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

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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 ($Na^{99m}$TcO₄) 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. $Na^{99m}$TcO₄ (7.4 MBq) was administrated via the ocular-plexus and, 20 minutes before sacrifice, blood was withdrawn for radiolabeling assay (BRL).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 ($p<0.0001$).

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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 250–350g, 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].

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.
The animals from G1 were free from needle insertions, while in G2 the rats were treated by a 5 mm guide-tube puncture at ST.36 bilaterally with a stainless needle measuring 0.25 × 20 mm (Sterile Acupuncture Needles — Wujiang Shenli Medical & Health Material Co., Ltd, China) but without any additional manipulation. In G3 and G4 the animals were treated in same manner as G2 but with associated electrostimulation.

2.3. Electroacupuncture procedure

In G3 and G4, the body of the needles, inserted at ST.36 from both sides of the rat, were attached to an electrostimulator (Sikuro DS100C, Rio de Janeiro, RJ, Brazil) equipped with small electrodes. The electrostimulator apparatus delivered an asymmetric biphasic waveform with the first pulse being a positive square wave of 0.7 millisecond duration followed by a second pulse with a negative sharp exponential wave with a duration of 6 milliseconds. The electrostimulation duration was 20 minutes in continuous mode with an intensity regulated between 2−4 V, measured by an independent oscilloscope (LG Precision OS-5020, 20 MHz Analog Oscilloscope, Korea). In G3, the frequency was set at 2 Hz, while in G4 it was 100 Hz [12].

2.4. Bioavailability method

Ten minutes after needle insertion, the rats received am 0.3 mL dose of radiobiocomplex Na\(^{99m}\)TcO\(_4\) (7.4 MBq) recently eluted from a molybdenum-99/technetium-99m column-type generator with a 0.9% sodium chloride solution (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil) via the ocular plexus. The electrodes and needles were withdrawn 20 minutes later, and the rats rapidly killed by cervical dislocation [4]. The desired organs and tissues were identified, removed, and isolated according to the Zang-Fu Organs Theory [4]; the heart, kidney, liver, lung, and pancreas were included in the Zang organs category and the stomach, small intestine, large intestine, and urinary bladder included in the Fu organs category. The brain, bone, and testis were included in the Extra-Fu organs category, while some tissues were collected which were not directly related to Zang-Fu organs: the thyroid, muscle, and blood. These samples were weighed by analytic balance (Bioprecisa, Electronic Balance, FAZ104N, São Paulo, Brazil) and the radioactivity assessed by a gamma-counter NaI (TI) (Cobra Auto-gamma, Packard Instrument Co.; Downers Grove, Illinois, USA). The radioactivity uptake was expressed as the percentage of the injected dose per gram of organ or tissue (%ID/g) and the percentage of the injected dose in the total organ or tissue mass weight (%ID/ot) (Figure 1). Feces and urinary excretions were excluded. The %ID/g was calculated by dividing the %ID/ot in each organ or tissue by the mass of each organ or tissue.

2.5. Distribution of the radiobiocomplex in blood constituents

Before killing an animal, a 2 mL blood sample was withdrawn into a heparinized tube (0.2 mL of Heparin\(^\circledast\)) via cardiac puncture between the fifth and sixth intercostal space of the rat’s left hemithorax. One mL of blood was centrifuged (clinical centrifuge, Bio-Eng 4000—Indústria e Comércio Ltd., São Paulo, Brazil) at room temperature for 5 minutes and the plasma (P) and blood cells (BC) isolated. Twenty \(\mu\)L samples of P and BC were precipitated with 5% trichloroacetic acid and the soluble (S) and insoluble (I) fractions (F) of each were isolated by centrifugation. This procedure was used to assess the ability of the acupuncture stimuli to induce fixation of Na\(^{99m}\)TcO\(_4\) on blood constituents without using stannous chloride [13]. The percentages of injected dose in blood (%ID/b) in the P, BC, IF-P, IF-BC, SF-P, and SF-BC fractions were determined together with the organ and tissue samples (Figure 1).

2.6. Statistical analysis

Statistical analyses involved one-way ANOVA, followed by the Tukey-Kramer Multiple Comparisons Test (TKMCT), with the significance level set at \(p < 0.05\). The GraphPad InStat software, version 3.01, and GraphPad Prism, version 3.00 (GraphPad Software Inc, San Diego Ca, USA), were used to perform these analyses.

