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Brain, Behavior, and Immunity 19 (2005) 318–324
BRAIN, BEHAVIOR, and IMMUNITY
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Short communication

Repeated acupuncture treatment affects leukocyte circulation in healthy young male subjects: A randomized single-blind two-period crossover study

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Received 15 June 2004; received in revised form 6 September 2004; accepted 1 October 2004
Available online 25 November 2004

Abstract

Acupuncture is the most popular component of traditional Chinese medicine in western countries, which has been widely used in the treatment of numerous medical conditions, e.g., pain, emesis or asthma. However, the effects of acupuncture on neuroendocrine and immune functions in humans remain unclear. Therefore, the present study was performed to analyse whether acupuncture treatment affects leukocyte circulation as well as plasma levels of cortisol and norepinephrine in humans. Ten healthy young male subjects were enrolled in a randomized single-blind two-period crossover study. Each period contained three sessions of either acupuncture or sham acupuncture (placebo) treatment. After randomisation, the group 1 ($n = 5$) received acupuncture treatment at acu-points ST36, LI11, SP10, and GV14, while sham acupuncture was performed for group 2 ($n = 5$). Two weeks later, each group received the alternative treatment. Blood samples were taken before needling, 10 min after, and 30 min after removing the needles in the first and the third session. In addition, blood pressure and heart rate were determined simultaneously. Although acupuncture treatment did not affect leukocyte circulation in peripheral blood after the first session, we observed a significant decrease in leukocyte and lymphocyte values after the third session. In contrast, cortisol and norepinephrine plasma levels remained unchanged by acupuncture. These data indicate that repeated acupuncture treatment can affect leukocyte circulation in healthy humans by still unknown mechanisms.

Keywords: Acupuncture; Traditional chinese medicine; Leukocyte circulation; Neuroendocrine system; Immune system; Cortisol; Norepinephrine

1. Introduction

Acupuncture has been used for thousands of years in China to treat numerous medical conditions, from common ailments to specific diseases, including pain, emesis, asthma, etc. The effects of acupuncture on central nervous system (CNS) (Hsieh et al., 2001; Okumura et al., 1999; Wu et al., 1999) and endocrine system (Akimoto et al., 2003) have been recently documented, and evidence accumulated during the last decade shows that acupuncture can affect humoral as well as cellular immune functions in both animals (Lundeberg et al., 1991; Okumura et al., 1999; Yu et al., 1998) and humans (Joos et al., 2000; Kho et al., 1990). Although the effects of acupuncture on neuroendocrine and immune systems

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doi:10.1016/j.bbi.2004.10.001

has been demonstrated in mice (Lundeberg et al., 1991; Okumura et al., 1999; Yu et al., 1998), in rats (Choi et al., 2002; Hahm et al., 2004), and in humans (Akimoto et al., 2003), whether and to what extent acupuncture treatment affects neuroendocrine and immune functions in healthy human subjects are still unclear.

Within traditional Chinese medicine (TCM) theory, acu-points ST36, LI11, SP10, and GV14 have been related to immune functions and often used in clinical practice for disorders of the immune system, e.g., allergic diseases, infections, immunodeficiency-syndromes, etc, (Cheng, 1987; Rogers et al., 1992). Additionally, ST36 and LI11 has been widely used in clinical and experimental studies investigating the immunomodulatory effects of acupuncture both in humans and animals (Huang et al., 2004; Karst et al., 2002; Ye et al., 2002; Yu et al., 1998). Therefore, we carried out the current study to analyse the effects of acupuncture at acu-points ST36, LI11, SP10, and GV14 on leukocyte circulation in peripheral blood, plasma levels of cortisol and norepinephrine and cardiovascular parameters in healthy young male subjects in a randomized single-blind crossover design.

