Electroacupuncture alleviates intraoperative immunosuppression in patients undergoing supratentorial craniotomy

Guoyan Li,1 Shuqin Li,1 Lixin An,1 Baoguo Wang2

ABSTRACT

Background Clinical experience suggests that anaesthesia using a combination of acupuncture and drugs can reduce the dosage of anaesthetics required for craniotomy, decreasing both the disturbance in physiological functions during the operation and postoperative complications and improving the rate of recovery. The aim of the present study was to investigate the impact of electroacupuncture (EA) on the dynamic equilibrium of the immune system and immune cell populations during the pericraniotomy period.

Methods A total of 56 patients undergoing craniotomy were randomised into three groups: control (C, n=18), EA (A, n=19) and sham acupuncture (S, n=19) groups. Blood samples were collected before anaesthesia (T0) and 30 min, 2 h and 4 h after induction of anaesthesia (T1, T2 and T3, respectively,) to measure the levels of tumour necrosis factor α (TNFα), interleukin (IL)-8, IL-10, IgM, IgA, IgG and full blood count.

Results There was no significant difference between the measurements in groups A and S during craniotomy. The levels of IgM and IgA decreased significantly in group C compared with groups A and S at T2 and T3 time points. The levels of total T cells and suppressor T cells in group C decreased significantly compared with groups A and S at T1 and T2, and the level of natural killer cells in group C decreased significantly compared with groups A and S at T1. No significant differences between groups were found in the levels of TNFα, IgG, IL-10, IL-8, leucocytes, neutrophils, monocytes, Th cells or B cells.

Conclusions EA appears to reduce immunosuppression of both the humoral and cellular components during surgery.

INTRODUCTION

The immunological response of the body to injury has been a subject of much research in recent years,1 and it is now well-established that surgery suppresses the immunological response. The degree of immunosuppression and the risk of subsequent infection are associated with the duration of surgery and the number of procedures performed.2 The immune responses include innate and adaptive responses. The innate responses primarily use phagocytic cells such as neutrophils, monocytes and macrophages, which are the first line of immediate defense against various infections. Adaptive responses primarily use B and T cells, which are responsible for the specificity of the response.3 Both comprise cellular and humoral components.4 Serious injury upsets the immune system resulting in progressive suppression of the immune responses during the first week after surgical trauma, which is thought to contribute significantly to the development of sepsis and the multi-organ dysfunction syndrome.5

Electroacupuncture (EA) has been used to alleviate pain in clinical practice.6 Previous studies have investigated the innate and adaptive immune response including total lymphocyte counts and lymphocyte subset proliferation during and after surgery.7 Cumulative evidence suggests that EA may enhance immune function after surgical trauma both in vitro and in vivo.8,9 However, no studies have investigated the effect of EA on immune function during surgery.

Many studies have shown that EA administered at sham acupuncture points has similar effects to that given at verum acupuncture points. Some researchers have therefore concluded that the results were not consistent with the concept of point specificity for this effect. On the
METHODS

Patients
All the operations and medical examinations were conducted in Beijing Tiantan Hospital (Beijing, China). A randomised controlled trial was performed in 56 patients aged 18–60 years undergoing supratentorial craniotomy. The American Society of Anesthesiologists physical status of these patients were grade I and II. Patients with immune, renal or CNS dysfunction and those with congestive heart failure, exogenous hormone therapy (including steroids), prior experience of acupuncture, pregnancy, malnutrition, diabetes, malignancy, infection or inflammation were excluded from the study. No analgesics or tranquillizers were administered before the operation. After the induction an intravenous infusion was given and non-invasive blood pressure (NIBP), heart rate (HR), oxygen pulse saturation (SPO2) and the bispectral index (BIS) were monitored. Patient characteristics and details of the operation are shown in table 1.