3. Results

In bioavailability studies of Na\(^{99m}\)TcO\(_4\), the statistical analyses, carried out by one-way ANOVA followed by TKMCT, showed significant differences among the following groups: (a) in the %ID/g of some organs and tissues; the 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 (\(p = 0.0409\)), urinary bladder (\(p = 0.0271\)), thyroid (\(p < 0.0001\)), muscle (\(p = 0.0187\)) (Tables 1–3); (b) in the %ID/ot of the spleen (\(p = 0.0349\), Table 1); and (c) in the %ID/b of the blood sample (\(p = 0.0235\), Table 4) were also significant (Figures 2 and 3).

Stimulating ST.36 with 2Hz-EA increased radioactivity uptake in spleen (0.67 ± 0.33 to 0.99 ± 0.24% ID/g), liver (0.72 ± 0.29 to 1.23 ± 0.23% ID/g) (Table 1); stomach (3.49 ± 1.43 to 4.23 ± 0.79% ID/g),
small-intestine (0.91±0.37 to 1.32±0.68% ID/g) (Table 2); and thyroid (5.20±1.25 to 7.72±2.08% ID/g) compared with the control at the level of significance p<0.05 (Table 4).

Stimulating ST.36 with 100Hz-EA increased the %ID/g radioactivity uptake in stomach (3.49±1.43 to 6.59±2.12 %ID/g) and urinary-bladder (1.50±0.74 to 2.59±0.94 %ID/g) (Table 2), and thyroid (5.20±1.25 to 8.84±1.01 %ID/g) compared with the control (Table 4). 100Hz-EA appeared to induce more radioactivity uptake than MA in thyroid and more than 2Hz-EA in stomach (Tables 2 and 4) at the level of significance p<0.05.

Table 5 and Figure 4 show the fixation of Na99mTcO4 on blood constituents. EA at low and high frequencies induced significant increases (p<0.0001) of radioactivity on IF-BC (34.57±7.29 to 70.43±3.17 and 34.57±7.29 to 62.22±10.98, respectively) compared with the control.

4. Discussion

The bioavailability of Na99mTcO4 can be changed in many circumstances by intrinsic factors; blood flow, capillary permeability, membrane transport, intracellular interactions, biotransformation, and others. Extrinsic factors include radiobiocomplex quality, a patient’s health status, disease states, drugs (natural or synthetic), dietary factors, invasive medical procedures, and complementary therapies such as acupuncture [4,20]. In the scientific
perspective, acupuncture's effects are considered nociceptive stimuli, which may interfere in the bioavailability of radiobiocomplexes by changing or influencing intrinsic factors [14,15]. Acupuncture may: (a) induce the aseptic inflammatory process through microtrauma by the stainless steel needle at a low electrical resistance cutaneous point [16–21], (b) increase blood circulation [22] and

<table>
<thead>
<tr>
<th>Zang organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.88±0.18</td>
<td>0.94±0.22</td>
<td>0.92±0.26</td>
<td>0.93±0.15</td>
<td>ns</td>
</tr>
<tr>
<td>Lung</td>
<td>1.20±0.30</td>
<td>1.79±0.37*</td>
<td>1.47±0.33</td>
<td>1.41±0.27</td>
<td>0.0013</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.67±0.33</td>
<td>1.01±0.19*</td>
<td>0.99±0.24*</td>
<td>0.90±0.23</td>
<td>0.0085</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.85±0.28</td>
<td>1.19±0.18*</td>
<td>1.04±0.22</td>
<td>0.92±0.29</td>
<td>0.0167</td>
</tr>
<tr>
<td>Liver</td>
<td>0.72±0.29</td>
<td>1.25±0.33*</td>
<td>1.23±0.23*</td>
<td>1.03±0.28</td>
<td>0.0003</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.24±0.28</td>
<td>1.31±0.33</td>
<td>1.27±0.36</td>
<td>1.36±0.28</td>
<td>ns</td>
</tr>
</tbody>
</table>

*The treated group (G2, G3 or G4) had significant increased (p<0.05) compared to the control group (G1). MA = manual acupuncture; EA = electro-acupuncture; %ID/g = percentage of injected dose per gram organ (tissue); %ID/ot = percentage of injected dose of total organ/tissue mass weight; TKMCT = Turkey-Kramer Multiple Comparisons Test.

