The Effect of Acupuncture on Proinflammatory Cytokine Production in Patients with Chronic Headache: A Preliminary Report

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Abstract: Acupuncture has been widely used as a treatment for various conditions like headache and stroke, especially in Asian countries such as Korea and China. But few scientific investigations have been carried out. The aim of the present study is to investigate the effect of acupuncture on the production of inflammatory cytokines in patients with chronic headache (CH). Patients with CH were treated with acupuncture during the acute stage. Clinical signs of CH disappeared markedly after three months of treatment with acupuncture. Peripheral blood mononuclear cells obtained from a normal group and those from the patients with CH,
before and after treatment with acupuncture, were cultured for 24 hours in the presence or absence of lipopolysaccharide (LPS). The amount of interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α) in LPS culture supernatant was significantly increased in the patients with CH compared to the healthy control group (p < 0.05). But those cytokines came down toward the levels of the healthy group (p < 0.05) after treatment with acupuncture, although the levels still remained elevated. Plasma cytokine levels were analyzed to evaluate any change due to acupuncture treatment. There was little difference in the levels of IL-1β or IL-6 due to the treatment with acupuncture in the patients with CH, but significantly reduced plasma levels of TNF-α were observed. These data suggest that acupuncture treatment has an inhibitory effect on pro-inflammatory cytokine production in patients with CH.

**Keywords:** Acupuncture; Chronic Headache; Interleukin; Tumor Necrosis Factor-Alpha.

**Introduction**

Acupuncture has been used for treatment of complex chronic diseases in China and Korea for centuries, but scientific studies on this topic have only recently started to emerge. The theoretical basis of this style of acupuncture rests firmly on the theories of Yin and Yang, the five elements, the 12 viscera and the meridian system of energy flow (Park et al., 2001; Joos et al., 2000).

Headache, which is believed to be associated with multiple components, is one of the most common neuroimmunological diseases. Seventy-seven percent of the patients experienced the onset of headache before the age of 30. The daily headaches were present on awakening in the morning or occur in the course of the morning in 79% of the patients. In 53%, they were worse in the afternoon or evening. The headaches interrupt the patients’ sleep at night at least once per week in 36%. At least twice per week, they were associated with nausea in 35% of the patients and with vomiting in 9% (Purdy, 2001). Common aggravating factors included light, physical activity, bending over, noise, stress or tension, and menstruation. Ninety-four percent of the patients experienced severe headaches in addition to the daily headaches. In 63%, the severe headaches occurred 10 days per month or less. There is no therapeutic method yet for perfect cure in these cases, only a few treating agents including caffeine, analgesics and aspirin are applied to relieve the symptoms temporarily (Schoenen, 2000).

Many newly isolated or recombinantly synthesized protein with therapeutic properties have the capacity to induce an acute inflammatory reaction in patients. These reactions include fever, chills, headaches and nausea which were very often mediated by a number of endogenously released proinflammatory cytokines, most notably IL-1, IL-6 and TNF-α (Vial and Descotes, 1995; Zaremba et al., 2001). Proinflammatory cytokines are proposed as the common mediators of headache (Martelletti et al., 1999). This unifying concept not only can account for headache, but also the prostaglandins, leukotrienes, platelet activating and vasoactive substances linked with headache, the varied symptoms associated with headache and the high incidence of headache with depression, infectious disease, trauma and in premenopausal women (Smith, 1992).
Among proinflammatory cytokines involved in hemostatic and immunological imbalance leading to enlargement of brain damage, the release of TNF-α is especially emphasized (Feuerstein et al., 1994; Feuerstein et al., 1998; Kim, 1996). Rapid increases in TNF-α levels within and surrounding the focus of damaged brain in experimental animal models of cerebral ischemia have been observed (Buttini et al., 1996; Gong et al., 1998).

In the present study, we investigated the release of proinflammatory cytokines, IL-1β, IL-6 and TNF-α from LPS-stimulated peripheral blood mononuclear cells (PBMC) taken from a normal group and a patient group with CH before and after treatment with acupuncture. We also measured the proinflammatory cytokines in the plasma.