The 56 patients were randomly divided into three groups. Group C was a control group without EA during the operation. In group A, EA was applied to LI4 (Hegu), TE5 (Waiguan), BL63 (Jiemei), LR3 (Taichong), ST36 (Zusanli), GB40 (Quchi), BL10 (Tianzhui), GB20 (Fengchi), BL2 (Cuanzhu) and EX-HN4 (Yuyao) on the same side as the craniotomy. For LI4, TE5, BL63, LR3, ST36 and GB40, each acupuncture point was treated with one needle. LI4 and TE5, BL63 and LR3, ST36 and GB40 were connected with the acupuncture point nerve stimulator in pairs. BL10 and GB20 were penetrated by a single needle, as were BL2 and EX-HN4, and the pairs of needles were then connected with the acupuncture point nerve stimulator to start the EA. Group S was a sham acupuncture point group. In this group, EA was applied at 9 and 12 Cun above BL60 (Kunlun), 7 and 10 Cun above KI3 (Taixi) and 7 and 9 Cun above HT7 (Shenmen) on the side of the craniotomy. After intravenous infusion was commenced, NIBP, HR, SPO2 and BIS were monitored and EA was started. The needles were inserted at a depth of 0.75–1.5 cm at the acupuncture points. EA stimulation was delivered via a LH202H HANS acupuncture point nerve stimulator (Beijing Huawei Co Ltd, China) using dense-dispersed wave, 2 Hz/100 Hz in frequency, alternating every 3 s. The waveform was symmetric biphasic. The stimulation intensity was at the level of maximal tolerance of each patient and stimulation lasted from the induction of anaesthesia until the end of the operation.

Target concentration infusion anaesthesia was performed using propofol and sufentanil. The induction plasma concentration of propofol was 5 μg/ml and of sufentanil was 0.5 ng/ml. When the patients were unconscious, the plasma concentration of propofol was reduced to 3.2 μg/ml, the concentration of sufentanyl was reduced to 0.3 ng/ml and vecuronium bromide 0.1 mg/kg was administered. After muscle relaxation, tracheal intubation was performed. Mechanical ventilation was applied with 10 ml/kg tidal volume, 12 times/ min respiratory frequency and 1 l/min oxygen flow. Intermittent administration of 0.05 mg/kg vecuronium bromide was given to maintain muscle relaxation. The concentration of sufentanil was adjusted to maintain the mean arterial pressure (MAP) and HR in the basic range of +10% to −20%. In cases of hypotension (MAP<20% of baseline), bradycardia (HR<50 beats/ min) or hypertension (MAP>10% of baseline values), 6 mg ephedrine, 0.5 mg atropine or 0.2–0.5 mg nicardipine, respectively, was administered.

Sampling
Blood samples were taken in SSTII advance tubes (Becton Dickinson, UK) before anaesthesia (T0) and 30 min, 2 h and 4 h after induction of anaesthesia (T1, T2 and T3, respectively) for measurement of cytokine and immunoglobulin concentrations. For blood cell counts and lymphocyte analysis, blood samples were taken in EDTA tubes (Becton Dickinson, USA) at T0, T1 and T2.

Evaluation of cellular immunity
A complete blood count was performed using an automated haemoanalyser to determine the white blood cell count and differential white blood cell counts including neutrophils, monocytes and lymphocytes. Lymphocyte subsets were analysed by flow cytometry.
Assessment of humoral immunity
A cytometric bead assay kit (Becton Dickinson, USA) was used to measure levels of tumour necrosis factor α (TNFα), interleukin (IL)-8, IL-10, IgM, IgA and IgG in plasma according to described protocols11: 3 ml blood was taken from each patient at each time point. IL-10 is an anti-inflammatory cytokine and IL-8 and TNFα are pro-inflammatory cytokines; they represent the balance of the inflammatory reaction.