<table>
<thead>
<tr>
<th>Fu organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>3.49±1.43</td>
<td>6.28±1.72*</td>
<td>4.23±0.79*</td>
<td>6.59±2.12†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.91±0.37</td>
<td>1.13±0.35</td>
<td>1.32±0.68*</td>
<td>1.12±0.54</td>
<td>0.0181</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.59±0.21</td>
<td>0.74±0.21*</td>
<td>0.70±0.24</td>
<td>0.73±0.24</td>
<td>0.0409</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>1.50±0.74</td>
<td>2.39±1.04</td>
<td>2.01±0.54</td>
<td>2.59±0.94*</td>
<td>0.0271</td>
</tr>
</tbody>
</table>

*The treated group (G1, G2 or G3) had significant increased (p<0.05) compared to the control group (G4); †the differences between 2Hz-EA (G2) and 100Hz-EA (G3) were significant (p<0.05). MA = manual acupuncture; EA = electro-acupuncture; %ID/g = percentage of injected dose per gram organ (tissue); %ID/ot = percentage of injected dose of total organ/tissue mass weight; TKMCT = Turkey-Kramer Multiple Comparisons Test.
microvascular permeability [23], (c) enhance intracellular Ca2+ [24] and ATP production [25], (d) influence tissue oxygenation and metabolites exchange [25], and (e) have anti-inflammatory actions [26], amongst other effects.

Li et al (2007) suggested that EA may modulate organ functions by inducing activation of the somatovisceral reflexes (SVR) and change of ANS [15]. Acupoints are considered as somatic afferent pathways and to be located at skin sites with low electric resistance [16], which are closely related to the cutaneous, muscle spindle and Golgi receptors [17]. The organs and tissues are considered as efferent pathways and the central nervous system (CNS) as the neuromodulation center that controls the main decisions of the ANS activities through SVR [18,19].

Therefore, in accordance with a previous study [4], the use of a Na99mTcO4 bioavailability method to study the actions of acupuncture may help us to understand better the acupoints’ potential actions in different organs or tissues. However, from the results of radiographic images, acupuncture may also cause changes since it can interfere with

Table 3  Bioavailability of Na99mTcO4 of extra organs after stimulating ST.36 in rats

<table>
<thead>
<tr>
<th>Extra organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.09±0.06</td>
<td>0.11±0.06</td>
<td>0.09±0.01</td>
<td>0.10±0.00</td>
</tr>
<tr>
<td>Testis</td>
<td>0.35±0.10</td>
<td>0.37±0.08</td>
<td>0.35±0.07</td>
<td>0.40±0.10</td>
</tr>
<tr>
<td>Bone</td>
<td>0.44±0.16</td>
<td>0.50±0.14</td>
<td>0.49±0.11</td>
<td>0.55±0.14</td>
</tr>
</tbody>
</table>

ANOVA (mean±SD)

<table>
<thead>
<tr>
<th>Extra organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.11±0.04</td>
<td>0.14±0.04</td>
<td>0.16±0.06</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>Testis</td>
<td>0.44±0.11</td>
<td>0.50±0.17</td>
<td>0.48±0.11</td>
<td>0.48±0.16</td>
</tr>
<tr>
<td>Bone</td>
<td>0.25±0.11</td>
<td>0.35±0.31</td>
<td>0.27±0.11</td>
<td>0.30±0.09</td>
</tr>
</tbody>
</table>

ANOVA p-value

MA = manual acupuncture; EA = electro-acupuncture; %ID/g = percentage of injected dose per gram organ (tissue); %ID/ot = percentage of injected dose of total organ/tissue mass weight; TKMCT = Turkey-Kramer Multiple Comparisons Test.

Table 4  Bio-availability of Na99mTcO4 of other organs after stimulating ST.36 in rats

<table>
<thead>
<tr>
<th>Other organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>5.20±1.25</td>
<td>6.38±1.32</td>
<td>7.72±2.08*</td>
<td>8.84±1.01*†</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.23±0.07</td>
<td>0.30±0.07*</td>
<td>0.24±0.06</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>Blood</td>
<td>5.14±1.28</td>
<td>5.63±1.49</td>
<td>5.63±1.49</td>
<td>5.25±1.27</td>
</tr>
</tbody>
</table>

ANOVA p-value

<table>
<thead>
<tr>
<th>Other organs</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>0.71±0.42</td>
<td>1.11±0.74</td>
<td>1.17±0.54</td>
<td>1.46±0.81</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.12±0.06</td>
<td>0.15±0.07</td>
<td>0.16±0.05</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>Blood</td>
<td>2.57±0.64</td>
<td>3.66±1.05*</td>
<td>2.81±0.74</td>
<td>2.62±0.63</td>
</tr>
</tbody>
</table>

