manipulation, which increased the perfusion values compared with a single manipulation.

Finally, in the main experiment, the results from three sets of manipulations—insertion only (INS), single manipulation of 27 rotations (MAN), and three repeated manipulation (RMAN) sessions—were compared.

Main experiments: study design and experimental procedures

Twelve young healthy volunteers (mean age, 22.8 ± 0.6 years; 6 women and 6 men) were included in the main ex-

periments. Participants received three sets of acupuncture interventions (INS, MAN, and RMAN; Fig. 3) in a random order. The session orders were randomly determined with a random table in Excel (Microsoft, Redmond, WA). Each experiment was separated by approximately 4 days (median). Case report forms that included basic demographic data were collected from all participants. Demographic data consisted of sex, age, height, and weight. Participants rested for 10 minutes and then pressure pain threshold (PPT) was measured before they received each intervention. The participants were placed on a bed in the supine position for the entire duration of the experiment; participants were comfortable enough to remain in that position but not so comfortable that they fell asleep. Baseline skin blood perfusions were obtained (baseline measurements), and the acupuncture needle was subsequently inserted into LI4 of the right hand. The needle was manipulated manually according to the assigned intervention, and the skin blood perfusion values were obtained (acupuncture measurements). The needle was removed after 21 minutes of acupuncture sessions, and the final skin blood perfusion value was obtained (postacupuncture measurements). After the final LDPI measurement, PPT was measured (Fig. 3). The subjective sensations of acupuncture and safety were also evaluated at the end of the experiments. Assessor measuring LDPI and PPT was blinded to the group of acupuncture manipulations.

LDPI measurements

A PIM3 system was used to measure skin blood perfusion in both the pilot and the main experiments. A moving laser beam sequentially scanned the tissue surface in a stepwise fashion, and the backscattered light was detected by a photo detector and analyzed for Doppler components that were generated by blood cells moving in the superficial microvascular network. In addition to the blood perfusion image, a normal photo was taken to locate the area of interest. Color-coded images showing the spatial distributions of tissue perfusions were created. Upon interaction with a moving particle, normally, in a red blood cell, a fraction of the individual photons becomes shifted in frequency according to the Doppler principle. When the scan was finished, the changes in the mean blood perfusion during 5 minutes were used to evaluate and quantify skin blood perfusion using the PIM software. A region of interest was selected to obtain the mean blood perfusion. The perfusion unit was used to calculate average perfusion values. The area of interest was a $3.5\text{-cm} \times 3.5\text{-cm}$ square centered on LI4 between the first and second metacarpal bones (Fig. 4). The distance between the detector and the tissue was fixed at 25 cm, and a normal resolution was used. Regions of interest were selected within an ellipse of an area of 100 mm^2 centered on LI4. To minimize movement artifacts, the right hands of the participants were stabilized with a kapok-filled vacuum cushion.

Measurement of PPTs

Changes in pain sensitivity were assessed by measuring PPTs, which were used to quantify subjective pain sensations. PPTs were measured in the ipsilateral hand (LI5), arm (LI10), abdomen (ST25), and leg (SP9) by using a handheld electronic pressure algometer (JTech Medical, Salt Lake

FIG. 3. Schematic protocol depicting the experiment procedures. Each black arrow indicates the insertion and withdrawal of acupuncture needle, respectively. White arrows indicate the measurement points of pressure pain threshold (PPT). Gray boxes indicate the measurement of laser Doppler (LD) perfusion imaging. Mean blood perfusion during 5 minutes were used as data.

City, UT) with a 0.7-cm diameter hard rubber probe that was calibrated in kilopascals. The exact locations are shown in Figure 4.

All participants were asked to indicate when the pressure became painful, at which time the pressure was immediately removed. Before measurement of PPTs, several measure-

ments were performed on the contralateral arm for training purposes. Readings were recorded for three measurement cycles by the same examiner, and the median values of these measurements were used in further calculations to increase the meanings of the representative values. Change in PPT was calculated by postacupuncture PPT minus baseline PPT.

Acupuncture sensation and safety

The subjective sensations of acupuncture were evaluated by using the retaining-acupuncture-needle items on the Acupuncture Sensation Questionnaire developed by Kim *et al.*¹⁸ Adverse events were assessed during and after acupuncture stimulations on every visit.

Statistical analysis

SPSS software, version 18.0 (SPSS Inc., Chicago, IL) was used for the statistical analyses. All data are expressed as the mean $\pm$ standard error (SEM). The time courses of changes in skin blood perfusion during the three interventions were assessed by repeated-measures analyses of variance (ANOVAs). ANOVAs with Tukey honest significant difference *post hoc* tests were used to analyze changes in skin blood perfusion and PPTs between groups at each point. Correlations between perfusions and PPTs were calculated as Spearman correlation coefficients. In all analyses, $p < 0.05$ was considered to indicate significant differences.