Statistical analysis
Data input and statistical analysis were performed in the department of epidemiology and hygienic statistics of Capital Medical University, Beijing, China using SPSS 13.0 statistical software. The results are presented as means±SD. Data were analysed using repeated-measures analysis of variance and separate effect analysis. The Maunchly test was used to judge whether there were relations between the repeatedly measured data. When p<0.05, the Greenhouse–Geisser correction was used to correct the results. Differences were considered significant at p<0.05. The baseline analysis showed significant differences in the baseline levels of TNFα, IL-10, IgM and IgA, but no statistical differences were observed in the changes in TNFα and IL-10 levels. There were no significant differences in the baseline level or changes in levels of IgG among the three groups. In the comparison of immunoglobulin levels during craniotomy we found that the peripheral blood IgM and IgA levels in group C were significantly decreased compared with those of groups A and S at T2 and T3 (figure 1).

Comparison of cellular immunity
As shown in table 3 and figure 2, lymphocytes, total T cells, Ts cells and NK cells in peripheral blood decreased significantly at T1 and T2 compared with T0 in group C (lymphocytes decreased 36.5% and 37.8% at T1 and T2, respectively; total T cells decreased 27.8% and 34.4%, respectively; Ts cells decreased 36.4% and 40.9%, respectively; and NK cells decreased 77.5% and 35% at the same time points). The decrease in Ts cells might be responsible for the decrease in total T lymphocytes, and the

Table 2 Cytokine and immunoglobulin levels during surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Before anaesthesia</th>
<th>2 h after anaesthesia</th>
<th>4 h after anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-10 (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.04±0.29</td>
<td>2.01±2.02*</td>
<td>5.04±3.73*</td>
</tr>
<tr>
<td>A</td>
<td>3.58±0.28</td>
<td>8.26±1.97*</td>
<td>13.8±3.63*</td>
</tr>
<tr>
<td>S</td>
<td>3.00±0.28</td>
<td>3.64±1.97</td>
<td>4.12±3.63</td>
</tr>
</tbody>
</table>

|       | IL-8 (pg/ml)       |                       |                       |
| C     | 9.25±2.47          | 7.2±1.11              | 12.02±1.69*           |
| A     | 8.92±2.40          | 10.27±1.08            | 14.85±1.64*           |
| S     | 14.33±2.4          | 10.45±1.08            | 16.03±1.64            |

Values are expressed as mean±SD. *p<0.05 vs before anaesthesia in group.

Comparison of the three groups showed that there were differences in the baseline levels of TNFα, IL-10, IgM and IgA, but no statistical differences were observed in the changes in TNFα and IL-10 levels. There were no significant differences in the baseline level or changes in levels of IgG among the three groups. In the comparison of immunoglobulin levels during craniotomy we found that the peripheral blood IgM and IgA levels in group C were significantly decreased compared with those of groups A and S at T2 and T3 (figure 1).

RESULTS
Fifty-six subjects participated in this randomised controlled trial and no subject withdrew from the trial. There were no differences in arterial blood pressure or HR among groups before, during or after EA stimulation. There was no operative morbidity or mortality.

Comparison of humoral immunity
As shown in table 2, IL-10 in peripheral blood increased significantly 2 h and 4 h after induction of anaesthesia in groups A and C compared with T0 (group A: increase of 130% at T2 and 290% at T3; group C: increase of 93.3% at T2 and 420% at T4). IL-8 in peripheral blood increased significantly at T3 in group A (66.5%) and group C (29.9%) compared with T0. The levels of TNFα and IgG did not change in the three groups (data not shown). IgM and IgA in peripheral blood decreased significantly in group C at T2 and T3 compared with T0 (IgM decreased 28.2% at T2 and 20.6% at T3; IgA decreased 28.2% at T2 and 23.7% at T3; figure 1).

Comparison of the three groups showed that there were differences in the baseline levels of TNFα, IL-10, IgM and IgA, but no statistical differences were observed in the changes in TNFα and IL-10 levels. There were no significant differences in the baseline level or changes in levels of IgG among the three groups. In the comparison of immunoglobulin levels during craniotomy we found that the peripheral blood IgM and IgA levels in group C were significantly decreased compared with those of groups A and S at T2 and T3 (figure 1).

