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Intraoperative Low-frequency Electroacupuncture Under General Anesthesia Improves Postoperative Recovery in a Randomized Trial

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Running title: Intraoperative electroacupuncture
Abstract
Neuronal stimulation improves physiological responses to infection and trauma, but the clinical potential of this strategy is unknown. We hypothesized that transdermal neural stimulation through low-frequency electroacupuncture might control the immune responses to surgical trauma and expedite postoperative recovery. However, the efficiency of electroacupuncture is questionable due to the placebo effect. Here, electroacupuncture was performed on anesthetized patients to avoid any placebo interference. This is a prospective double-blinded pilot trial to determine whether intraoperative electroacupuncture on anesthetized patients improves postoperative recovery. Subjects with electroacupuncture required 60% less postoperative analgesic, but they had pain scores similar to those in the control patients. Electroacupuncture prevented postoperative hyperglycemia and attenuated serum ACTH in the older and heavier group of patients. From an immunological perspective, electroacupuncture did not affect the protective immune responses to surgical trauma, including the induction of IL6 and IL10. The most significant immunological effect of electroacupuncture was enhancing TGFβ1 production during surgery in the older and lighter group of patients. These results suggest that intraoperative electroacupuncture on anesthetized patients can reduce postoperative use of analgesics and improve immune and stress responses to surgery.

KEYWORDS
electroacupuncture;
inflammation;
physiological stress;
cytokines;
pain;
surgery

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Abbreviations
ACTH, adrenocorticotrophic hormone; ELISA, enzyme-linked immunoassay; PACU, post-analgesia care unit; TGFβ1, transforming growth factor β1.

Author contributions
DG, EK, and AB planned the clinical trial, obtained IRB approval, recruited patients, obtained informed consent, administered anesthesia and electroacupuncture, performed the surgery, and monitored patients. DDK contributed to the plan for the clinical trial, the IRB approval, and the experimental protocol, including the acupuncture point selection and the electroacupuncture method. ZL analyzed the cytokine data, performed statistical analyses, and prepared the figures. PM performed the ELISA to analyze the blood samples and performed statistical analyses. LU directed the analyses and wrote most of the manuscript. All authors approved the final version of the article.

Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this article.
**Introduction**

The CDC estimates over 51 million surgical procedures performed annually in the USA. Over 85% of the surgical patients report significant postoperative pain, which higher incidence in female patients [1]. Postoperative pain is treated with opioids that have multiple adverse side effects including respiratory depression and decreased intestinal motility [2]. These side effects increase the risk of surgical complications and delay postoperative recovery [3, 4]. Furthermore, surgical trauma induces hyperglycemia, physiological stress and inflammation that cause cardiovascular, renal, and neurological complications contributing to postoperative mortality [5-7]. Postoperative hyperglycemia is an insulin resistance process that exacerbates inflammation, delays wound healing, and infections. Therefore, there is a clinical need of novel strategies to reduce hyperglycemia and improve postoperative recovery.

Neuromodulation represents efficient systems selected by evolution to control physiological homeostasis [8-10]. Thus, neural stimulation can be a promising strategy to attenuate surgical trauma. We reported that electrical stimulation of the vagus nerve improves physiological responses to infection and trauma [9, 10]. These results were confirmed by other investigators reporting that vagal stimulation improved physiological responses to experimental ischemia and reperfusion, hemorrhage and resuscitation, pancreatitis, colitis, endotoxemia, septic shock, and severe sepsis [9-12]. In humans, surgical implantation of vagus nerve stimulators was first approved by the FDA in 1997 for the treatment of refractory epilepsy [13]. However, these studies have limited clinical implications because they were performed through a surgical stimulation of the vagus nerve. Recently, we reported that transdermal neuronal stimulation with electroacupuncture also regulates physiological responses to infection and trauma [9]. Thus, transdermal neuronal stimulation with electroacupuncture can represent a promising clinical approach to alleviate surgical trauma and improve postoperative recovery.