Results

Changes in skin blood perfusion at the acupoint LI4

Figure 5 shows that acupuncture stimulation induced significant increases in skin blood perfusion compared with baseline values in the INS, MAN, and RMAN conditions during AP1 ($367.3\% \pm 27.6\%$, $482.2\% \pm 41.0\%$, and $496.8\% \pm 85.3\%$ of the baseline value, respectively). However, only RMAN produced sustained elevations in skin blood perfusion values ($506.6\% \pm 92.8\%$ and $556.5\% \pm 105.1\%$ of baseline during AP2 and AP3), whereas the INS and MAN conditions decreased continuously to $246.1\% \pm 29.1\%$ and $212.3\% \pm 22.8\%$ (INS) and to $314.0\% \pm 40.5\%$ and $239.3\% \pm 23.1\%$ (MAN) of baseline, although the needle was still inserted.

FIG. 4. The experimental set-up, including the locations of acupuncture and measurement sites (laser Doppler perfusion imaging [LDPI] and pain pressure threshold). The black circle indicates acupoint LI4, where the acupuncture stimulation was applied and skin blood perfusion was measured by LDPI. Gray circles indicate the acupoints ST25, LI5, LI10, and SP9 where pain pressure thresholds were measured.

Comparison analyses revealed that the greatest difference in skin blood perfusion between the RMAN condition and the INS condition occurred during the AP2 phase ($p=0.014$) and that the RMAN condition showed increased blood perfusion levels compared with the MAN and INS conditions during the AP3 phase ($p=0.006$ and $p=0.004$, respectively). After the needle was removed, the blood flow perfusion values of all groups decreased continuously (Fig. 5).

FIG. 5. The changes in perfusion before, during, and after acupuncture manipulations around acupoint LI4. (A) Representative perfusion imaging from a 23-year-old female volunteer obtained via PIM3 software. (B) The graph shows the changes in perfusion around LI4. At each point in the image, skin blood perfusion had been color-coded by using a scale that ranges from dark blue (lowest value) to red (highest value). The picture on the right represents acupoint LI4, where acupuncture stimulation was applied and skin blood perfusion was measured with LDPI. The area of interest (white square) was a 3.5-cm $\times$ 3.5-cm square centered on LI4 between the first and second; $*p<0.05$ and $**p<0.01$ compared with the INS, and $##p<0.01$ compared with the manipulation (MAN) condition by analysis of variance followed by Tukey honest significant difference *post hoc* tests. Color images available online at www.liebertpub.com/acm

FIG. 6. Changes in PPTs. The changes in PPTs were calculated by postacupuncture PPT minus baseline PPT. Values are presented as the mean $\pm$ standard error. $*p<0.05$ compared with the INS condition analyzed by an analysis of variance followed by Tukey honest significant difference *post hoc* tests.

Changes in PPTs

The relative PPT changes are shown in Figure 6. The RMAN condition elicited significantly enhanced PPT values at ST25 compared with the INS and MAN conditions (each $p<0.05$). Interestingly, Spearman correlations analysis revealed significant correlation between changes in local perfusion and PPTs ($r=0.393$; $p=0.018$) (Table 1).

For the PPTs measured at LI10, LI5 and SP9, the MAN and RMAN conditions showed trends toward increased PPTs compared with the INS condition; however, these differences were not statistically significant.

Assessment of acupuncture sensations and adverse events

None of the factors of acupuncture sensation significantly differed across conditions (Table 2). Nevertheless, heavy

TABLE 1. CORRELATIONS BETWEEN CHANGES IN LOCAL MICROCIRCULATION PERFUSION AND PAIN PAIN THRESHOLDS INDUCED BY REPEATED ACUPUNCTURE MANIPULATIONS

Measurement points/region (corresponding acupoint)	r-Value	p-Value
Abdomen (ST25)	0.3928	0.0178 ^a
Hand (LI5)	0.2990	0.0764
Arm (LI10)	0.2115	0.2156
Leg (SP9)	0.08454	0.6240

^aStatistically significant.

TABLE 2. ASSESSMENT OF ACUPUNCTURE SENSATIONS

<i>Sensation</i>	<i>INS</i>	<i>MAN</i>	<i>RMAN</i>
Heavy	3.8±0.6	3.8±0.6	5.2±0.7
Compressing or pressing	3.1±0.6	3.1±0.6	4.3±0.8
Spreading out	4.4±0.4	4.4±0.4	4.8±0.8
Surging opening flow of stuffed or choked feeling	4.3±0.6	4.3±0.6	4.2±0.8
Activated blood circulation	3.8±0.6	3.8±0.6	4.0±0.8
Refreshing or relieving	3.5±0.6	3.5±0.6	3.4±0.6
Warm	3.0±0.7	3.0±0.7	3.1±0.7

Values are presented as the mean±standard error.