Materials and Methods

Patients

Patients with CH were examined at the Jeonju Oriental Medical Hospital, Wonkwang University (Iksan, Korea) from June 2001 to January 2003. They had been suffering from “tension headaches” for about 20 years and had been taking analgesic drugs twice a day. Without medication, however, the patients’ headaches persisted. The patients complained of pain in different body regions (head, neck, shoulders, upper back and lower back), thus they were requested to undergo further diagnostic and psychological tests (usually the MMPI) in order to identify cases of psychosis or neurosis. The results showed that they were suffering from chronic tension headache resulting from cervical orthopedic spondylosis. They were not treated with immunosuppressants or plasmapheresis for 1 year prior to acupuncture treatment. The period of acupuncture treatment was 3 months. It was performed twice a week for 30 minutes. For cytokine assay, blood was obtained before the treatment and 3 months after beginning the treatment from four patients (one male, three females, mean age 58 years, range 54–69 years) with CH and ten healthy adults (five males and five females, mean age 62.5 years, range 41–68 years) with no medically diagnosable illness as a control group. The patients had suffered from tension headache for about 2, 6, 10 and 20 years, respectively.

Blood was taken just once from each donor. Informed consent was obtained from all subjects before performing these studies. All samples were collected in a sterile glass tube and allowed to clot spontaneously for 15 minutes. Serum was then collected by centrifugation and quickly frozen and stored in aliquots at −80°C until assay.

Acupuncture Treatment

An experienced qualified acupuncturist performed acupuncture for a period of 30 minutes. Acupuncture points were selected on the site of GB20, SI3 and UB60 bilaterally. The needles were 2.5 cm long, made of stainless steel and sterilized before use. Insertions were made to depths between 0.6 and 1.3 cm after the usual skin sterilizing procedure. Stimulation was brought about by manual rotations of the needles, which then evoked a tingling, non-painful
sensation (de Qi). The procedure was then repeated for 10 seconds every 5 minutes by further rotations. And the acupuncturist was blind to the experiment, while the subjects knew nothing about the details of the treatment.

**PBMC Isolation and Culture**

PBMC from heparinized venous blood were isolated by Ficoll-gradient centrifugation, washed three times in phosphate-buffered saline (PBS) solution and resuspended in RPMI 1640 medium (GIBCO) supplemented with 2 mM L-glutamin, 100 U/ml penicillin G, 100 µg/ml streptomycin and 10% FBS inactivated for 30 minutes at 56°C. PBMC were adjusted to a concentration of $2 \times 10^6$ cells/ml in 30 ml falcon tubes, and 100 µl aliquots of cell suspension were placed in a four-well cell culture plate. PBMC were cultured for 24 hours in 95% humidified air containing 5% CO$_2$ (37°C), in the presence or the absence of lipopolysaccharide (LPS) (10 ng/ml), and the supernatants were collected by centrifugation and stored at $-20°C$.

**ELISA of IL-1β, IL-6 and TNF-α**

Sandwich ELISA for IL-1β, IL-6 and TNF-α was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark), each coated with 100 µl aliquots of mouse anti-human IL-1β, IL-6 and TNF-α monoclonal antibodies (R&D Systems, Minneapolis, MN, USA) at 1.0 µg/ml in PBS at pH 7.4. Plates were incubated overnight at 4°C and then washed in PBS containing 0.05% Tween-20 (Sigma, St. Louis, MO, USA) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN$_3$ for 1 hour. After additional washes, sample or IL-1β, IL-6 and TNF-α standards were added and incubated at 37°C for 2 hours. After a 2-hour incubation at 37°C, the wells were washed and then each of 0.2 µg/ml of biotinylated anti-human IL-1β, IL-6 and TNF-α were added and again incubated at 37°C for 2 hours. After washing the wells, streptavidin-peroxidase was added and the plates were incubated for 20 minutes at 37°C. Wells were again washed and ABTS substrate (Sigma) was added. Color development was measured at 450 nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using recombinant IL-1β, IL-6 and TNF-α (R&D Systems) in serial dilutions.

**Statistical Analysis**

Levels of cytokines among the clinical groups were compared using the two-tailed Student’s t-test; a value of $p < 0.05$ was accepted as statistically significant. Values of cytokines are given in the text as mean ± standard deviation (SD).
Results

IL-1β Production in Culture Supernatants

Spontaneous IL-1β production, in the culture supernatants of non-stimulated PBMC, obtained from the patients with CH before or after acupuncture treatment (0.28 ± 0.11 ng/ml and 0.27 ± 0.11 ng/ml, respectively), was similar to that in the culture supernatants from healthy controls (0.27 ± 0.02 ng/ml). However, when LPS stimulated PBMCs, the level of IL-1β production before the treatment (7.08 ± 3.11 ng/ml), which was significantly higher than that of the healthy controls (0.48 ± 0.060 ng/ml), dropped significantly toward that of the healthy group after the treatment (2.23 ± 1.09 ng/ml) (Fig. 1).

