Laser stimulation of the acupoint ‘Zusanli’ (ST.36) on the radiopharmaceutical biodistribution in Wistar rats

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Laser used to stimulate acupoints is called laser acupuncture (LA). It is generally believed that similar clinical responses to manual acupuncture can be achieved. Here we analysed the effects of the laser (904 nm) at the ‘Zusanli’ acupoint (ST.36) of the stomach meridian on the biodistribution of the radiopharmaceutical Na\textsuperscript{99m}TcO\textsubscript{4}. Wistar rats were divided into control (CG) and experimental groups (EG). The EG were exposed daily to the laser (904 nm) at ST.36 with 1 joule/min (40 mW/cm\textsuperscript{2}) for 1 min. The animals of the CG were not exposed to laser at all. On the 8th day after LA, the animals were sedated and Na\textsuperscript{99m}TcO\textsubscript{4} was administered. After 10 min, the animals were all sacrificed and the organs removed. The radioactivity was counted in each organ to calculate the percentage of radioactivity of the injected dose per gram (%ATI/g). Comparison of the %ATI/g in EG and CG was performed by Mann-Whitney test. The %ATI/g was significantly increased in the thyroid due to the stimulation of the ST.36 by laser. It is possible to conclude that the stimulation of ST.36 does lead to biological phenomena that interfere with the metabolism of the thyroid.

1. Introduction

Laser, an acronym for ‘light amplification by stimulated emission of radiation’, is a form of electromagnetic radiation. The main physical properties are its (i) monochromacity, (ii) polarization, and (iii) high coherence (Basford 1993; Litscher and Opitz 2012; Round et al. 2013). Low-intensity-level laser (LLL) has potency ranging from 1 to 100 mW and has been used for therapeutic purposes without major damage of the tissues (Niemz 2007). Laser with low potency is between 600 and 1300 nm (Karu 1999). Its effects are considered to be associated with biostimulation or biomodulation of the synthesis of DNA, RNA and proteins, as well as the release of anti-inflammatory factors (Karu 2013). Kim (2002) has reported that LLL therapy at low energy has been utilized to treat some diseases due to the biostimulative effect of this electromagnetic radiation.

LLL has also been effective to wound healing (Basford 1993; Litscher and Opitz 2012; Round et al. 2013) and to treat several clinical conditions, such as (a) musculoskeletal

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pain (Chow et al. 2009), (b) rheumatoid arthritis (Ekim et al. 2007), and (c) facial swelling (Aras and Gängörmüş 2009). Carroll et al. (2014) have reported that the potential benefits of LLL therapy have been demonstrated in many healthcare fields including improved healing, reduced inflammation, and pain control. These results suggest its considerable potential in oral tissues. Besides the use in the general clinical procedures, laser has been used to stimulate the acupoints (acupuncture points) such as laser acupuncture (LA), which was discussed in line with the concepts of Traditional Chinese Medicine (TCM) (Whittaker 2004; Litscher and Opitz 2012; Round et al. 2013). In LA, the wavelengths of the laser used are usually between 405 nm and 904 nm (Anderson and Parrish 1981; Litscher 2012; Litscher and Opitz 2012). LA has been used to treat various clinical conditions, such as osteoarthritis (Yurtkuran et al. 2007; Shen et al. 2009), chronic low back pain (Glazov 2010; Glazov et al. 2014), burning mouth syndrome (Brailo et al. 2013), childhood bronchial asthma (Elseify et al. 2013), temporomandibular dysfunction (Ferreira et al. 2013), depression (Quah-Smith et al. 2013), monosymptomatic nocturnal enuresis on bladder reservoir function and nocturnal urine output (Radvanska et al. 2011), menopausal symptoms (Beyazit et al. 2010), myofascial pain of the masticatory muscles (Katsoulis et al. 2010), blood pressure and body weight (Zhang et al. 2008). The benefit of LA is that this technique is not invasive and is suitable for patients who have fear of needles. In addition, children can be treated with LA. Moreover, there are low risks of local bleeding and infections such as perichondritis and chondritis (Baxter et al. 2008). Furthermore, in general, LA is performed in less time than acupuncture with needles (Rubach 2001).

The understanding of TCM has also increased because of the development of different experimental models (Senna-Fernandes et al. 2009; Cheng et al. 2014; da Silva et al. 2015). Some of them use radionuclide, such as technetium-99m ($^{99m}$Tc) and sodium pertechnetate (Na$_2^{99m}$TeO$_4$), which are the most widely used radionuclides to label radiopharmaceuticals used in Single Photon Emission Computed Tomography (SPECT) examinations (Senna-Fernandes et al. 2009; Saha 2010). Besides the clinical approaches (Saha 2010), $^{99m}$Tc-radiopharmaceuticals have also been used in studies to evaluate the effects of drugs (Vallabhajosula et al. 2010), physical agents (Meyer et al. 2013), plant extract (De et al. 2009) and stimulation of acupoints (Senna-Fernandes et al. 2009).

Na$_2^{99m}$TeO$_4$, as a radiopharmaceutical, when it is administered in a subject, is distributed through the vascular and interstitial fluids and generally shows preferential uptake in the thyroid, stomach, intestinal tract, and salivary glands (Saha 2010). Besides the diseases, factors such as drug therapy (synthetic and natural products), radiation therapy, surgical procedures, diet conditions and diseases can affect the biodistribution of different radiopharmaceuticals. The alteration on the expected bioavailability of the radiopharmaceutical is associated with changes in the local metabolism due to some factor (Bernardo-Filho et al. 2005; Vallabhajosula et al. 2010). Following the recent concept of the meridian system, i.e. the neurovascular bundle and its smaller branches, it is interconnected with visceral organs (Zan-Fu in TCM). The same authors have reported that the stimulation of specific acupoints with needles can also alter the biodistribution of radiopharmaceuticals (Senna-Fernandes et al. 2009).

