Electroacupuncture effect at the LI 4 Hegu point on the plasma β-endorphin level of healthy subjects

A L Setiawardhani, A Srilestari and C Simadibrata*
Department of Medical Acupuncture, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
*E-mail: akupunturrcm@yahoo.com

Abstract. As a therapy modality, acupuncture is becoming popular for treating diseases. Nevertheless, the acupuncture mechanism of action remains unclear until now. Some studies suggest that acupuncture works by stimulating the β-endorphin release, whereas other studies show the opposite. This study aims to determine whether electroacupuncture at the LI 4 Hegu point could increase the plasma β-endorphin level as a basic of acupuncture mechanism of action. Thirty-six healthy subjects were involved and divided randomly into two groups, namely, intervention (n=18) and control (n=18). In the intervention group, electroacupuncture was applied at the LI 4 Hegu point with a low frequency for 30 min. In the control group, sham electroacupuncture was applied at a non-acupoint for 30 min. The plasma β-endorphin was examined before and after intervention using the ELISA method. A significant difference was found between the intervention and control groups in increasing the plasma β-endorphin level (9(50%) vs. 1(5.6%); p = 0.009). A significant difference was also observed in the plasma β-endorphin level after intervention between the two groups (35.1 ±3.4 vs. 10.3±1.8; p=0.003). Electroacupuncture at the LI 4 Hegu point was found to increase the plasma β-endorphin level in healthy subjects.

1. Introduction
Acupuncture is an important healthcare practice in Asian cultures, especially in China. Currently, the use of acupuncture is increasing among clinicians in the world, particularly in the United States. However, the acupuncture mechanisms of action remain unexplained. Some studies suggest that acupuncture stimulates the release of neurotransmitters and endogenous opioid-like substance as well as activates c-FOS in the central nervous system. One endogenous component of the opioid-like substance is β-endorphin [1-4]. In using acupuncture in the treatment of pain; the release of endogenous opioid neuropeptides (i.e., endorphins) in the cerebrospinal fluid is increased through the hypothalamic–pituitary activity. Some studies support the endorphins theory that the administration of opioid antagonists, such as naloxone, decreases the analgesic effects induced by acupuncture. A study conducted on patients with recurrent pain proved that the β-endorphin levels in the cerebrospinal fluid, not the methencephaline levels, increased after electroacupuncture (EA) [5].

Although the above evidence supports the theory of endorphins that forms the basis of acupuncture mechanisms, many studies still doubt the theory. Studies in Japan found an increase in the threshold of excitatory pain, but the plasma β-endorphins and ACTH levels in the cerebrospinal fluid did not increase after EA. The study concluded that other endogenous opioid peptides could be involved in acupuncture analgesia other than β-endorphins [6]. Harbach and colleagues, who examined the effects
of acupuncture on patients with chronic low back pain, found that acupuncture had no effect on plasma β-endorphin levels [7]. The inconsistencies in previous studies motivated the researchers to examine the effect of EA on the plasma β-endorphin levels of healthy subjects. This study is expected to verify the hypothesis that EA at the LI 4 Hegu point could increase plasma β-endorphin levels in healthy subjects in comparison with placebo puncture and can serve as reference on the acupuncture mechanism of action.

2. Materials and Methods
The research design used was randomized controlled trial. The study was conducted on 36 healthy subjects who met the acceptance criteria of physical health, did not consume drugs or foods containing caffeine, morphine, and alcohol, did not exercise at least 24 h before the research, and were willing to follow the research procedure by filling in the informed consent form. The exclusion criteria included pregnancy, subjects with pacemakers, and subjects who experienced pre-shock condition during blood sampling or during the research. The study was conducted after passing the ethical review and obtained the approval from the Ethics Committee. The participants agreed to be involved in this research by signing the informed consent of confidentiality and voluntary non-coercion.

In this study, the acupuncture needle used had a size of 0.25 mm × 25 mm (Huangqiu®) and was an electrostimulator Multiple Electronic Acupunctoscope® type WQ-10C2. The plasma β-endorphin content was examined using the ELISA method of β-endorphin ELISA (MD Bioproducts®, Division of MD Biosciences, Inc. 2575 University Ave. W.Ste.100 St. Paul, MN 55114 North America, Catalog number M056011). The subjects were divided into two random groups, with each group consisting of 18 participants for the intervention group and 18 for the control group. In the intervention group, EA was performed at the LI 4 Hegu point on the left and right using a continuous wave with a low frequency on scale 2 and intensity adjusted to the comfort level of the research subjects. In the control group, EA sham was performed at the points that were not acupuncture points on the left and right inner forearms: four fingers from the wrist and midway between the ulnar flexor muscle tendon and the longitudinal palmar muscle tendon. The electrostimulator was installed but not turned on. The treatment time was 30 min for each subject in all groups.