IL-6 Production in Culture Supernatants

Spontaneous IL-6 production, in the culture supernatants of non-stimulated PBMC, obtained from the patients with CH before or after acupuncture treatment (0.24 ± 0.09 ng/ml and 0.25 ± 0.07 ng/ml, respectively), was higher than that in the culture supernatants from healthy controls (0.085 ± 0.007 ng/ml). In addition, in the culture supernatants of LPS-stimulated PBMC, before the treatment, IL-6 production (0.71 ± 0.12 ng/ml) was significantly higher than that of the healthy controls (0.335 ± 0.045 ng/ml), but it decreased significantly after the treatment (0.53 ± 0.09 ng/ml) (Fig. 2).
Figure 2. Production of IL-6 in culture supernatants of non-stimulated and LPS-stimulated PBMC from healthy controls, and the patients (n = 4) with CH before and after acupuncture treatment. Culture supernatants were collected from non-stimulated and LPS-stimulated PBMC which were cultured for 24 hours. IL-6 levels in culture supernatants were measured using ELISA. Culture supernatants of LPS-stimulated PBMC from acupuncture-treated CH patients showed significantly decreased IL-6 production compared with that before the treatment (*p < 0.05).

Figure 3. Production of TNF-α in culture supernatants of non-stimulated and LPS-stimulated PBMC from healthy controls, and the patients (n = 4) with CH before and after acupuncture treatment. Culture supernatants were collected from non-stimulated and LPS-stimulated PBMC, which were cultured for 24 hours. TNF-α levels in culture supernatants were measured using ELISA. Culture supernatants of LPS-stimulated PBMC from acupuncture-treated CH patients showed significantly decreased TNF-α production compared with that before the treatment (*p < 0.01).
TNF-α Production in Culture Supernatants

Spontaneous TNF-α production in the culture supernatants of non-stimulated PBMC from patients with CH before or after acupuncture treatment (0.34 ± 0.18 ng/ml and 0.21 ± 0.11 ng/ml, respectively) was higher than that in the culture supernatants from the healthy controls (0.122 ± 0.020 ng/ml). TNF-α production in the culture supernatants of LPS-stimulated PBMC from the patients with CH before the treatment (4.12 ± 1.50 ng/ml) was significantly increased compared with that of the healthy controls (0.35 ± 0.008 ng/ml), but decreased significantly after treatment with acupuncture (0.86 ± 0.28 ng/ml) (Fig. 3).

IL-1β, IL-6 and TNF-α Levels in Plasma

Blood was obtained from the patients with CH. Higher levels of plasma IL-6 than the normal controls (0.069 ± 0.02 ng/ml) were observed in patients with CH. Plasma levels of IL-1β and IL-6 after acupuncture treatment were not significantly changed. Higher levels of plasma TNF-α were detected in patients with CH (1.83 ± 0.03 ng/ml) than normal controls (0.087 ± 0.01 ng/ml) but was significantly decreased (0.89 ± 0.13 ng/ml) after acupuncture treatment (Table 1).

| Table 1. Effect of Acupuncture Treatment on Plasma IL-1β, IL-6 and TNF-α Level |
|-----------------|-----------------|-----------------|
|                 | IL-1β (ng/ml)   | IL-6 (ng/ml)    | TNF-α (ng/ml)   |
| Healthy control | ND              | 0.069 ± 0.02    | 0.087 ± 0.01    |
| Before treatment| 2.95 ± 2.62     | 0.26 ± 0.11     | 1.88 ± 0.03     |
| After treatment | 2.38 ± 1.53     | 0.27 ± 0.11     | 0.89 ± 0.13*    |

Four patients with CH were treated by acupuncture for 3 months. Data are shown as mean ± SD.
*There are significant differences before and after acupuncture treatment by paired t-test at p < 0.05.
ND: Not detected.

Discussion

The present study demonstrated the effect of acupuncture on LPS-stimulated PBMC culture supernatant from the patients with CH. Production of IL-1β, IL-6 and TNF-α was significantly higher in the culture supernatants of the patient with CH than in those of healthy controls. After acupuncture treatment, these cytokines decreased toward the levels of healthy controls but still remained elevated (Figs. 1 to 3).