Electroacupuncture is currently endorsed by the National Institutes of Health and the World Health Organization. Previous studies analyzed the potential of electroacupuncture to alleviate postoperative pain and nausea [14-16]. However, the results from these studies were contradictory [16]. Many investigators question these results because the patients were conscious and therefore susceptible to placebo [14, 15, 17-19]. Indeed, many clinical studies on electroacupuncture were not conclusive as the results were statistical similar to the placebo group [18, 20]. We recently reported that electroacupuncture regulated physiological responses to infection and trauma in anesthetized mice [9], which are not susceptible to placebo effect. Similar studies also indicated that ST36 stimulation induced antinociceptive effects via adenosine A1 receptors [17]. The use of electroacupuncture on anesthetized patients has been previously avoided assuming that general anesthesia may conceal the analgesic effects of electroacupuncture. We hypothesized that low frequency electroacupuncture may prevent physiological stress and improve postoperative recovery. Low frequency electroacupuncture acts on the arcuate nucleus of the hypothalamus, and converges in the periaqueductal grey matter to induce...
endomorphin/beta-endorphin/encephalin. The effects of endomorphin/beta-endorphin/encephalin in low-frequency electroacupuncture are mediated by the mu/delta opioid receptors [21]. Thus, low frequency electroacupuncture can induce analgesic effect that depends on the activation of the opioidergic system. Here we performed a prospective double-blinded randomized pilot study to determine whether intraoperative electroacupuncture (using acupoints LI-4, LI-11, and ST-36) on anesthetized patients undergoing thyroid or parathyroid surgery could reduce the use of analgesic, pain score, physiological stress or immune cytokine responses. Given that our previous studies indicated that electroacupuncture inhibited the production of inflammatory cytokines [9], and surgical trauma induces inflammatory cytokines, we also analyzed whether intraoperative electroacupuncture also regulated the immune cytokine responses.

Materials and Methods

Clinical Trial. Prospective pilot study approved by Institutional Review Board (Pro2012002417) of the Rutgers New Jersey Medical School, and registered at clinicaltrials.gov (code NCT01937520). This is a prospective double-blinded study with 20 patients undergoing thyroid and parathyroid surgery randomized in two groups: electroacupuncture (EA, n=11) or sham (control, n=9) group. Participation was voluntary without economical compensation, and each participant signed a written consent. Exclusion criteria include pre-existing diabetes, cardiovascular conditions, or elevated levels of blood glucose, insulin or TNF. Patient #11 was excluded because of the preexisting levels of TNF > 1 ug/mL prior to surgery. All patients underwent the induction of anesthesia with midazolam 1-2.5 mg, propofol 1.0-2.0 mg/kg, fentanyl 1-3 mcg/kg, lidocaine 1 mg/kg. Then, anesthesia was maintained with sevoflurane at a minimal alveolar concentration (MAC) of 1.0-3.0 mixed with 50% Oxygen and 50% Air.

Electroacupuncture (EA). Low frequency electroacupuncture consisted of two 30 minute treatments stimulating simultaneously the He Gu (LI-4), Qu Chi (LI-11), Zu San Li (ST-36) acupoints simultaneously during the maintenance phase of general anesthesia (Fig.1a). LI-4 and LI-11 acupoints lie on the large intestine meridian (pathway). LI-4 point is located in the radial side of the hand at the middle of the 2nd metacarpal bone; LI-11 point is located at the lateral end of the transverse cubital crease toward the elbow. ST-36 point is located on the outside of the anterior crest of the tibia and just below the knee. Electroacupuncture was performed by a licensed anesthesiologist and acupuncturist delivered by a stimulator (Digital Electronic Acupunctoscope 4-C, Model AWQ-104L™ Hong Kong, Dist by Lhasa Medical, Weymouth, MA) at 10 Hz frequency with continuous electrical current of wave through 30 gauge electroacupuncture needles. A symmetrical biphasic wave will be delivered to the electrodes so that the electrode will be alternately positive and negative and the bilateral LI-4, LI-11, and ST-36 acupoints will be stimulated alternately. Mild muscle twitching will be observed. This setting showed significant anti-hyperalgesic effects in a rat inflammation model (22, 23) and also inhibited the up-regulation of IL-1β and its mRNA compared to the sham control in a rat model of bone cancer pain (24).
Clinical End-points. All analgesic treatments were converted to morphine equivalents of milligrams. Pain score (0-10) was determined using the Visual Analog Scale (VAS). The Quality of Recovery scores (QoR), a 9-item validated tool (with a maximum best possible score of 18), was used to assess patient oriented outcomes postoperatively.