Before and after treatment, 3 ml of a venous blood sample was taken, placed in a tube containing EDTA anticoagulants, and then stored in plasma format −200 °C. Plasma β-endorphin levels were analyzed using the ELISA method. Data were analyzed using T-test to compare the plasma β-endorphin levels before and after treatment in each group. Fisher’s exact test, which is an alternative to the chi-square test, was used to assess differences in the plasma β-endorphin levels between the two groups. A p value < 0.05 indicated a significant difference in the two groups being compared. Data were processed using SPSS version 20.

3. Results and Discussion
3.1 Results
This study was conducted on 36 healthy subjects randomly divided into two groups: intervention group and control group. The day before the study was conducted, the research subjects were gathered for a briefing on the course of the research. All the participants successfully completed the research (i.e., no one was cancelled) (Table 1). The effects of EA on the plasmaβ-endorphin levels are presented in Table 3. Plasma β-endorphins had a statistically significant increase in the intervention group in comparison with the control group (Figure 1). Table 4 shows the comparison of the plasmaβ-endorphin levels before and after treatment.
### Table 1. Subjects’ characteristics by sex, education, and occupation

| Characteristics                | Intervention n (%) | Control n (%) | Total n (%) | p   |
|--------------------------------|--------------------|---------------|-------------|-----|
| Sex                            |                    |               |             | 0.462* |
| Male                           | 11 (61.1)          | 11 (61.1)     | 22 (61.1)   |     |
| Female                         | 7 (38.9)           | 7 (38.9)      | 14 (38.9)   |     |
| Education                      |                    |               |             | 0.395* |
| Primary School                 | 3 (16.7)           | 3 (16.7)      | 6 (16.7)    |     |
| Junior High School             | 7 (38.9)           | 7 (38.9)      | 14 (38.9)   |     |
| Senior High School             | 8 (44.4)           | 7 (38.9)      | 15 (41.7)   |     |
| Bachelor’s Degree              | 0 (0)              | 1 (5.6)       | 1 (2.8)     |     |
| Occupation                     |                    |               |             | 0.610* |
| Laborer                        | 2 (11.1)           | 1 (5.6)       | 3 (8.3)     |     |
| Housewife                      | 6 (33.3)           | 7 (38.9)      | 13 (36.1)   |     |
| Employee                       | 2 (11.1)           | 1 (5.6)       | 3 (8.3)     |     |
| Entrepreneur                    | 4 (22.2)           | 2 (11.1)      | 6 (16.7)    |     |
| College student                | 0 (0)              | 1 (5.6)       | 1 (2.8)     |     |
| Unemployed                     | 4 (22.2)           | 6 (33.3)      | 10 (27.8)   |     |

*Fisher’s exact test

### Table 2. Subjects’ characteristics by age, blood pressure, pulse frequency, temperature, and plasma β-endorphin level before intervention

| Characteristics                  | Intervention (X ± SD) | Control (X ± SD) | p   |
|----------------------------------|-----------------------|------------------|-----|
| Age (year)*                      |                       |                  | 0.825** |
| Blood pressure (mmHg)*           |                       |                  |     |
| Systolic                         | 115 ± 5.8             | 110 ± 5.4        | 0.213** |
| Diastolic                         | 75 ± 5.0              | 82 ± 5.6         | 0.633** |
| Pulse frequency (per minute)*    | 69.8 ± 7.0            | 72.8 ± 8.0       | 0.144** |
| Temperature (°C)*                | 35.9 ± 0.39           | 36.0 ± 0.56      | 0.809** |
| Plasma β-endorphin level (pg/ml)*| Before intervention   |                  | 0.454** |

*Mean ± SD, **Mann–Whitney U test

### Table 3. Effects of electroacupuncture on the plasma β-endorphin level*

| β-Endorphin | Intervention n(%) | Control n(%) | Total n(%) |
|-------------|-------------------|--------------|------------|
| Decreased   | 7 (38.9)          | 14 (77.8)    | 21 (58.3)  |
| Constant    | 2 (11.1)          | 3 (16.7)     | 5 (13.9)   |
| Increased   | 9 (50.0)          | 1 (5.6)      | 10 (27.8)  |