2. Methods

Subjects were recruited by the use of public advertisement at the University Hospital in Essen, Germany. They had to meet the following inclusion criteria: (1) male; (2) 18–35 years old; (3) non-smoker; and (4) no vaccinations in the past three months. Any medication during the experiment was considered as the exclusion criterion. Additionally, the subjects were requested to not perform any overly exhausting sports during the experiment. The study was carried out in the department of Internal Medicine V, Kliniken Essen-Mitte, Essen, Germany. All subjects were identified as healthy following a medical history and physical examination by a physician. The study protocol was approved by the ethics committee for human investigation of the University of Duisburg-Essen, and written informed consent was obtained from all subjects. Before enrollment, the subjects had no previous contact or relationship with any person involved in the experiment, to avoid possible psychological influences. A computer-generated random allocation sequence was prepared, and the assignments were placed in serially numbered, sealed, opaque envelopes. After enrollment, subjects were randomized into two groups and received acupuncture or sham acupuncture (placebo) treatment in a two-period crossover design. Group 1 were first treated with acupuncture and group 2 with sham acupuncture. Two weeks later, each group received the alternative treatment (Fig. 1). Each period of the study contained three sessions of either acupuncture or sham acupuncture treatment, at acupoints ST36 and SP10 on the leg, LI11 on the arm bilaterally and GV14 on the neck back unilaterally, which were performed around 8:00 a.m. on Mondays, Wednesdays, and Fridays, both with sterilized acupuncture needle (Seirin, B type, Dreieich, Germany). Upon arrival, subjects were seated and rested for 20 min, and then subjects lie down (face up) before, and during the 30 min of acupuncture treatment. In acupuncture treatment, all needles were manipulated to induce the special needle sensation (DeQi) (Gareus et al., 2002), whereas in sham acupuncture, needles were placed away from, but contiguous to classical acu-points, manipulated without inducing DeQi (Lux et al., 1994). In both cases a further manipulation for each acupoint occurred at the middle of the treatment (15 min). All treatments were performed by the same acupuncturist who was aware of the group assignment. However, the subjects, the physician drawing blood, and the investigators measuring immunological as well as biochemical variables were fully blinded to the group assignments. Blood pressure (BP) and heart rate (HR) were determined each 30 s by a Finapres BP Monitor (Ohmeda, Louisville, USA) continually from 10 min before to 10 min after the treatment both in the first and the third session. BP and HR values were calculated 10 min before the needle insertion (baseline/0 min), 15 min after the insertion (15 min), 15 min after the further manipulation (30 min) and 10 min after the needle removing (40 min). Blood samples were collected from the cubital vein (4–5 cm away from acupoint LI11) prior to needling (0 min), 10 min after (40 min), and 30 min after needle removing (60 min) in the first and the third session.

White blood cell (WBC) count and flow cytometry were performed as described previously (Goebel et al., 2002). Cortisol plasma concentration was determined using Bayer ADVIA Centaur immunoassay system (Bayer, Tarrytown, NY). Norepinephrine plasma concentration was determined using commercially available radioimmunoassay (RIA) (Diagnostic Systems Laboratories, Texas, USA). Data were analysed using repeated measures analysis of variance (ANOVA) and paired t test (SPSS, Chicago, IL, USA). In order to minimize inter-individual variation, leukocyte and leukocyte subset numbers were analysed as percent change from baseline, which was calculated by the following formula: Percent change of 40 or 60 min = (absolute cell numbers of 40 min or 60 min − absolute cell numbers of baseline) × 100/absolute cell numbers of baseline. Any value which was out of the range of means ± 3 standard deviations were excluded from analysing. All data are presented as means ± standard error of mean (SEM).

3. Results

Eleven subjects were recruited from April 2003 until September 2003, with one subject refused to participate.