Serum analyses. All blood samples were collected during general anesthesia from the radial arterial catheter. Blood samples were coagulated for 120 min and centrifuged. Serum was aliquoted, and stored at −80 ºC. Hormones and immune cytokines were analyzed by ELISA as previously described [12], using the human ACTH (CalBiotech, Spring Valley, CA, Cat#AC018T) and cortisol (CalBiotech, Cat#CO103S). Glucose was analyzed using the One Touch Ultra test (LifeScan Inc., Milpitas, CA) [22]. TNF was analyzed using recombinant TNF (eBioscience Cat #88-7346) as a standard curve and the capture (RRID:AB315249) and detection (Ab RRID:AB315255) antibodies (Cat#430201, Biolegend, San Diego, CA). Human cytokines were analyzed using the Biolegend: IL2 (Cat#431801), IL6 (Cat #430501), IL4 (Cat #430301), IL10 (Cat#571009, 501401 & 501501, Biolegend), and TGFβ1 with the capture (21C11) and detection (19D8) antibodies (Cat#580709, 525301 & 521705, Biolegend). Plates were read with the 450 nm with the VersaMax plate reader (Sunnyvale, CA) and values were interpolated with the Open SoftMax Pro 3.5 point-to-point regression software.

Statistical analyses. We hypothesized that intraoperative electroacupuncture may prevent hyperglycemia, physiological stress or inflammation. The principal outcome was analyzing serum levels of glucose, ACTH, cortisol, and immune (TNF, IL2, IL4, IL6, IL10 & TGFβ1) cytokines. The second outcome was analyzing the request of analgesic and the pain score. Sample size was determined using standard deviation values and power analyses of our previous studies on electroacupuncture in surgical induced trauma and infection [9]. Statistical analyses were performed using the Graphpad Prism 5.0 (La Jolla, CA). Continuous variables were expressed as mean ± standard error (SE). Normality and homogeneity of variance were confirmed with Graphpad Prism 5.0 using the D'Agostino-Pearson omnibus K2 test and the F-ratio of variances, respectively. Results with non-normal distributions were analyzed with the nonparametric Mann–Whitney U test. Mean values with normal distribution of two experimental groups were analyzed using the parametric unpaired homoscedastic student’s t-test; The Welch's correction was used for samples with different variances. Statistical analyses of more than two groups were performed with ANOVA with the Bonferroni’s adjustment for multiple hypothesis testing. Two-way ANOVA was used to analyze the two factors of electroacupuncture and time. Linear regressions were performed with Graphpad including the calculation of p values and squared correlation coefficients.
Results

This prospective pilot study enrolled patients undergoing thyroid and parathyroid surgery to analyze whether electroacupuncture improves postoperative recovery. The demographics of the subjects (age, body weight, and surgical procedure) were similar in the control and electroacupuncture group (Table 1a,b). All subjects were under general anesthesia during the electroacupuncture and the blood collection, and thus they were blinded to the treatment to avoid any placebo (Fig.1a). The patients with electroacupuncture had similar use of analgesic and the pain score when the heterogeneous group (with both males and females) of electroacupuncture was compared with the control group (with females only) (Fig.1b,c). Since gender affect the threshold for analgesic and pain and both groups had the same number of females, we analyzed the effects of electroacupuncture in females only. Electroacupuncture-treated females required 60% less analgesics than control females at the PACU (Post-Analgesia Care Unit), but both groups had similar pain scores (Fig.1d,e). We also compared the use of analgesic and the pain scores in the group of patients under or above the average age (45 years) or body weight (75 kg). The request for analgesic and pain scores were similar in the subgroups of control patients with age (<45 years vs >45 years) or body weight (<75 kg vs >75kg). However, electroacupuncture significantly reduced the request for analgesic in the younger (<45 years) but not in the older patient group and induces a similar effect in the heavier and lighter patient groups (Fig.1f). After the hospital discharge, subjects with or without electroacupuncture had the same use of analgesic, pain scores and quality of recovery during the three days after the surgery (Fig.1g-i).