*Fisher’s exact test, p = 0.009
Figure 1. Effects of electroacupuncture on the plasma β-endorphin level

Table 4. Plasma β-Endorphin level before and after intervention

| Plasma β-Endorphin (pg/ml) | Intervention | Control | p   |
|----------------------------|--------------|---------|-----|
| Before                     | 34.6 ± 3.5a  | 43.6 ± 3.3b | 0.454* |
| After                      | 35.1 ± 3.4a  | 10.3 ± 1.8b  | 0.003* |

1Mean ± SD,*Mann–Whitney U test, Wilcoxon Signed Rank a p = 0.897; b p = 0.001

3.2 Discussion
This study on the EA effect on the plasma β-endorphin level of healthy subjects was conducted on 36 subjects randomly divided into two groups: 18 in the intervention group and 18 in the control group. No side effects were observed during the study either because of needle stabbing or excitation of the electrostimulator and during blood taking. No side effects from needling stabbing, electrostimulator stimulation, or blood taking were observed. The equivalent tests performed on both groups in terms of age, sex, education, occupation, blood pressure, pulse frequency, temperature, and baseline β-endorphin levels resulted in p > 0.05. Thus, both groups were in equivalent conditions at the start of the study and worthy to be compared. The results showed that 50% (n = 9) of the healthy subjects had increased plasma β-endorphin levels after EA at the LI 4 Hegu point for 30 min at a low frequency. This study is consistent with an experimental study on rats that received low-frequency EA at the LI 4.
Hegu point and had an increased β-endorphin level [8]; only 5.6% (n = 1) of the subjects in the control group had an increased β-endorphin level. Using Fisher’s exact test, which is an alternative test to chi-square, the p value obtained was 0.009 (p < 0.05). This result suggests a significant difference between the intervention group and the control group in increasing the plasma β-endorphin levels. In one study, the subjects (5.6%) in the control group showed elevated plasma β-endorphin levels. This result is consistent with the suggestion that a placebo effect is induced by acupuncture sham on acupuncture analgesia, especially in cases of chronic pain, the effects of which can reach 33%–50% [9]. In this study, the plasma β-endorphin levels before and after intervention in the control group showed a significant decrease. This result is consistent with those of several previous studies that found a decrease in plasma β-endorphins levels in the control group [5,10]. This decrease is possible because β-endorphin secretion follows a 24 h cycle of circadian rhythm and has a short half-life [11].

The acupuncture point used in this study is the LI 4 Hegu point. This point was chosen because experimental studies on rats proved the increase in β-endorphin levels by stimulating this point [8]. An initial study using functional magnetic resonance imaging in the brain concluded that EA stimulation at the LI 4 Hegu point increased the activity of several brain cerebral signals including the insula area, the activity of which always increases when overcoming pain [12]. The acupuncture point of LI 4 Hegu increases plasma β-endorphins, especially through the central pathway. Local stimuli through afferent somatic nerve fibers (A-delta and C) are passed to the marginal cells in the spinal cord to be transmitted to the ventral thoracic nucleus of the thalamus and projected onto the cerebral cortex to activate the hypothalamic–pituitary pathway, thus releasing β-endorphins into the blood and cerebrospinal fluid [13]. β-endorphins are the result of the breakdown of the pro-metomelanocortin precursor hormone (POMC) formed in the hypothalamic arcuate nucleus. POMC splits into ACTH hormone and β-lipoprotein. In the pituitary, β-lipoprotein is converted into β-endorphin and is released together with ACTH by the anterior pituitary into the circulatory and cerebrospinal fluid. The use of low-frequency EA in this study is based on the evidence that EA (especially at low frequencies) produces more signal boosts in the brain than manual acupuncture and control [14]. In addition, the use of low-frequency EA selectively releases enkephalin and β-endorphins and that of high-frequency EA releases dinorphine [8].