Figure 1 Levels of IgM and IgA during surgery. *p<0.05 vs before anaesthesia in group; #p<0.05 vs group C. A, electroacupuncture group; C, control group; S, sham acupuncture group.
decrease in total T cells and NK cells might be responsible for the decrease in lymphocytes. The leucocyte, neutrophil, monocyte, Th cell and B cell counts did not change in the three groups.

Figure 2 shows the differences in the baseline levels of lymphocytes, total T cells, Ts and NK cells in the three groups. In a comparison of the immune cell counts during craniotomy we found that peripheral blood lymphocytes, total T cells, Ts cells and NK cells in group C were decreased significantly compared with groups A and S at T1. Peripheral blood lymphocytes, total T cells and Ts cells in group C were also significantly decreased compared with groups A and S at T2. However, there was no significant difference between groups A and S.

**DISCUSSION**

Point specificity is an important underlying principle used for applying acupuncture treatment in traditional Chinese medicine. According to the theory, stimulation of acupuncture points can elicit functional responses which can be used to treat diseases. Non-acupuncture points are points located some distance from verum acupuncture points or halfway between two parallel meridians. Non-acupuncture points are thought to have no therapeutic influence. However, recent systematic reviews have suggested that stimulation of non-acupuncture points can elicit effects similar to those of stimulation of verum acupuncture points. In addition, Yeh et al reported that auricular acupuncture intervention was acceptable to children and their parents to prevent/treat chemotherapy-induced nausea and vomiting (CINV), but there were no statistically significant differences between the appropriate and sham auricular acupuncture points groups in the prevention/treatment of CINV. In our study we selected sham acupuncture points that were in muscle bulk far away from meridians; several main and collateral channels are located in the head so, for control points for craniotomy, we chose sham acupuncture points in the upper and lower limbs instead of the head.

Lymphocytes are important immune cells. They can be divided into four subpopulations: B cells, T cells, NK cells and NK-T cells. B cells express B cell receptor for antigen on their surface, respond to specific antigens and produce different antibodies. T cells express T cell receptor for antigens on their surface and are divided into several subsets. This study showed that lymphocytes, total T cells, Ts cells and NK cells were significantly decreased 30 min and 2 h after induction of anaesthesia in the control group, which was consistent with a previous study. In groups A and S, lymphocyte counts, total T cells, Ts cells and NK cells were not significantly decreased, suggesting that EA could alleviate immunosuppression during surgery. Furthermore, this study also found that stimulation of verum acupuncture points and non-acupuncture points elicited similar effects, which is consistent with recent systematic reviews and studies.

T cells are a critical component of the adaptive immune response. Our investigation of circulating T cells showed that the number of T cells was decreased during craniotomy. Other studies have also shown that T cells were decreased for some days after surgical trauma. Previous studies showed that surgical trauma suppressed T lymphocyte proliferation and EA administration restored suppressed lymphocyte proliferation. Cheng et al noted that EA at ST36 acupuncture point might increase lymphocyte proliferation of surgically traumatised rats. These results also suggested that acupuncture might improve

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**Table 3** Blood cell populations test result

<table>
<thead>
<tr>
<th>Group</th>
<th>Before anaesthesia</th>
<th>30 min after anaesthesia</th>
<th>2 h after anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.3±0.19*</td>
<td>1.46±0.13*</td>
<td>1.43±0.12*</td>
</tr>
<tr>
<td>A</td>
<td>1.37±0.27</td>
<td>1.12±0.19**</td>
<td>1.19±0.16**</td>
</tr>
<tr>
<td>S</td>
<td>1.18±0.27</td>
<td>0.99±0.19**</td>
<td>1.19±0.16**</td>
</tr>
<tr>
<td>Total T cells (x10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.51±0.13</td>
<td>1.09±0.10*</td>
<td>0.99±0.08*</td>
</tr>
<tr>
<td>A</td>
<td>0.84±0.18</td>
<td>0.73±0.14**</td>
<td>0.67±0.11**</td>
</tr>
<tr>
<td>S</td>
<td>0.71±0.18</td>
<td>0.68±0.14**</td>
<td>0.82±0.11**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. *p<0.05 vs before anaesthesia in group; **p<0.05 vs group C. A, electroacupuncture group; C, control group; S, sham acupuncture group.
immunosuppression after surgical trauma. A comparison with previous studies indicated that a similar effect of EA was also observed during craniotomy.