ANOVA p-value

*The treated group (G2, G3 or G4) had significant increased (p<0.05) compared to the control group (G1). †the differences between MA (G1) and EA group (G2 or G3) were significant (p<0.05). MA = manual acupuncture; EA = electro-acupuncture; %ID/g = percentage of injected dose per gram organ (tissue); %ID/ot = percentage of injected dose of total organ/tissue mass weight; TKMCT = Turkey-Kramer Multiple Comparisons Test.
Effect of Zusanli (ST.36) electroacupuncture on radiobiocomplex uptake in various organs and tissues by changing cell activities [4].

4.1. Effect of EA at ST.36 on the endocrine regulation and on the thyroid uptake

In organ intracellular interactions, a high level of radioactivity uptake of $^{99m}$TcO$_4^-$ is usually found in the thyroid gland. Various mechanisms may be involved, including the active membrane transport of the Na$^+$/K$^+$ ATPase system and a mechanical entrapment of $^{99m}$TcO$_4^-$ into the thyrocyte, instead of iodine (I$^-$) as both have a similar tetrahedral shape [27]. In our study, the ability of the thyroid gland to uptake $^{99m}$TcO$_4^-$ was significantly enhanced when rats were stimulated by EA at either frequency, compared with the control ($p<0.0001$) (Table 3). In particular, the uptake values of 100 Hz-EA were superior to MA ($p<0.05$). Gao et al (2000) and Lai et al (2008) hypothesized that EA at ST.36 may result in some regulatory effects on the nervous, endocrine, and immune systems [5, 14]. According to Liu et al (2005), EA effects may be involved in the process of iodine binding into the thyrocyte [28]. Several studies have pointed out that acupuncture could improve some signs and symptoms of Graves’ disease, endogenous depression, thyroiditis, and others [29–31]. However, as the present results suggest that acupuncture
therapy itself may have interfered with the bioavailability of radiobiocomplexes, this may lead to misdiagnoses from such evaluation tools as scintigraphic images. Otsuka et al (1997) reported that a patient with thyroid carcinoma under acupuncture therapy had abnormal accumulation of 131I in the regions of both hands and feet in whole body scanning and that the sites of abnormal accumulation of 131I were similar to those observed with bone scintigraphy [32].

4.2. Effect of EA at ST.36 on the neuromodulation response and on the gastrointestinal uptake

In the gastrointestinal tract, 99mTcO4− is actively concentrated mainly in the gastric mucosa (including ectopic) and secondarily in different parts of the intestines. The secretion of 99mTcO4− in gastric mucosa is by mucoid (not chief or parietal) cells and then reabsorbed and recycled into the circulatory system from the stomach and upper intestine [27]. Some brain peptides can change the uptake of 99mTcO4− by the gastrointestinal mucosa [33]. Various authors have suggested that EA could mediate some brain-gut peptides responses in the gastric mucosa, including gastrin, motilin, somatostatin, and beta-endorphin [34−36], which play important roles in gastric acid secretion. EA may protect the gastric mucosa of stressed rats against gastric acid secretion by enhancing gastric mucosal barrier, stabilizing gastric mast cells and inhibiting gastrin levels in the gastric mucous [37]. Zhou et al (1984) suggested that the EA effect on gastric secretion involves a somatic afferent-visceral reflex mechanism [48]. Moreover, other authors have also pointed out that EA at ST.36 may modulate the vagal and splanchnic nerve responses on the activity of the gastric acid secretion, gastric motility, and emptying from the

### Table 5  Effect of the Zusanli (ST.36) point electro-acupuncture and manual acupuncture on the blood radiolabeling of Na99mTcO4 in rats