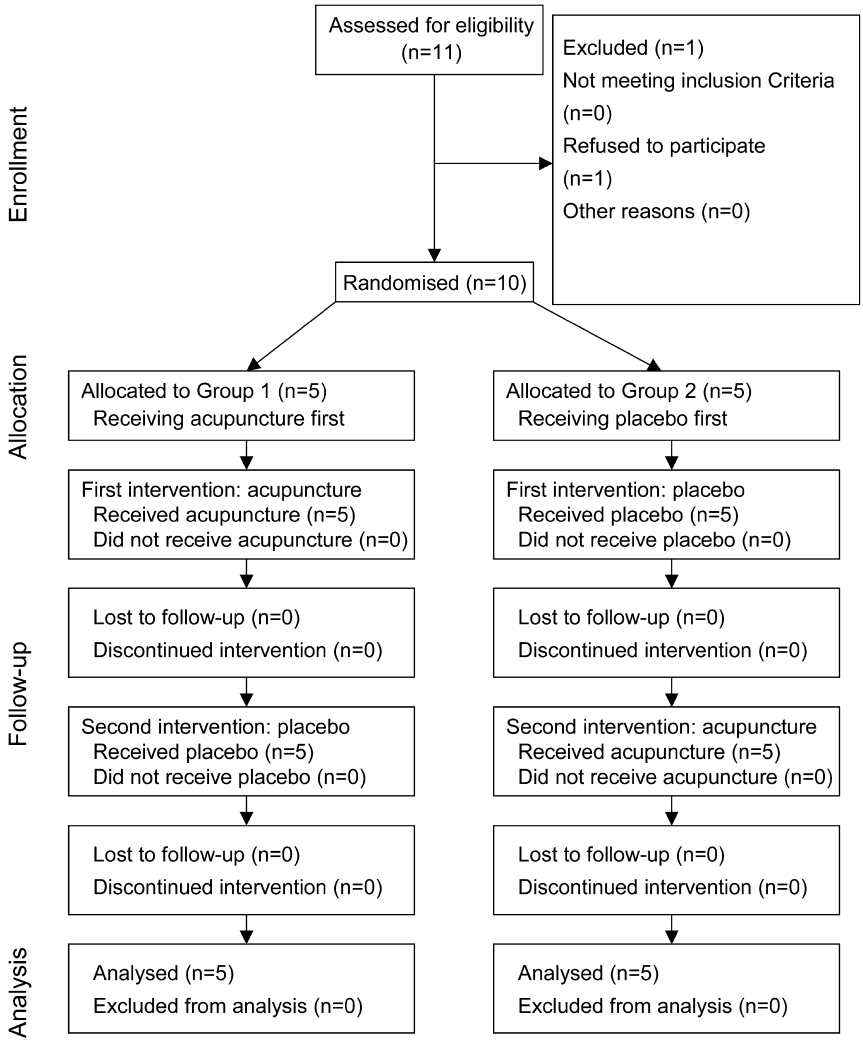

Fig. 1. Flow diagram of subjects through the trial.

Ten subjects (age 25.80 ± 1.03 years) were enrolled, randomly assigned, and completed the study (Fig. 1). The primary analysis was intention-to-treat and involved all these subjects. No significant baseline differences between the two groups were found except the absolute numbers of granulocytes in the first session (data not shown). After the first session, acupuncture did not affect leukocyte circulation, plasma levels of cortisol or BP and HR compared to sham acupuncture (data not shown).

At third acupuncture session, absolute numbers of leukocytes was reduced after treatment, peaking at 40 min time point and still reduced at 60 min time point (Table 1). However, these changes did not reach statistical significance; therefore we reanalyse the data as percent change from baseline trying to minimize the inter-individual difference, showing that after the third acupuncture session leukocyte values were significantly reduced at 40 and 60 min in comparison to sham acupuncture ($p < .05$, respectively; Fig. 2A). In addition, lymphocyte values decreased at 40 min ($p < .01$, Fig. 2B) as well as at 60 min ($p < .05$, Fig. 2B), whereas the granulocytes and monocytes values remained unchanged by acupuncture (data not shown). Moreover, when analysing lymphocyte subpopulations, we observed a significant reduction in values of $CD3^+$ lymphocytes at 40 min ($p < .05$, Fig. 2C), $CD3^+CD4^+$ lymphocytes at 40 min ($p < .01$, Fig. 2D), and $CD3^+CD8^+$ lymphocytes at both 40 and 60 min ($p < .05$, respectively; Fig. 2E). In contrast, no significant changes were found for $CD3^-CD20^+$ lymphocyte (Fig. 2F) and $CD3^-CD16^+$ lymphocyte values (data not shown). Interestingly, we observed significantly higher systolic BP (SBP) and diastolic BP (DBP) levels in the acupuncture compared to the sham acupuncture group (Table 1). HR (Table 1), plasma concentrations of cortisol remained unaffected by acupuncture (data not shown). For plasma levels of norepinephrine, subjects treated by acupuncture showed an increase while subjects in sham acupuncture group showed a decrease at 40 min in the first as well as in the third session, both of which returned to the baseline levels at