NK cells are a first line of defence against various infections and malignancies and are an important component of the innate immune system. Major surgery suppresses NK cell cytotoxic activity, and NK cell function during the perioperative period is associated with an increased risk of mortality in patients with cancer. Previous studies reported that EA increased NK cell activity in the spleen by approximately 44%. In our study, NK cell counts decreased significantly in group C during surgery but not in groups A or S, indicating that EA can regulate immunity in order to prevent a decrease in NK cells and attenuation of anaesthesia- and surgery-induced immunosuppression.

Reliable measurements of endogenous mediators such as TNFα, IL-8 and IL-10 have enabled clarification of the pathway of the inflammatory response, providing an important tool in the clinical setting. The plasma levels of TNFα, IL-8 and IL-10 were found to increase significantly after cardiopulmonary bypass, and our study results are consistent with this. We found that the IL-10 level increased significantly 2 h and 4 h after induction of anaesthesia in groups A and C, while the IL-8 level increased significantly 4 h after induction of anaesthesia in groups A and C. This may be due to the injury of surgery.

There are five types of immunoglobulins in serum, but the amounts of IgE and IgD are very small so we only determined the levels of IgA, IgM and IgG. A previous study reported that EA can enhance the levels of serum IgM and IgA significantly in postoperative rats with gastric carcinoma. Our study showed that IgM and IgA decreased significantly in group C during surgery but were not decreased in the EA groups. This indicated again that EA could regulate immunity to prevent the decrease in IgM and IgA levels and upregulate the anaesthesia- and surgery-induced immunosuppression.

By detecting the levels of the humoral immunity index (including serum IgG, IgM and IgA), the levels of the cellular immunity index (including CD4/CD8 T cells in the peripheral blood), the counts of the innate immunity index (including monocytes and NK cells) and the counts of the adaptive immunity index (including B cells and T cells), we found that EA could improve humoral and cellular immunity, innate and adaptive immunity and alleviate anaesthesia- and surgery-induced immunosuppression.

In summary, this study shows that EA, in addition to its analgesic effects, could prevent the decrease of immune cells and deleterious immunological changes during operation. Also, it seems likely that point specificity is important for some indications but not for others, since the study also suggested that stimulation of non-acupuncture points can elicit effects similar to those of stimulation of verum acupuncture points. Further studies in this area are needed to analyse the underlying mechanisms. If replicated elsewhere, our results suggest that EA might be useful in the operation to decrease morbidity and mortality and to improve the management and prognosis of those patients at risk of developing postoperative complications.

**Summary points**

- 56 patients undergoing craniotomy were randomised to receive EA, sham point EA or no EA (control).
- Stimulation was set at maximum tolerance at induction (ie, patients were aware of either having EA or not).
- The fall in markers for immune function was significantly less in both EA groups compared with control (but note baseline differences).
- There were no significant differences between real and sham point EA.

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**Contributors** The work presented here was carried out in collaboration between all authors. BW defined the research theme. GL designed the methods and experiments, carried out the laboratory experiments, analysed the data, interpreted the results and wrote the paper. SL and LA worked together on associated data collection and their interpretation. All authors have contributed to, seen and approved the manuscript.

**Competing interests** None.

**Ethics approval** The study protocol was approved by the Institutional Review Board of Beijing Tiantan Hospital. The study was approved and registered by our hospital Ethics Committee, including related screening, treatment and data collection of follow-up of these patients. All studies were undertaken following the provisions of the Declaration of Helsinki.

**Patient consent** All subjects signed an informed consent form.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**REFERENCES**


Original paper


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