INS, insertion-only condition; MAN, single manipulation condition; RMAN, repeated manipulation condition.

and pressure sensations, which are traditionally known to result from acupuncture, were greatest in the RMAN condition. No adverse events were reported during this study.

Discussion

The local reactions (i.e., *de qi* sensations) induced by acupuncture manipulations are believed to be among the important factors in the induction of the effects of acupuncture; however, this connection has not been clarified by using standardized tools. This study demonstrated that repeated manipulation of acupoint LI4 enhanced local microcirculation and increased analgesic effects compared with single manipulations or the insertion alone. In addition, these two parameters were correlated, indicating that local increases in microcirculation might influence the analgesic effects of acupuncture.

Acupuncture alters skin microcirculation. The current study showed that the insertion of a needle triggered an increase in skin blood perfusion and that, as the number of rotation was incremented, the local perfusion values were also enhanced (27 rotations>9 rotations>insertion only). These results agree with Sandberg's report that acupuncture manipulations enhanced blood flow in the skin and muscle to a greater extent than does insertion alone. The current study extends these finding by showing that these increases did not continue in a linear fashion; 54 rotations produced lower perfusion values than did 27 rotations. These data indicate that there is an optimal dose window of rotation to the changes of local microcirculation and that when the number of rotation cycle was increased beyond peak dose, the physiologic reaction also can be declined. Previously, Langevin et al. also found similar dose effects of acupuncture rotation: the cellular response to rotation was non-monotonic, and maximal responses were occurred within a specific range of cycle number.¹⁹

Moreover, the current study also demonstrated that repeated manipulations produced greater elevations in perfusion than did insertion only or single manipulation. Acupuncture manipulation is important for acupuncture analgesia. The local physiologic changes induced by acupuncture manipulations (*de qi*) are considered important factors that trigger the effects of acupuncture. In the current study, RMAN, which showed higher microcirculatory changes, significantly decreased the pressure pain at ST25. Of note, Spearman correlations analyses revealed significant

correlation between changes in local perfusion and PPTs. The current study is believed to be the first to show that repeated manipulations can produce greater increase in microcirculation levels as well as greater decrease in pressure pain at the relevant sites than single manipulation or needle insertion.

Acupoint LI4, a source point of the large intestine meridian, is a strong analgesic acupoint, and several studies have shown that acupuncture at acupoint LI4 could alter PPTs in healthy volunteers.^{16,17} In this study, repeated acupuncture manipulations at LI4 decreased the pressure pain only at ST25 on the abdomen. According to East Asian medicine, acupoint ST25 is the representative responsive abdominal point (i.e., the mu point), which corresponds to the large intestine meridian. These results might suggest the possible correlation between acupoint LI4 and ST25. However, to understand the interrelationship among remote areas of the body, more well-designed studies are warranted.

In the current study, although local microcirculatory changes by acupuncture have significant correlations with the decrease of pressure pain at the relevant area, they are still limited in their contribution to understanding how these changes in the skin relate to the analgesic response. Both central and peripheral mechanisms have been proposed for the mechanism of acupuncture analgesia.^{2,20} Central modulation is important to understand this remote effects of acupuncture analgesia: Acupuncture has been reported to activate some brain areas participating in descending inhibitory control, such as arcuate nucleus, locus coeruleus, nucleus raphe magnus, and periaqueductal gray.^{2,20} Further studies are necessary to elucidate the mechanism connecting the local and central effects of acupuncture. In addition, acupuncture stimulation results in the local changes of various biological factors, including nitric oxide,^{7,8} calcitonin gene-related factor,⁹ histamine,²¹ phosphorylated extracellular signal-regulated kinase,²² and adenosine triphosphate and its metabolite adenosine. Recently, Goldman et al.²³ and Park et al.²² reported that local release or molecular changes such as adenosine or phosphorylated extracellular signal-regulated kinase play an important role in triggering the acupuncture analgesia. The exact mechanism to explain the link between the increase of microcirculation and the regulation of central control needs to be elucidated further.

In conclusion, this study found that repeated manipulations enhanced microcirculatory effects and induced decreases in pressure pain at the relevant sites. In addition, these two changes were significantly correlated, which indicates that local microcirculatory changes may be used as indices of proper acupuncture stimulation.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Nos. 2005-0049404 and 2012R1A1A2006793) and a grant of the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (HI13C0540). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Disclosure Statement

No competing financial relationships exist.