The responses of the subjects in the plasma β-endorphin enhancement after EA at the LI 4 Hegu acupuncture point compared with those of the control subjects, who mostly exhibited a decreased level, indicated that acupuncture was involved in the β-endorphin-releasing process. Although the mean plasma β-endorphin levels did not increase significantly, EA was able to maintain the plasma β-endorphin levels to prevent them from decreasing. Therefore, acupuncture can be useful in conditions requiring the role of β-endorphins to improve the body’s system disorders. The result of Harbach et al. [7] study, which found that acupuncture did not cause β-endorphin release, should be reviewed because the number of samples and the frequency of stabbing of only onetime produced unfavorable results. Moreover, the study used a comparator in the form of non-steroidal anti-inflammation analgesic drugs, which work through the intermediate prostaglandin and not β-endorphins. The limitation of this research is that it did not determine the most optimal time during β-endorphin examination, as β-endorphin exposure is affected by the circadian rhythm. In addition, the venous blood taking time should be carefully calculated and the duration should not be too long, especially after intervention given the short half-time of β-endorphins.

4. Conclusion
A significant difference was found in the increased plasma β-endorphin levels between the intervention group and the control group (9 (50%) vs. 1 (5.6%), p = 0.009). A significant difference was also observed in the plasma β-endorphin levels between the two groups after intervention (35.1 ± 3.4 vs. 10.3 ± 1.8, p = 0.003). Thus, the effect of EA at the LI 4 Hegu point on the plasma β-endorphins levels increased in healthy subjects. This study should be extended by using a larger sample, multiple acupuncture points, and repeated administrations of EA to determine if a significantly greater increase in the plasma β-endorphin levels will occur. Moreover, other samples
such as cerebrospinal fluid should be used to examine the effect of acupuncture on the β-endorphin levels to determine the acupuncture mechanism action clearly.

References

[1] Wang S M, Kain Z N and White P 2008 Acupuncture analgesia: the scientific basis. *Anesth. Analg.* **106** 602-10.

[2] Sharma A and Verma D 2010 Endorphins: Endogenous Opioid In Human Cells. *World. J. Pharm. Pharm. Sci.* **4** 357-74.

[3] Bonta I L 2002 Acupuncture beyond the endorphin concept? *Med. Hypotheses.* **58** 221-4.

[4] Han J S 2004 Mini-review Acupuncture and endorphin. *Neurosci. Lett.* **361** 258-61.

[5] Zhipeng N, Lixing L 2015 Acupuncture for Pain Management in Evidence-based Medicine *Journal of Acupuncture and Meridian Studies* **8** 270-273.

[6] Taguchi R 2008 Acupuncture anesthesia and analgesia for clinical acute pain in Japan. *eCAM.* **5** 153-8.

[7] Harbach H, Moll B, Boedeker R H, et al. 2007 Minimal immunoreactive plasma b-endorphin and decrease of cortisol at standard analgesia or different acupuncture techniques. *Eur. J. Anaesth.* **24** 370-6.

[8] Huang C, Wang Y, Han JS, Wan Y 2002 Characteristics of electroacupuncture-induced analgesia in mice: variation with strain, frequency, intensity and opioid involvement *Brain Res.* **945** 20-5.

[9] Zhang J, Zhang N 2007 Study on mechanisms of acupuncture analgesia *Zhongguo Zhen Jiu* **27** 72-5.

[10] Lazarou L, Kitsios A, Lazarou I, Sikaras E, Trampas A 2009 Effects of intensity of Transcutaneous Electrical Nerve Stimulation (TENS) on pressure pain threshold and blood pressure in healthy humans: A randomized, double-blind, placebo-controlled trial *Clin J Pain.* **25** 773-80.

[11] Foley K M, Kourides I A, Inturrisi C E, et al. 1979 β-endorphin: Analgesic and hormonal effects in humans. *Proc. Natl. Acad. Sci. USA.* **76** 5377-81.

[12] Kong J, Ma L, Gollub R L, et al. 2002 A pilot study of functional magnetic resonance imaging of the brain during manual and electroacupuncture stimulation of acupuncture point (LI-4 Hegu) in normal subjects reveals differential brain activation between methods. *J. Altern. Compl. Med.* **8** 411-9.

[13] Kenji K, Kaoru O 2014 Acupuncture therapy: mechanism of action, efficacy, and safety: a potential intervention for psychogenic disorders? *Biopsychosoc Med.* **8** 4.

[14] Napadow V, Makris N, Liu J, et al. 2005 Effects of electroacupuncture versus manual acupuncture on the human brain as measured by fMRI. *Human. Brain. Mapping.* **24** 193-205.