<table>
<thead>
<tr>
<th>Constituents</th>
<th>G1 (Control)</th>
<th>G2 (MA)</th>
<th>G3 (EA-2Hz)</th>
<th>G4 (EA-100Hz)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>59.34±6.47</td>
<td>58.45±7.89</td>
<td>60.55±2.72</td>
<td>59.98±6.92</td>
<td>ns</td>
</tr>
<tr>
<td>BC</td>
<td>40.66±6.47</td>
<td>41.55±7.89</td>
<td>39.45±2.72</td>
<td>40.02±6.92</td>
<td>ns</td>
</tr>
<tr>
<td>IF-P</td>
<td>5.78±2.39</td>
<td>10.63±3.03</td>
<td>8.21±3.01</td>
<td>7.42±0.33</td>
<td>ns</td>
</tr>
<tr>
<td>SF-P</td>
<td>94.22±2.39</td>
<td>89.37±3.03</td>
<td>91.79±3.01</td>
<td>92.58±0.33</td>
<td>ns</td>
</tr>
<tr>
<td>IF-BC</td>
<td>34.57±7.29</td>
<td>25.22±5.34</td>
<td>70.43±3.17*</td>
<td>62.22±10.98*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SF-BC</td>
<td>65.43±7.29</td>
<td>74.78±5.34</td>
<td>29.57±3.17*</td>
<td>37.78±10.98*</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The uptake of Na99mTcO4 in the blood constituents after stimulating ST.36 with different acupuncture therapies showed that insoluble and soluble fractions of the red blood cells were significant (p<0.0001) among the groups by one way ANOVA followed by Tukey-Kramer Multiple Comparisons Test. %ID/b = Percentage of Injected Dose of blood.

**Figure 4** Blood radiolabeling of Na99mTcO4 in rats.
somato-parasympathetic and somato-sympathetic stimuli, respectively [15,39–41]. These ST.36 acupuncture effects may be correlated to our data which suggested that 100Hz-EA could increase the uptake of $^{99m}$TcO$_4^-$ in the stomach, while 2Hz-EA was similarly effective in the small intestine, and no differences were found in the large intestine with either EA, at the $p<0.05$ level of significance.

### 4.3. Effect of EA at ST.36 on immunomodulation action and on spleen uptake

In immunomodulation response, EA stimulation of the ST.36 is cited by many studies to enhance splenic natural killer (NK) cells and cytokine activities, decreasing splenic lymphocytes apoptosis via inhibiting Fas protein expression and increasing the induction of interleukin-2 production of spleen lymphocytes; consequently preventing deleterious immunological changes and improving the immunosuppression after operative trauma stress in rats. As acupuncture may play an important role in affecting the CNS, the anterior and lateral hypothalamic areas, in particular, are capable of modulating the cytoplastic activity of adherent splenic NK cells [42–45]. In this study, the significant increased uptake in spleen induced by 2Hz-EA, compared with the control ($p<0.05$), may be correlated to the potential immune-modulation action of the ST.36 point [14].

### 4.4. Effect of EA at ST.36 on the elimination process and on the urinary bladder

As the hydrophilic radiobiocomplex, $^{99m}$TcO$_4^-$, is pharmacokinetically eliminated from the kidney by passive glomerular filtration, with more than 80% reabsorbed in the proximal tubules, only 30% of injected doses are excreted in urine in 24 hours [31]. Ben and Zhu (1995) have pointed out that EA can play a role in regulation of the functional state of the urinary bladder, changing the pressure-volume of the bladder, controlled by the wall of the bladder itself, and Ben and Zhu (1995) have pointed out that EA may play a role in regulation of the functional state of the urinary bladder by changing the pressure-volume of this organ, and the control of the parasympathetic nerve [46]. Tanaka et al (2002) suggested that acupuncture stimulation affects both the bladder activity and the sleep-arousal system [47]. Our data showed that 100Hz-EA significantly increased the uptake of $^{99m}$TcO$_4^-$ in the urinary bladder ($p<0.05$). The results from the study may correlate with the potential action of ST.36 stimulated by 100Hz-EA in different conditions of the urinary bladder suggested by those authors.

### 4.5. Effect of EA at ST.36 on the hepatic metabolism and on the liver

The present study showed that 2Hz-EA induced significant increases in the uptake of $^{99m}$TcO$_4^-$ in the liver ($p<0.05$), an effect which may be correlated to its therapeutic potentials, as observed in animal models and clinical trials. According to Huang et al (1995), ST.36 might play a certain role in improving hepatic metabolism and promoting membrane transport [48]. While Shimoju-Kobayashi et al (2004) showed that EA stimulation to a hindlimb point in rats can increase hepatic glucose output. This action may be promoted by acupuncture effects on activation of the somatic afferents and sympathetic efferent pathways, involving adrenal medulla [49]. However, hepatic glucose output also can be reduced simultaneously by parasympathetic nerves responses as a feedback mechanism.