References

1. Weidenhammer W, Linde K, Streng A, et al. Acupuncture for chronic low back pain in routine care: a multicenter observational study. *Clin J Pain* 2007;23:128–135.
2. Zhao ZQ. Neural mechanism underlying acupuncture analgesia. *Prog Neurobiol* 2008;85:355–375.
3. Takakura N, Yajima H. Analgesic effect of acupuncture needle penetration: a double-blind crossover study. *Open Med* 2009;3:e54–61.
4. Li QQ, Shi GX, Xu Q, et al. Acupuncture effect and central autonomic regulation. *Evid Based Complement Alternat Med* 2013;2013:267959.
5. Litscher G. (2006). Bioengineering assessment of acupuncture, part 2: monitoring of microcirculation. *Crit Rev Biomed Eng* 2006;34:273–294.
6. Sandberg MT, Lundberg T, Lindberg LG, Gerdle B. Effects of acupuncture on skin and muscle blood flow in healthy subjects. *Eur J Appl Physiol* 2003;90:114–119.
7. Tsuchiya M, Sato EF, Inoue M, Asada A. Acupuncture enhances generation of nitric oxide and increases local circulation. *Anesth Analg* 2007;104:301–307.
8. Jou NT, Ma SX. Responses of nitric oxide-cGMP release in acupuncture point to electroacupuncture in human skin in vivo using dermal microdialysis. *Microcirculation* 2009;16:434–443.
9. Shinbara H, Okubo M, Kimura K, et al. Participation of calcitonin gene related peptide released via axon reflex in the local increase in muscle blood flow following manual acupuncture. *Acupunct Med* 2013;31:81–87.
10. Knowles RG, Moncada S. Nitric oxide as a signal in blood vessels. *Trends Biochem Sci* 1992;17:399–402.
11. Itoh K, Minakawa Y, Kitakoji K. Effect of acupuncture depth on muscle pain. *Chin Med* 2011;6:24.
12. Kim JH, Min BI, Schmidt D, et al. The difference between electroacupuncture only and electroacupuncture with manipulation on analgesia in rats. *Neurosci Lett* 2000;279:149–152.
13. Wardell K, Jakobsson A, Nilsson GE. Laser Doppler perfusion imaging by dynamic light scattering. *IEEE Trans Biomed Eng* 1993;40:309–316.
14. Zhang W, Wang L, Huang T, et al. Laser Doppler perfusion imaging for assessment of skin blood perfusion after acupuncture. *Med Acupunct* 2008;20:109–118.
15. Hsiu H, Hsu WC, Hsu CL, et al. Assessing the effects of acupuncture by comparing needling the hegu acupoint and needling nearby nonacupoints by spectral analysis of microcirculatory laser Doppler signals. *Evid Based Complement Alternat Med* 2011;2011:435928.
16. Zaslawski CJ, Cobbin D, Lidums E, Petocz P. The impact of site specificity and needle manipulation on changes to pain pressure threshold following manual acupuncture: a controlled study. *Complement Ther Med* 2003;11:11–21.
17. Li W, Cobbin D, Zaslawski C. A comparison of effects on regional pressure pain threshold produced by deep needling of LI4 and LI11, individually and in combination. *Complement Ther Med* 2008;16:278–287.
18. Kim Y, Park J, Lee H, et al. Content validity of an acupuncture sensation questionnaire. *J Altern Complement Med* 2008;14:957–963.
19. Langevin H, Bouffard N, Churchill DL, Badger DJ. Connective tissue fibroblast response to acupuncture: dose-dependent effect of bidirectional needle rotation. *J Altern Complement Med* 2007;13:355–360.
20. Zhang R, Lao L, Ren K, Berman BM. Mechanism of acupuncture-electroacupuncture on persistent pain. *Anesthesiology* 2014;120:482–503.
21. Yu X, Ding G, Huang H, et al. Role of collagen fibers in acupuncture analgesia therapy on rats. *Connect Tissue Res* 2009;50:110–120.
22. Park JY, Park JJ, Jeon S, et al. From peripheral to central: the role of ERK signaling pathway in acupuncture analgesia. *J Pain* 2014;15:535–549.
23. Goldman N, Chen M, Fujita M, et al. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nat Neurosci* 2011;13:883–888.

Address correspondence to:

Hi-Joon Park, KMD, PhD

*Studies of Translational Acupuncture Research
Acupuncture and Meridian Science Research Center
Kyung Hee University
26 Kyungheedaero
Dongdaemun-gu, Seoul 130-701
Republic of Korea*

E-mail: acufind@khu.ac.kr