

Effect of acupuncture on TNF- α , IL-1 β and IL-10 concentrations in the peritoneal exudates of carrageenan-induced peritonitis in rats

Efeito da acupuntura sobre a concentração de TNF- α , IL-1 β e IL-10 no exsudato peritoneal de ratos com peritonite induzida por carragenina

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ABSTRACT

Acupuncture is an ancient and empirical therapeutic procedure known by its efficacy in the treatment of pain. However, the influence of acupuncture on inflammatory process is still poorly understood and additional research is needed. In this work, we investigated the mechanism of action of manual acupuncture on the inhibition of neutrophil migration to the peritoneal cavity induced by the inflammatory stimulus carrageenan in Wistar rats. Previous results from our laboratory showed that this anti-inflammatory effect is not due to endogenous corticoid release. Furthermore, the concentration of IL-1 β , but not of TNF- α or IL-10 in the carrageenan-induced exudates was reduced in the acupuncture group. Further research will be needed to elucidate the mechanisms involved in the anti-inflammatory action of acupuncture as described here.

Key words: acupuncture, inflammation, cytokines, neutrophils, rats, carrageenan.

RESUMO

A acupuntura é método terapêutico milenar reconhecido por sua eficácia no tratamento da dor, porém seu efeito sobre processos inflamatórios é ainda pouco conhecido e maiores estudos são necessários. Neste trabalho, é investigado o mecanismo de ação da acupuntura manual sobre a inibição na migração de neutrófilos para a cavidade peritoneal induzida por carragenina em ratos Wistar. Resultados prévios indicam que esse efeito antiinflamatório não depende de hormônios corticóides. Entretanto, as

concentrações de IL-1 β no exsudato induzido por carragenina foram reduzidas pelo tratamento com acupuntura. Por outro lado os níveis de TNF- α e IL-10 não foram afetados pelo tratamento. Mais pesquisas poderão elucidar os mecanismos envolvidos na ação antiinflamatória da acupuntura.

Palavras-chave: acupuntura, inflamação, citocinas, neutrófilos, ratos, carragenina.

INTRODUCTION

Acupuncture is the insertion of needles in cutaneous specific locations of the body, known as acupoints, for the treatment or prevention of several diseases (SCHOEN, 1993; ERNST & WHITE, 1999). It is a reflex therapy where the nociceptive stimulus given in an area acts on another one (FARBER & TIMO-IARIA, 1994).

Although this technique is increasingly used for the treatment of pain and other conditions, the rational basis underlying its use remains unclear. Improvement of knowledge in acupuncture therapeutic mechanism is essential to validating it since acupuncture is difficult to test under double-blind, placebo-controlled conditions (STREITBERGER & KLEINHENZ, 1998;

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TUKMACHI, 2000; LANGEVIN, et al., 2001; LANGEVIN et al., 2001; SHERMAN et al., 2002). In fact, few reports concerning the effect of acupuncture on inflammatory models are available, although clinical trials claim the success of acupuncture in the treatment of pruritic dermatosis, bronchial asthma, carpal tunnel syndrome, rheumatoid arthritis and other inflammatory disorders in humans and animals (SCOGNAMILLO-SZABÓ & BECHARA, 2001). However, these observations are limited by methodological considerations such as small sample size, placebo effects and lack of control groups (STREITBERGER & KLEINHENZ, 1998; CASIMIRO et al., 2002, MCCARNEY et al., 2004). Therefore, research on the use of acupuncture in experimentally induced inflammatory process will bring new perspectives towards investigations in this area, including on the mechanisms of action of such technique.

The inflammatory response involves a complex set of highly orchestrated events. An initial inflammatory stimulus (chemical or mechanical trauma, microorganism invasion) triggers the release of chemical mediators from plasma or connective tissue cells. Such soluble mediators, acting together or in sequence, amplify the initial inflammatory response and influence its evolution by regulating the subsequent vascular and cellular responses. This is a benign process if there is a suitable regulation of the inflammatory response (TRACEY & WARREN, 2004).