### 4.6. Effect of EA at ST.36 on labeling of blood constituents

In general, the intracellular processing of Na$^{99m}$TcO$_4^-$ occurs in the BC where $^{99m}$TcO$_4^-$ is reduced by the stannous chloride (SnCl$_2$) and reacts with hemoglobin to form $^{99m}$Tc-hemoglobin via the $\beta$-chain of the globin fraction [29]. Callahan and Rabito (1990) stated that the transport of $^{99m}$TcO$_4^-$ into the RBC depends on the band-3 protein located in the cell membrane [50]. In the present study, we were able to observe that EA in either frequency had the ability to induce radiolabeling of $^{99m}$TcO$_4^-$ on the blood components without using stannous chloride. The unexpected changes in the bioavailability of the Na$^{99m}$TcO$_4^-$ in IF-BC and SF-BC (Table 5 and Figure 4) might suggest that EA in either frequency interfered with the entrance of $^{99m}$TcO$_4^-$ through the BC membrane. It is also possible to speculate that EA may have had the potential to modify the oxidation state of the blood cells. According to Li et al (2005) [51], acupuncture is effective in antagonizing oxygen stress and not only has a chain-blocking effect, but also the preventive and repairing effects of antioxidation against free radicals and other oxidation mechanisms that participate in the pathological process of multiple diseases in organisms.

### 4.7. General discussion

In this experimental model, the authors did not include the ‘sham acupuncture’ group as an ‘inactive’ control, since needling non-acupoints (any cutaneous location of the body surface) could produce unpredictable somatovisceral reflex responses, and therefore could complicate the interpretation of the results [52].
Thiopental may affect a variety of processes in the cerebral cortex, including oxygen consumption by the mitochondria, and the interrelation between mitochondrial function and anesthetics may cause a significant, dose-dependent decrease in blood flow and a significant decrease in extracellular levels of potassium, with no significant changes in NADH levels in normoxic and ischemic rats [53].

Cervical dislocation is an acceptable method for euthanasia as it causes minimal distress and is quick, safe, efficient, economic, and easy to perform [54]. The injection of NaTcO₄ via ocular plexus is a frequently used procedure in studies of the bioavailability of radiocomplexes [20,52]. These procedures were chosen for the experimental model since there are no studies indicating that they could cause gross histological or histochemical changes that would adversely affect experimental results.

Although the effect of acupuncture on the bioavailability of radiobiocomplex is not yet fully understood, this experimental model demonstrated that different types of acupuncture stimuli on the same acupoint (ST.36) in rats could produce different results in terms of the uptake of the radioactive pertechnetate anion, ⁹⁹mTcO₄⁻, (described as %ID/g and %ID/ot) in certain organs and tissues (lung, spleen, pancreas, liver, stomach, small-intestine, large-intestine, urinary-bladder, thyroid, muscle, and blood), but not others (heart, brain, kidney, testis, and bone). Under specific conditions, the radiobiocomplex itself may be responsible for the bioavailability differences in a specific rat organ. For instance, in this study significant changes of radioactivity uptake were not observed in brain among the groups. It is known that the blood-brain barrier, under normal conditions, hinders ⁹⁹mTcO₄⁻ entering into brain cells but, under certain circumstances, it can be altered. Therefore, Na⁹⁹mTcO₄ is frequently used to study brain tumors as the protective function of the blood-brain barrier may be damaged or there is still a minimal amount of radioactivity of Na⁹⁹mTcO₄ which can be detected from the brain circulation [55].

On the other hand, we observed in this study that significant effect was often lost when %ID/ot measures were used instead per gram, %ID/g. This difference may have been related to the calculation of %ID/ot in which the radioactivity uptake of a given organ or tissue was measured directly from its total mass weight which may have varied in weight and dimension among the organs or tissues samples and animals of the same group. However, %ID/g appeared to be a more refined parameter, because it could be used to examine the radioactivity uptake per gram in comparisons of the weight of organ or tissue samples among the groups [27].

The bioavailability of radiobiocomplexes in rat organs and tissues after stimulating the acupoints used in this study demonstrated that it could be a valuable and promising method for correlating radioactivity uptake abilities with the potential actions of acupoints stimulated by the different types of acupuncture therapy that have already been described in several studies in TCM, animal experiments, and clinical trials.

In the present study, ST.36 under stimulation in rats by EA or MA showed that EA may have potential therapeutic actions on some types of organs and tissues, which are thought to be useful not only in complementary-alternative therapies, but also in the perspective of Western medicine. However, further rigorous experimental studies to examine their effectiveness in other acupuncture therapies need to be performed.

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References