The molecular mechanisms of electroacupuncture were studied by analyzing the serum collected at three time points during anesthesia: (Pre) Preoperative before the surgery and electroacupuncture; (Intra) intraoperative after the electroacupuncture; (Post) postoperative during anesthesia but right after surgery (Fig.1a). Surgery increased the serum levels of both ACTH and cortisol in control patients by 10 and 3-fold, respectively (Fig. 2a,c). Mean ACTH serum levels were higher in the heavier (>75kg) patient group but similar between the two age subgroups (Fig.2b). Cortisol serum levels were statistically similar among all the patient subgroups (Fig.2d). Electroacupuncture reduced mean serum ACTH levels at the PACU by over 70% without affecting cortisol (Fig. 2a,c). Electroacupuncture reduced serum ACTH levels by over 80% in the older and heavier patient groups without affecting the younger or lighter patient subgroups (Fig. 2b). One of the most significant effects of electroacupuncture was preventing hyperglycemia. Surgery gradually increased hyperglycemia in the control patients without affecting serum insulin levels (Fig.2e,g) inducing a similar effect in all the patient subgroups. However, electroacupuncture prevented hyperglycemia (Fig.2e) being more significant in the older and heavier patient groups (Fig.2f).

Electroacupuncture also modulates the immune responses. TNF and IL6 are critical pyrogen and inflammatory cytokines produced during the surgical trauma. IL2 and IL4 are critical cytokines to induce cellular versus humoral immunity. Neither surgery nor electroacupuncture affected the serum
levels of TNF, IL2, or IL4 (Fig.3a-c). However, surgery increased serum IL6 levels by sevenfold at post-surgery in the control and electroacupuncture groups regardless of the age and body weight (Fig.3d,e). We also analyzed the critical anti-inflammatory cytokines IL10 and TGFβ1. Surgery increased serum IL10 levels by twofold at post-surgery in the control and electroacupuncture groups regardless of the age and body weight (Fig.3f,g). The most significant immunological effect of electroacupuncture was to increase serum TGFβ1, a pivotal factor regulating the inflammation and wound healing. Surgery increased serum TGFβ1 levels by fourfold at post-surgery in the control patients (Fig.3h). Serum TGFβ1 levels were similar among the two control age subgroups but 65% higher in the older than in the younger control group (Fig.3i). Electroacupuncture induced threefold higher serum TGFβ1 levels at post-surgery (Fig.3h). Electroacupuncture enhanced TGFβ1 serum levels in the older and lighter patient groups but had no significant effect in the younger and heavier patient groups (Fig.3i).

**Discussion**

Previous studies of electroacupuncture were performed on conscious patients before or after the anesthesia and therefore susceptible to placebo [14-16, 19]. To our knowledge, our study describes the first clinical trial of electroacupuncture performed on anesthetized patients with all blood samples collected under general anesthesia. Low frequency electroacupuncture on anesthetized patients during surgery attenuated hyperglycemia, physiological stress, immune responses, and postoperative request for analgesic. Despite their similar demographics and pain scores, females with electroacupuncture required 60% less analgesic at the PACU. These results are important for four reasons: (1) all subjects were under general anesthesia, and therefore blinded to the treatment to avoid any placebo effect. Despite the general anesthesia, electroacupuncture induced an antinociceptive effect. (2) The lower amount of analgesic in the electroacupuncture group did not cause a higher pain score. It is logical to expect a lower pain score in the electroacupuncture group, if both groups would have received the same amount of analgesics. (3) Female subjects have lower threshold for pain and acupuncture than male subjects. Thus, our results in females are likely to be replicated in male subjects. (4) After the discharge, the groups have similar analgesic requirements and pain score, and quality of recovery during the three days after the discharge. Thus, the lower request for analgesic in the electroacupuncture group was a transient effect, and not due to a demographic difference in age, gender or body weight among the groups. In all, these results support the analgesic potential of electroacupuncture even when performed under general anesthesia.