Table 1
The effects of the third session of acupuncture (A) or sham acupuncture (SA) treatment on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and absolute leukocyte numbers

	0 min	15 min	30 min	40 min	60 min
SBP (mmHg)					
A	121.35 ± 5.31	131.02 ± 3.97*	135.52 ± 5.86*	135.79 ± 5.66**	n.d.
SA	108.72 ± 9.08	117.88 ± 5.52	119.62 ± 5.34	118.38 ± 6.13	n.d.
DBP (mmHg)					
A	78.25 ± 2.14*	79.41 ± 2.78*	80.86 ± 4.11*	79.86 ± 4.88*	n.d.
SA	65.78 ± 4.73	69.73 ± 4.01	69.80 ± 3.64	69.49 ± 3.75	n.d.
HR (beats/min)					
A	67.64 ± 2.73	65.35 ± 2.69	65.24 ± 3.08	65.02 ± 2.94	n.d.
SA	64.26 ± 3.16	64.72 ± 2.49	64.55 ± 2.54	66.75 ± 2.13	n.d.
Leukocytes (cells/μl)					
A	5494 ± 441	n.d.	n.d.	4810 ± 369	5145 ± 369
SA	4843 ± 334	n.d.	n.d.	4480 ± 289	4901 ± 353

Data are presented as means ± SEM.
$n = 10$ in each group.
* $p = .05$ compared to sham acupuncture treatment in paired t test.
** $p = .01$ compared to sham acupuncture treatment in paired t test.

60 min; however no statistical significance was found. Additionally, it is important to indicate that mean baseline values for BP and leukocyte absolute numbers at third session were more distant each other compared to baseline values of the first session, being statistically different for DBP (Table 1). Apart from some common complaints about blood draws, no subject declared any discomfort caused by the acupuncture or sham acupuncture treatments.

4. Discussion

This study investigated the effects of acupuncture treatment on neuroendocrine, immune, and cardiovascular functions simultaneously in young healthy male humans. Our data indicate that although acupuncture does not affect BP, HR, and leukocyte circulation in humans after the first treatment session, repeated acupuncture treatments induces a significant reduction in leukocyte and lymphocyte values. These changes occurred in parallel to BP (both systolic and diastolic) increments.

The modulation of electroacupuncture on granulocyte and lymphocyte circulation in healthy humans has been recently reported (Mori et al., 2002), however our data indicate for the first time that repeated applied acupuncture, without the combination of electro-stimulation, can influence leukocyte circulation in young healthy humans as well. We did not observe any immediate effects of acupuncture on immune parameters after one single session of 30 min treatment, which is in agreement with previous investigations (Karst et al., 2002). However, the decreases in leukocyte and lymphocyte values occurred after the third session of the treatment illustrates that repeatedly applied acupuncture affects leukocyte circulation in humans. Supporting our results, it has been proposed that the total number of acupuncture treatments is an important feature for the final outcome (Ezzo et al., 2000). Moreover, repetition is also an indispensable feature for a successful acupuncture treatment from the TCM theory. Interestingly, mean baseline values of leukocyte absolute numbers and BP at the third session were more distant each other, compared to mean baseline values from the first session. We consider that this effect at baseline present just at third session could be related to an accumulative acupuncture effect from the first and second acupuncture sessions. However, further studies are necessary to clearly distinguish between the effects of short and long term acupuncture treatments on immune system and cardiovascular functions.