The neutrophil migration observed in the inflammatory process is mediated by several chemotactic mediators (BODOLAY et al., 2002). Among them, the cytokines TNF- α and IL-1 β have a pivotal role (BURGER, DAYER, 2002). On the other hand, IL-10 down-modulates the neutrophil migration into inflammatory exudates (KOTENKO, 2002).

Acupuncture treatment reduces both the number of inflammatory cells and volume of exudate in several inflammatory models, such as carrageenan injection in the air pouch of rats, turpentine in rabbit ears, carrageenan-induced paw oedema, carrageenan-induced pleurisy, Freund's adjuvant-induced rheumatoid arthritis, sepsis and chemical peritonitis in rats (SIN et al., 1983; SIN, GWEE & LOH, 1984; SIN, 1986; KENDALL, 1989; QINGLAN, 1991; CECCHERELLI et al., 1999; KWON, et al., 2001; KWON, et al., 2002; ZHANG et al., 2002; SCOGNAMILLO-SZABÓ et al., 2004; SCOGNAMILLO-SZABÓ et al., 2004). Supporting these data, clinical trials confirm the efficacy of acupuncture treatment

on allergic rhinitis, pruritic dermatosis, bronchial asthma, eczema, rheumatoid arthritis and other disorders (TOWNSEND et al., 1999; TUMACHI, 1999; CASIMIRO et al., 2002; PETTI et al., 2002; STEURER-STEYA; RUSSIB & STEURERC, 2002; BIELORY & HEIMALL, 2003; MADSEN et al., 2003; MCCARNEY et al., 2004).

Previous results demonstrated that acupuncture significantly suppressed neutrophil migration to the peritoneal cavity induced by intraperitoneal carrageenan injection in rats. It was found that the effect of acupuncture was not mediated by endogenous corticoids, as the administration of mifepristone (RU-486, an antagonist of the glucocorticoid receptor) did not interfere with it. Providing there was no difference in neutrophil migration between sham-acupuncture and acupuncture treated rats, it was clear that the anti-inflammatory effect of acupuncture was not due to the nonspecific effect of needling (SCOGNAMILLO-SZABÓ et al., 2004).

The present study investigated the effect of acupuncture treatment on cytokine concentration in the peritoneal exudates of carrageenan-induced peritonitis rats.

MATERIALS AND METHODS

Animals

Twelve male Wistar rats weighing 180-200g were used throughout the experiment. The animals were housed in cages in temperature controlled rooms and received water and food *ad libitum*. All experimental protocols were approved by the Animal Care and Use Committee of University of São Paulo in accordance with NIH guidelines on animal care.

Experimental peritonitis

Peritonitis was induced through an intraperitoneal injection of carrageenan in pyrogen-free saline (30mg mL⁻¹ cavity⁻¹). Negative control animals received 1mL i.p. of the vehicle. All experiments were performed in the mornings between 8:00 AM and 11:00 AM, in order to avoid circadian interference (CHEN & HE, 1989; STORCH et al., 2002). The inflammatory exudate was harvested 4h after carrageenan injection.

Treatments

Manual acupuncture and sham-acupuncture were performed twice: 10 minutes and two hours after inflammatory stimulus. Detailed description of treatments are shown on table 1.

Table 1 - Acupuncture and sham-acupuncture treatment protocols in rats submitted to intraperitoneal carrageenan injection.

Treatment protocols					
Acupuncture			Sham-acupuncture		
Time	Acupoint	Acupoint localization	Time	Stimulated area	Localization of the stimulated area
10 minutes	1 - Yin Tang	Glabella (between the eye-brows)	10min	1 - sham-Yin-tang	1cm laterally (left) to Yin Tang
	2 - Hou Hai	Midline between anus and tail		2 - sham-Hou Hai	1cm laterally (left) to Hou Hai
	3 - Bai Hui	Lumbo-sacral space		3 - sham-Bai Hui	1cm laterally (left) to Bai Hui
2 hours	The same as 10min	The same as 10min	2h	The same as 10min	The same as 10min