The most significant effects of electroacupuncture were preventing postoperative stress and hyperglycemia. To our knowledge, our study is the first evidence that electroacupuncture in anesthetized patients prevents postoperative hyperglycemia. Previous studies on electroacupuncture and glycemia were performed in conscious subjects susceptible to placebo. Most studies were performed in diabetic patients with electroacupuncture at the CV12 acupoint to induce insulin
production. However, postoperative hyperglycemia is an insulin resistance process that exacerbates inflammation, delays wound healing, and infections. Given that electroacupuncture reduced ACTH serum levels and that ACTH can induce insulin resistant hyperglycemia [23], electroacupuncture may prevent hyperglycemia by inhibiting ACTH. In agreement with this hypothesis, electroacupuncture attenuated both ACTH and hyperglycemia with the same pattern in the older and heavier patient groups. Electroacupuncture may inhibit ACTH production through a downstream neuronal network. LI4 and LI11 acupoints lie on the large intestine meridian that attenuates sympathetic signals in the dorsal periaqueductal gray (DPAG) and the rostral ventrolateral medulla that innervate the paraventricular nucleus of hypothalamus. Meanwhile, ST36 activates the afferent sciatic nerve [17], which regulates the rostral ventrolateral nucleus via innervations from the paratrigeminal (Pa5). Thus, LI4, LI11 and ST36 can converge at the paraventricular nucleus of hypothalamus to inhibit corticotropin-releasing hormone and thereby ACTH production from the pituitary gland. In turn, ACTH inhibition can then prevent surgical surgical-induced hyperglycemia. Our results concur with previous studies indicating that electroacupuncture affect neither intraoperative ACTH nor cortisol levels during surgery [24]. Our electroacupuncture did not affect intraoperative serum levels of ACTH or cortisol. But, electroacupuncture inhibited postoperative ACTH levels after the surgery. The specific regulation of ACTH without affecting cortisol has not been previously reported. By contrast, a recent study showed that electroacupuncture can regulate both ACTH and cortisol in conscious patients [25]. This correlation likely involves the hypothalamic-pituitary-adrenal (HPA) axis and the potential of ACTH to activate cortisol production in the adrenal cortex. The inhibition of ACTH without affecting cortisol reveals the other factors regulating cortisol production.

An important result was the potential of electroacupuncture to induce TGFβ1 serum levels especially in the older and lighter patient groups. This pattern differs from the analgesic effects of electroacupuncture in the younger patient groups, and the stress regulation of ACTH and hyperglycemia in the older and heavier patient groups. Although these results are limited by the small sample size, the effects of age and body weight by electroacupuncture are not well established. These different patterns may suggest different mechanisms induced by the stimulation of several acupoints to control TGFβ1, pain, and physiological stress. Indeed, simultaneous stimulation of several acupoints at the same time has stronger effects than the stimulation of single acupoints [26]. For example, concurrent stimulation of LI11, CV12 and ST40 significantly attenuated atherosclerosis in hyperlipemic rats, but single acupoint stimulation did not [26]. Simultaneous stimulations trigger different mechanisms, which makes it harder to determine the discrete mechanisms modulating a specific symptom. In addition to the neuronal networks described above for LI11, LI4 and ST36, these stimulations can trigger other mechanisms to control pain and the immune system. ST36 is the most common acupoint used to control pain and inflammation by inducing endorphins or via adenosine A1 receptor [17]. We reported that ST36 inhibits inflammatory cytokines TNF and IL6 in septic mice [9].
Likewise, LI4 stimulation in rats prior to endotoxemia prevents TNF, IL-6, and IL-1, without affecting IL-10 or glucocorticoid levels [27]. Here, electroacupuncture did not affect serum levels of TNF, IL2, or IL4 in humans. One possible explanation is that the human and rodent responses to electroacupuncture may be different. Another possible explanation is that there was no major surgical induction of TNF, IL2 or IL4. However, surgery significantly induced the production of IL6 and IL10 but they were not affected by electroacupuncture. These results suggest that electroacupuncture does not cause immunosuppression preventing the immune responses to trauma and wound healing. Indeed, electroacupuncture enhanced TGFβ1 production. TGFβ1 is a critical factor that limits inflammation to trauma and triggers wound healing. Our results warrant future studies to determine the potential of electroacupuncture to control wound healing and its mechanisms to control physiological stress and expedite postoperative recovery.

