J Acupunct Meridian Stud 2009;2(2):135–146

RESEARCH ARTICLE

Effect of Zusanli (ST.36) Electroacupuncture at Two Frequencies on the Bioavailability of ^{99m}Tc-Sodium Pertechnetate and on Labeling of Blood Constituents in Rats

Vasco Senna-Fernandes[1,2,4*], Daisy L França[1,2,4], Kelly CM Santos[2], Rafael S Sousa[2], Dilson Silva[3], Célia M Cortez[3], Mario Bernardo-Filho[2], Marco AM Guimarães[1]

[1]*Pós-Graduação de Ciências Médicas (PGCM), Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil*
[2]*Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcântara Gomes (IBRAG), UERJ, Rio de Janeiro, Brazil*
[3]*Departamento de Ciências Fisiológicas, UERJ, Rio de Janeiro, Brazil*
[4]*Academia Brasileira de Arte e Ciência Oriental/Colégio Brasileiro de Acupuntura (ABACO/CBA), Rio de Janeiro, Brazil*

KEY WORDS:
biological availability;
electroacupuncture;
frequencies;
sodium pertechnetate Tc 99m;
Zusanli-point (ST.36)

Abstract
Objectives: A study was performed on the effects of stimulation at Zusanli-point (ST.36) by electroacupuncture (EA) at two frequencies on the bioavailability of ^{99m}Tc-sodium pertechnetate ($Na^{99m}TcO_4$) in rats.
Methods: Forty Wistar rats were divided into four groups: untreated control, treated by manual acupuncture at ST.36 bilaterally, treated by EA at 2 Hz at ST.36 bilaterally, and the same site at 100 Hz bilaterally. $Na^{99m}TcO_4$ (7.4 MBq) was administrated via the ocular-plexus and, 20 minutes before sacrifice, blood was withdrawn for radiolabeling assay (BRL). In the bioavailability analysis, organs and tissues were isolated, their radioactivity determined, and the percentage of injected dose per gram of organ or tissue (%ID/g) and the %ID were calculated for each organ or tissue (%ID/ot). For BRL, the plasma and blood cells isolated, and the fractions also precipitated with 5% trichloroacetic acid to separate the soluble and insoluble fractions; these were assessed as percentage of injected dose (%ID) in blood (%ID/b).
Results: The results showed significant differences in the %ID/g in some organs and tissues in comparison with controls; lung ($p=0.0013$), spleen ($p=0.0085$), pancreas ($p=0.0167$), liver ($p=0.0003$), stomach ($p<0.0001$), small-intestine ($p=0.0181$), large-intestine ($p=0.04099$), urinary-bladder ($p=0.0271$), thyroid ($p<0.0001$), muscle ($p=0.0187$); %ID/ot in spleen ($p=0.0349$); and %ID/b in blood sample ($p=0.0235$). In the blood labeling analyses, EA in either frequency significantly increased insoluble fraction/blood cells ($p<0.0001$).

*Corresponding author. Pós-Graduação de Ciências Médicas (PGCM) — Universidade do Estado do Rio de Janeiro (UERJ); Av. 28 de setembro, 87, CEP: 20551-030, Rio de Janeiro, RJ, Brazil.
E-mail: vascosf888@yahoo.com.br

Conclusions: These findings suggested that acupuncture procedures at ST.36 could modulate responses in some organs, tissues, and blood in rats. Further rigorous experimental studies to examine the effectiveness in either acupuncture therapy need to be pursued.

1. Introduction

Electroacupuncture (EA) is a therapy in which the principles of electrical stimulation are integrated with traditional concepts of acupuncture. There are two electrical frequencies commonly used for EA research in acupuncture analgesia [1]; high (100 Hz) and low frequency (2 Hz). According to Chen et al (1996), the antinociception induced by 2 Hz electrical stimulation is mediated by both mu and delta opioid receptors and the antinociception by 100 Hz electrical stimulation is mediated primarily by kappa receptors [2]. However, other investigations of EA effects at different frequencies deal with non-pain control studies involving the autonomic nervous system (ANS), neuroimmune system, and hormonal regulation [3].

In rat experimental models, *Zusanli* point (ST.36) is of the most used acupuncture points (acupoints) in the investigation of several neurophysiological mechanisms of EA effects [4]. Comparative studies of EA where ST.36 has undergone electrostimulation at 2 and 100 Hz have suggested many kinds of effects. Gao et al (2000) suggested that EA at 2 Hz (2Hz-EA, low frequency) could lead to more effects on the regulation of internal organ systems as a network compared with EA at 100 Hz (100Hz-EA, high frequency) [5]. Chou et al (2003) demonstrated that EA effects on gastric myoelectrical activity were related to low instead of high frequency [6]. Kim et al (2005) observed that low frequency EA was more suitable for the treatment of cold allodynia than high frequency EA [7]. Tseng et al (2005) showed that EA in either frequency could induce a decrease in glucose, an increase in lactate metabolites, and a reduction in lactate/glucose ratios which lead to an increase in cellular anaerobic glucose metabolism [8]. Tian et al (2005) suggested that EA at both frequencies could induce a therapeutic effect on obesity by the stimulation of proopiomelanocortin neurons in the arcuate nucleus (ARC) of the hypothalamus to release alpha-MSH, which inhibits food intake, resulting in a decrease of rat body weights [9].

According to a previous study, a well stabilized experimental method for examining the bioavailability of the radiopharmaceutical (radiobiocomplex) sodium-pertechnetate ($Na^{99m}TcO_4$) was used as a means of investigating the effect of acupuncture in rats [4]. When some organs and tissues, directly or indirectly related to the *Zang-Fu* theory of traditional Chinese medicine (TCM), were stimulated by manual acupuncture (MA) at ST.36, they showed significantly increased uptake radioactivity compared with the control group. On the other hand, there are studies which suggest that acupuncture stimuli can induce changes in blood constituents [10–11]. De Vernejoul et al (1992) suggested that acupuncture stimuli at a specific acupoint has the ability to induce constant and reproducible changes in cellular physiology involving alterations in the membrane potential of erythrocytes and granulocytes [10]. Ye Xi et al (1992) suggested that ST.36 could enhance the membrane fluidity of red cells in rabbits, observed using a fluorescence polarization method [11].

This study was designed to compare the EA effects of different EA frequencies (2 and 100 Hz) on the bioavailability of $Na^{99m}TcO_4$ and on radiolabeling of blood elements in Wistar rats after stimulating ST.36.

2. Methods

2.1. Animals

The following study was performed according to the guidelines on the use of living animals in scientific investigations. All experiments were approved by the Ethical Committee of the Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro. Forty healthy albino male Wistar rats, weighing 250–350 g, aged 4–5 months were randomly chosen and divided into four groups ($n=10$): G1, the control group; G2, treated by MA at ST.36; G3, treated with 2Hz-EA at ST.36; and G4, treated with 100Hz-EA at ST.36.

2.2. Experimental design

The rats were anesthetized intraperitoneally (i.p.) with sodium thiopental 6.7% (Thiopentax®) at a dose of 60 mg/kg body weight/i.p. The anatomical location technique of ST.36 in rats was based on a previous study [4]. Rats were placed in a ventral decubitus position without immobilization in order to avoid animal stress and, thus prevent interference of emotional factors [5].