We also found that plasma IL-1β, IL-6 and TNF-α levels in patients with CH were higher than in the healthy controls. Acupuncture treatment decreased the TNF-α levels significantly but the IL-1β and IL-6 levels were not affected.
Endotoxin and LPS can initiate a variety of cell activation pathways. These unique macromolecules have been extensively studied to elucidate and define relevant pathophysiological parameters of endotoxin shock, a profound life-threatening consequence of bacterial sepsis (Tsuzuki et al., 2001). These bacterial products have generated intense interest due to their pluripotential immunostimulatory activity as manifested by the activation of host cells, B-lymphocyte and macrophage to differentiate functionally (Ohno and Morrison, 1989). Cells activated by LPS produce cytokines including interferons, IL-1β, IL-6, TNF-α, platelet-activating factor and procoagulant tissue factor. These cytokines induced by LPS are involved in inflammation and exert pathophysiological effects such as fever, shock or headache in mammals (Masihi et al., 1997).

Migraine is a moderate to severe headache evolving by stereotyped crises of a unilateral throbbing pain, which is aggravated by physical activity, and is associated with marked symptoms such as nausea and/or vomiting, photo- and phono-phobia (Jennum and Jensen, 2002). The migraine headache can occur with or without aura symptoms where the aura usually precedes the headache with a visual or sensory aura. Migraine is a common episodic headache disorder and the result of cortical hypersensitivity, hyperactivity and a lack of habituation (Kropp et al., 2002). Recently, research into the mechanisms of migraine and the progressive recognition that cortical hyperexcitability and an imbalance between neuronal inhibition [mediated by gamma-aminobutyric acid (GABA)] and excitation (mediated by excitatory amino acids) may play an important role in migraine pathophysiology, have lead to the identification of potential new agents for the prevention of migraine attacks. In this study, clinical signs of CH decreased markedly after patients were treated with acupuncture for 3 months.

IL-1β exerts multifunctional biological effects by promoting and increasing the molecular events of cellular inflammation (Suzuki et al., 2001). Higher levels of IL-1β were detected in the sera of cervicogenic headache patients (Martelletti, 2000). IL-6 has also been described in a variety of neuropathologies including brain injury (Maimone et al., 1991). IL-1β and IL-6 are released during inflammatory responses and can act synergistically in brain diseases. IL-1 initiates fever by increasing prostaglandin (PG) E2 synthesis in or near the anterior hypothalamus in the cat (Coceani et al., 1983). And IL-6 has also been shown to stimulate PGE2 synthesis in the brain of animals (Dinarello et al., 1991). These cytokines were decreased in LPS-stimulated PBMC culture supernatant of acupuncture-treated CH patients and clinical signs of CH patients decreased markedly. These results suggest that regulation of IL-1β and IL-6 might have a beneficial effect in the treatment of CH patients. But these cytokines in plasma were not significantly changed by acupuncture treatment on plasma levels. Therefore, further studies are necessary to clarify the mechanism of IL-1β and IL-6 reduction by acupuncture in patients with CH.

TNF-α is a pleiotropic cytokine and appears to be involved in blood-brain barrier, inflammatory, thrombogenic and vascular changes associated with brain injury. TNF-α levels in brain tissue, cerebrospinal fluid and plasma have been found to be elevated in several central nervous system disorders, including chronic headache, Alzheimer’s disease, multiple sclerosis, Parkinson’s disease, meningococcal meningitis and HIV infection (Barone et al., 1997). Recent studies have indicated that blocking TNF-α reduced brain injury and attenuated
intracellular adhesion molecule expression during transient cerebral ischemia (Yang et al., 1998). In fact, the major side effects of TNF-α, when injected into humans, have been chills, fever, headache, nausea, vomiting and hypotension (Moritz et al., 1989). In this study, the level of TNF-α in patients with CH was very high compared with the healthy controls in the PBMC culture supernatant after LPS stimulation. TNF-α in the PBMC culture supernatant was decreased significantly in the acupuncture-treated CH patients. Our results also showed that increased plasma TNF-α levels in patients with CH were decreased toward healthy control values after acupuncture treatment, although the levels were still elevated (Table 1). These results indicate that TNF-α levels were modified by acupuncture treatment and might also play an important role in patients with CH.

Overall, our results of this preliminary study suggest that acupuncture treatment modified the imbalance of cytokine production in CH. Therefore, we speculate that acupuncture treatment may improve immunotherapy and contribute to the development of successful and safe immunotherapy for the patient with CH. However, studies with large number of CH patients to evaluate this effect are warranted in the future.

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References