**Figure Legends.**

**Table 1. Demographics of the subjects.** (a) Individual demographics of the patients (C=Control, EA=electroacupuncture), sex (M=Male, F=Female), age, body weight and surgery (T = Thyroid, P = Parathyroid). (b) Distribution of the subjects including sample size (n), average age, average body weight, number of patients with thyroid (T) or parathyroid (P) surgery and number of female (F) subjects.

**Figure 1. Electroacupuncture attenuated post-operative use of analgesia.** (a) Control or two electroacupuncture (EA) treatments of 30 mins started ~15 mins after the induction of general anesthesia. Blood samples include: Pre (before EA and surgery), intra (after EA and during surgery), and Post (during anesthesia but right after surgery). (b-e) Use of analgesics and pain score in the control or EA group at the post-analgesia care unit (PACU) analyzing all the patients or female subjects. (f) Use of analgesics at the PACU in the age and body weight subgroups. (g-i) Use of analgesics, pain score and Quality of Recovery (QoR) mean values of the females with and without electroacupuncture at the PACU (P) and the three days after surgery. Graphs depict Mean ± SE. *, and ** represents p<0.1 and p<0.05, respectively.

**Figure 2. Regulation of physiological stress and glycemia.** Blood from female subjects with or without electroacupuncture (EA) were collected before (Pre), during (Intra) and after (Post) surgery to analyze serum levels of (a, b) ACTH, (c, d) cortisol, (e, f) glucose, and (g) insulin. (b,d,f) Postoperative serum levels of ACTH, cortisol, and glucose in the age and body weight subgroups. Graphs depict Mean ± SE. *, ** and *** represents p<0.1, p<0.05 and p<0.005, respectively.

**Figure 3. Regulation of the Immune responses to surgical trauma.** Serum levels of (a) TNF, (b) IL2, (c) IL4, (d) IL6, (e) glucose, (f, g) IL10 and (h, i) TGFβ1 in control and electroacupuncture (EA)
groups at the indicated time points. (e,g,i) Postoperative serum levels of IL6, IL10 and TGF\(\beta\)1 in the postoperative (post) samples. Graphs depict Mean ± SE. * and ** represents p<0.1 and p<0.05, respectively.
References.


### Table 1a.

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Grech et al. Tables
Grech et al. Fig. 1
**Fig 2**

- **a)** ACTH (pg/ml) levels before (Pre), during (Intra), and after (Post) EA administration. Significant differences are indicated by *p < 0.05 and **p < 0.01.
- **b)** Total and age-specific (under 45, over 45, under 75, over 75) ACTH levels post-EA administration, showing significant changes.
- **c)** Cortisol (ng/ml) levels with similar significant changes.
- **d)** Total and age-specific cortisol levels, showing consistent trends.
- **e)** Glucose (mg/dl) levels with significant decreases post-EA administration.
- **f)** Comparative analysis of glucose levels across different age groups, with marked decreases in the post-EA group.
- **g)** Insulin (pg/ml) levels before and after EA administration, showing negligible changes.

*Grech et al.*
Grech et al. Fig 3