The continuous circulation of immune cells from blood into various immune compartments, and back into blood, is essential for the maintenance of an effective immune defence network (Sprent and Tough, 1994). Herein, we show that repeatedly applied acupuncture in humans modulates leukocyte circulation, providing new evidence that acupuncture is able to affect immune functions. Although effects on circulation of leukocyte subsets in peripheral blood do not necessarily predict a clinical relevant immune response, it implicate a direct effect of acupuncture on immunocompetent cells, supporting previous reports where acupuncture have impacted the clinical outcome of immune related diseases (Xin et al., 2002; Ye et al., 2002). Further experiments will be necessary to test the clinical relevance of the results here reported, however our data provide new insights to understand the principles of acupuncture, that we consider will directly impact on a wider acceptance of it as an alternative or complementary therapy planned to support desired immune response

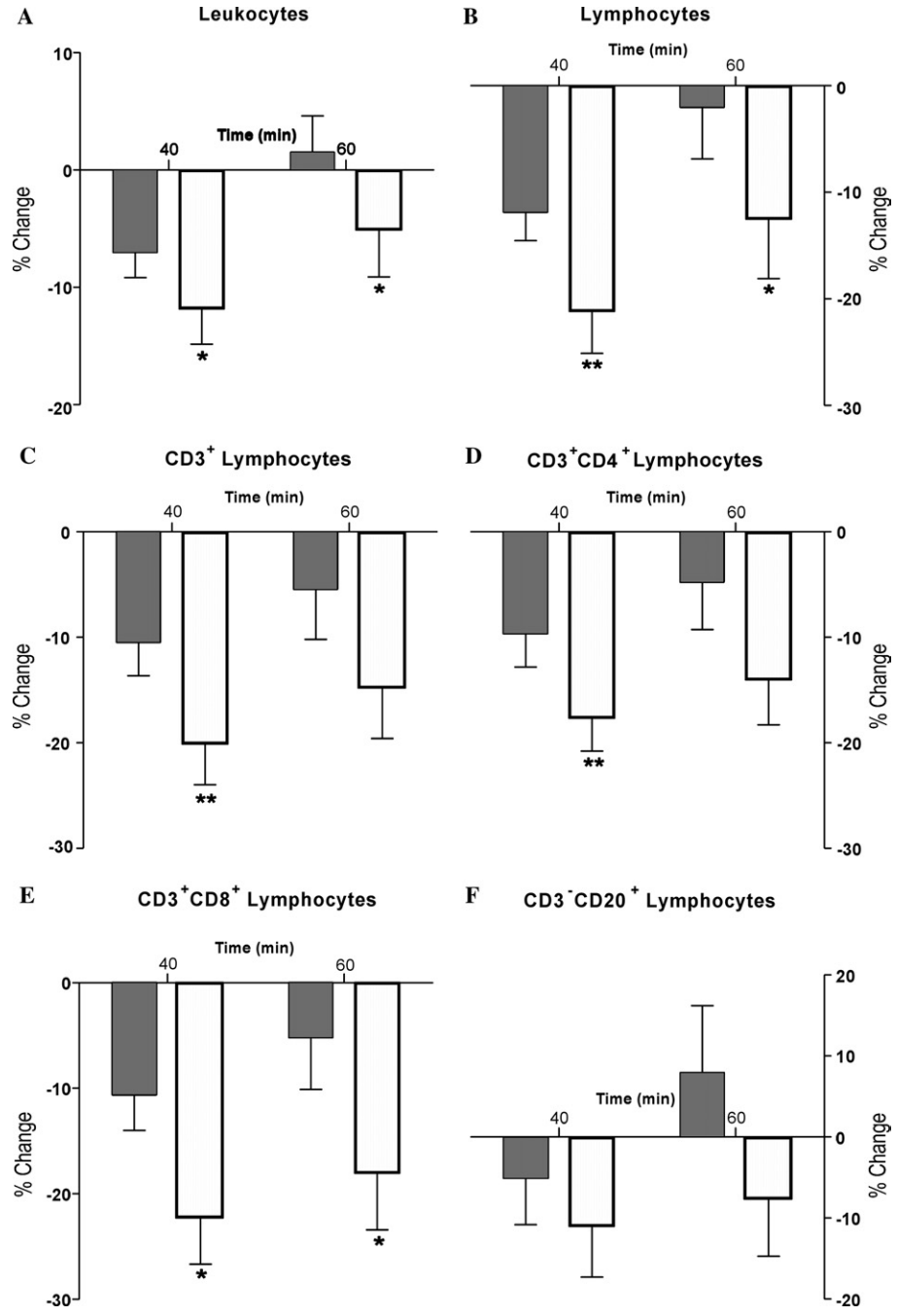

Fig. 2. The effects of the third session of acupuncture (□) or sham acupuncture (■) treatment on the percent changes to the baseline (0 min) in numbers of Leukocytes (A), Lymphocytes (B), $CD3^+$ Lymphocytes (C), $CD3^+CD4^+$ Lymphocytes (D), $CD3^+CD8^+$ Lymphocytes (E), and $CD3^-CD20^+$ Lymphocytes (F). Data are expressed as means ± SEM ($n = 10$ in each group). $^*p = .05$, $^{**}p = .01$ compared to sham acupuncture treatment at each time point.