Neutrophil migration into the peritoneal cavity

Neutrophil migration was assessed 4h hours after carrageenan injection. The animals received an over-dose of sub-cutaneous anaesthetic (2.5% tribromoethanol) and the cells present in the peritoneal cavity were harvested after local administration of 10mL of phosphate-buffered saline (PBS) containing 1mM EDTA followed by a gentle belly massage. Total cell counts were performed with a cell counter (Coulter AcT series analyzer; Coulter Corp., Miami, Fla.) and differential cell counts were carried out on cytocentrifuge slides (Cytospin 3, Shandon Southern Products, Astmoore, United Kingdom) stained by the May-Grunwäld Giemsa (Rosenfeld) method. The results are expressed as the number of neutrophils per cavity.

Cytokine measurements

The concentrations of tumor necrosis factor-alpha (TNF- α), interleukin-1 β (IL-1 β), and interleukin-10 (IL-10) in the peritoneal exudates were determined by using a double-ligand enzyme-linked immunosorbent assay (ELISA). Briefly, flat-bottomed 96-well microtiter plates were coated with 100mL per well of antibody specific to one of the above cytokines at a dilution of 2mg mL⁻¹ of coating buffer and incubated overnight at 4°C. Afterwards, the plates were washed and nonspecific binding was blocked for 120min at 37 °C with 1mL calf normal serum. Nondiluted samples and standards were loaded in plates and incubated at room temperature for 4h. Recombinant rat TNF- α , IL-1 β , and IL-10 standard curves were used to calculate cytokine concentrations. The plates were thoroughly washed and the appropriated biotinylated polyclonal or monoclonal anticytokine antibodies were added. The plates were washed 1h later, avidin-peroxidase (diluted 1:5000) was added to each well for 15min, and each plate was thoroughly washed again. In the sequence, substrate (0.4mg of o-phenylenediamine [OPD] for 1mL of substrate buffer) was added, the reaction was stopped with H₂SO₄ (1M), and the optical density was

measured in an ELISA plate scanner (Spectra Max 250, Molecular Devices) at 490nm. The results were expressed as picograms of TNF- α , IL-1 β , and IL-10 per mL of supernatant, comparing the optical density in the samples with standard curves.

RESULTS

It was observed that the concentration of IL-1 β in the peritoneal exudates of carrageenan injected rats was inhibited by the acupuncture. However the concentration of TNF- α and IL-10 in the exudates was not affected by the acupuncture (Figure 1).

DISCUSSION

Controlled clinical trials claim acupuncture efficacy in the treatment of inflammatory process, e.g., arthritis (TOWNSEND et al., 1999; TUMACHI, 1999; TUKMACHI, 2000; WHO, 1996; XUE et al., 2003). However, experimental studies concerning acupuncture and the inflammatory process are rare (SIN et al., 1984; KENDALL, 1989; ZHAO & ZHU, 1990; QINGLAN, 1991; ZHAO & ZHU, 1992; MA, 1992; SCOGNAMILLO-SZABÓ et al., 2004a; SCOGNAMILLO-SZABÓ et al., 2004b; SIN et al., 1984), highlighting the importance of a better understanding of mechanisms involved on acupuncture anti-inflammatory effects.

Among experimental models, carrageenan-induced peritonitis is largely used in the study of acute inflammatory process. Although it is a good model to use, there are no references to its use in the investigation of the anti-inflammatory mechanisms of action of the acupuncture.

There are several evidences demonstrating that the cytokines and chemokines mediate the neutrophil migration into the inflammatory site (SCHEIN, 2002). On the other hand, anti-inflammatory cytokine, such as IL-10,