(e.g., vaccination) or suppress undesired ones (e.g., autoimmune diseases).

A solid scientific framework is missing to analysis the precise mechanisms by which acupuncture affects peripheral immune parameters. We propose that these mechanisms can be investigated through the psychoneuroimmunology paradigm, in which acupuncture may stimulate the afferent and efferent neuroendocrine pathways to and from the CNS, thus indirectly affecting immune functions, including leukocyte circulation (Benschop et al., 1996; Dhabhar et al., 1996; Exton et al., 2001; Pacheco-López, 2003). This hypothesis is supported by data obtained by functional MRI (Wu et al., 1999) and positron emission tomography (Hsieh et al., 2001), suggesting that acupuncture has regionally specific effects on brain structures, e.g., limbic system, hypothalamus, and midbrain, indicating that acupuncture may treat immune disorders through the CNS

immunomodulatory capacities. Furthermore, the immunomodulatory effects of acupuncture in mice were abolished by addition of β-adrenergic blockers, demonstrating that acupuncture can modulate some immune responses by activation of sympathetic nervous system (SNS) (Lundeberg et al., 1991). All these suggesting data support our hypothesis that acupuncture may modulate leukocyte circulation, as observed in this study, through the neuroendocrine-immune network. Interestingly, we observed higher BP after repeated acupuncture treatment, indicating SNS might be involved in the acupuncture effects observed. In addition, we found that subjects treated by acupuncture tended to have higher norepinephrine plasma levels while subjects in the sham acupuncture group showed a trend towards decrease both in the first and the third session. Because no statistical significance was found, we consider that a bigger sample size is necessary to clarify this point.

DeQi is a feeling of heaviness, numbness, or tightness experienced by the subjects during acupuncture, and the acupuncturist should also feel tenseness around the needle (Lao, 1996). Although, in the present study, acupuncture and sham acupuncture introduce different sensations to the subjects (unpublished data), the lack of previous acupuncture experience did not allow the subjects to identify DeQi sensation. More importantly, the subjects were not aware that DeQi is specific for acupuncture and is a prerequisite for acupuncture to be effective (Cheng, 1987). Therefore, in this case, the experience of DeQi or the lack of it should not induce different psychological expectations that could affect the experimental outcome. Additionally, analysis of pain sensation and transient anxiety/tension status before and after the treatments (unpublished data) also indicate that acupuncture does not induce more pain or anxiety (stress), compared to sham acupuncture treatment. Although it can not be excluded that some effects here reported could be related to relaxation or position of the subjects during treatment, the difference observed between groups (A vs. SA) is independent of such effects since both treatments occurred on similar experimental conditions. Additionally, the crossover experimental design helps us to avoid anticipatory effects.

TCM conceptualize acupuncture as a treatment to restore the balance of body functions (Sung, 2002), implying that this treatment should be only effective in an unbalanced system (disease). In contrast, the present study shows that acupuncture has immunomodulatory effects even in healthy subjects (balanced system), suggesting that acupuncture, as any medical treatment, can also disturb the homeostasis of the body. This finding should be taken into account for further clinical or experimental trials.

In summary, our findings indicate that repeated acupuncture treatment affects leukocyte circulation and blood pressure in healthy young humans. However, future studies are necessary to investigate the underlying mechanisms and its clinical relevance.

Acknowledgments

We thank Dr. Li Feng Meng for her clinical expertise in performing the study. The study was supported by Ministery of Science and Research of the State of North Rhine-Westphalia. We thank Dr. Andreas Michalsen, Sven Baum, Björn Willmann for their help.

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