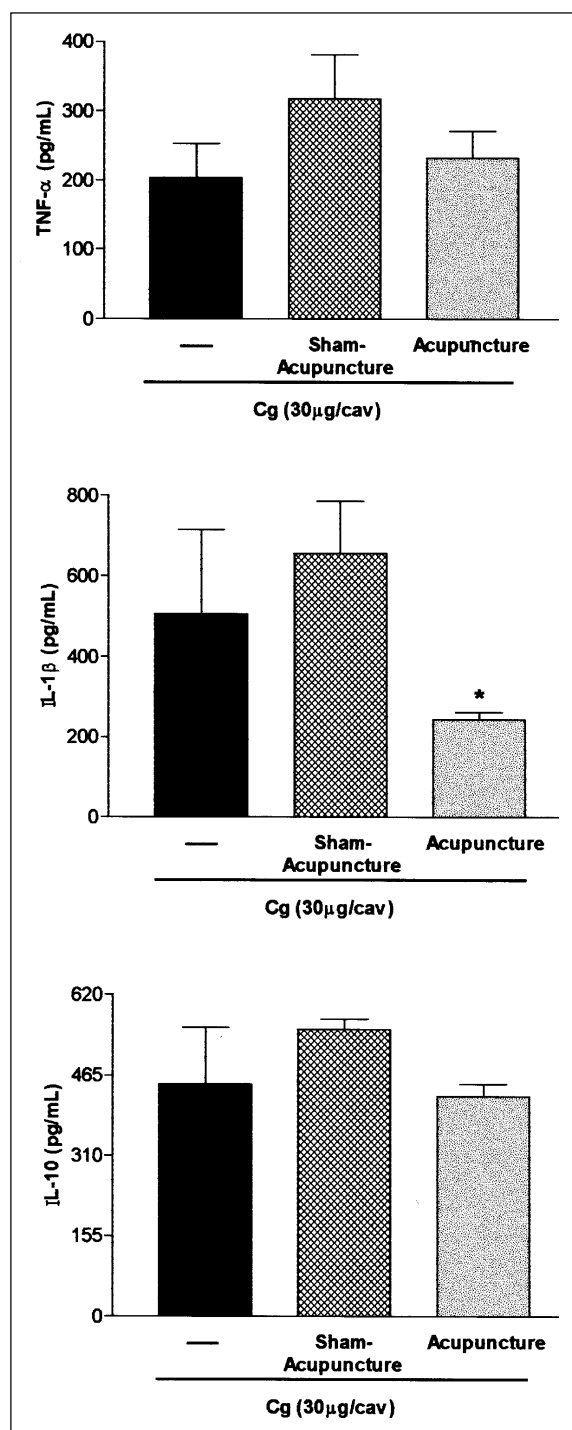


Figure 1 - Effect of acupuncture and sham-acupuncture on peritoneal exudate concentrations of TNF- α , IL-1 β and IL-10 in carrageenan (Cg - 30 μ L cavity⁻¹) injected rats. The measurements of the cytokines were realized 4h after Carrageenan injection. Results are expressed as mean \pm s.e. of cytokine concentration (n=4). *P<0.05 compared with control group (analysis of variance, followed by Bonferroni's test).

down-modulates the process (KOTENKO, 2002). In order to investigate whether the acupuncture inhibits the production of the chemotactic cytokines or increases the production of the anti-inflammatory cytokines, the effect of acupuncture on the IL-1 β , TNF- α and IL-10 concentration in the exudates of carrageenan-injected rats was investigated. IL-1 β and TNF- α mediate the neutrophil migration observed in several experimental models and also in human inflammatory diseases. On the other hand, IL-10 inhibits the neutrophil migration induced by several inflammatory stimuli and also by chemotactic mediators (LAICHALK et al., 1996; NAGANO et al., 2002).

Our results showed that the production of IL-1 β , but not TNF- α and IL-10 was partially inhibited by acupuncture treatment. Further studies need to be done to clarify whether the effect of acupuncture treatment is a consequence of the inhibition of IL-1 β production and also the possible participation of other chemotactic cytokines and/or chemokines.

In conclusion, these data show a measurable and significative interference of acupuncture on the inflammatory process. Since previous results indicate that anti-inflammatory effect of acupuncture does not involve either the release of corticoids nor the nonspecific effect of needling, it seems that the anti-inflammatory effect is due to specificity of the stimulated area: the acupoint, as charted by the ancient Chinese Medicine. The fact that the acupuncture inhibited the production of IL-1 β points out the importance of investigating the role of cytokines on the anti-inflammatory effect of acupuncture.

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