A search in the PubMed database on 19 January 2015 found 734 publications with the keyword ‘laser acupuncture’ and 21,812 publications with the keyword ‘acupuncture’. This indicates that is necessary to increase the investigations of LA using different experimental models. The evaluation of the patterns of the bioavailability of a radiopharmaceutical is very important to verify the presence of diseases in an organ or system (Saha 2010). In addition, the study of factors that can alter the biodistribution of radiopharmaceutical has been useful to try to understand the effect of physical and chemical agents in the body. Moreover, this evaluation of the biodistribution of radiopharmaceutical has been used to evaluate the stimulation of acupoints with needles (Senna-Fernandes et al. 2009).

Considering the limited number of publications in the PubMed database with investigations in laser acupuncture, and due to the importance of this technique in children and patients with the fear of needles, the aim of this study was to analyse the effect of the exposure to the laser with a wavelength of 904 nm in the acupoint Zusanli (ST.36) on the biodistribution of the radiopharmaceutical Na$_2^{99m}$TeO$_4$.

2. Materials and methods

2.1 Animals and Ethics Committee

Ten adult male Wistar rats (3 months aging, weighing between 250 and 300 g) were divided equally and randomly into control group (CG) (n=5) and experimental group (EG) (n=5). The animals were kept under care at average temperature of $25^\circ$C, relative humidity around 55% and light/dark cycle of 12 h and were fed with standard diet and water ad libitum. All experiments were conducted following the standards of the Comitê de Ética Para o Uso de Animais Experimentais (CEUA), Instituto de Biologia Roberto.
2.2 Laser equipment and laser exposition of the animals

The equipment that emits laser (Laser Fluence, HTM Industry Electrical and Electronic Equipment LTDA, São Paulo) at the wavelength of 904 nm and peak power of 25W. Manual shaving was done in the region of the acupoint ST.36 of the stomach meridian (de Souza Franco et al. 2012), that is at the outer edge of the tibial tuberosity (Stux and Pomeranz 1998). In figure 1, a region of the body (Stux and Pomeranz 1998) of the rat where it is possible to find the acupoint ST.36 is shown. This region was shaved and the arrow indicates the site of the ST.36.

The animals (EG) were exposed daily to the laser (904 nm) at acupoint ST.36 with 1 joule/min (40 mW/cm²) for 1 min. The animals of the CG were not exposed to the laser. The option to use a CG without laser exposition was based in other studies (El-Mekawy et al. 2015; Raith et al. 2015). On the 8th day after the laser exposure, the animals of the two groups were sedated intraperitoneally (i.p.) with 6.7% sodium thiopental (Thiopentax ®) at a dose of 60 mg/kg body weight (Radvanska et al. 2011) to the administration of the radiopharmaceutical.

2.3 Pathway of the stomach meridian

Figure 2 shows a general representation of the pathway of the Stomach meridian. It is possible to verify that, in a general representation, this meridian starts in the head, passes through the stomach and goes to the foot (Stux and Pomeranz 1998). Notice that the Stomach meridian is in symmetric pair, only the left branch of this meridian is shown.

2.4 Experimental procedure after the administration of the radiopharmaceutical

Just after the animals were under the effect of the anesthesia, the radiopharmaceutical Na⁹⁹mTcO₄ (3.7 MBq, 1 mL) was administrated via ocular plexus. After 10 min, the animals were sacrificed and the organs including thyroid, stomach, intestine, kidney, liver, brain, pancreas, lung, heart, muscle

Figure 1. Region of the animal's body that contains the acupoint ST. 36. The arrow indicates the ST. 36

Figure 2. General representation of the pathway of the stomach meridian. The arrow indicate that flow direction of the Qi
and left bone femur were removed. The radioactivity of each organ was then determined in a well counter, and the percentages of injected dose of radiation per gram (%ATI/g) in the organs were calculated as reported in (Senna-Fernandes et al. 2009).

2.5 Statistical analysis

Comparison of the %ATI/g in EG and CG was performed by Mann-Whitney statistical test ($p \leq 0.05$).

3. Results

In table 1 the %ATI/g of the Na$^{99m}$TcO$_4$ in the various organs isolated from the animals submitted to different treatments is shown. It is possible to verify that the uptake of studied radiopharmaceutical was only altered in the thyroid. The amount of changes in other organs was not significant, although in the stomach, pancreas and spleen, increase in the uptake of the radiopharmaceutical studied was verified.

4. Discussion

Our results in table 1 indicate that the LA acupuncture with 904 nm was also capable to increase the uptake of the radiopharmaceutical Na$^{99m}$TcO$_4$ in the thyroid due to the stimulation of the ST.36 acupoint. This finding could be associated with the bio-stimulative effect of the laser suggested by Kim (2002). Senna-Fernandes et al. (2009) have also verified that the ability of the thyroid gland to uptake $^{99m}$TcO$_4$ was significantly enhanced when rats were stimulated by electro-acupuncture at two frequencies compared with the control because of stimulation of the ST.36. In particular, the results are much similar to the 100 Hz case. Gao et al. (2000) hypothesized that EA at ST.36 may result in some regulatory effects on the nervous, endocrine, and immune systems. The high level of radioactivity uptake of the radiopharmaceutical Na$^{99m}$TcO$_4$ in the thyroid gland has important clinical applications, such as to verify the size and location of this gland (Saha 2010). Various mechanisms may be involved with the uptake of Na$^{99m}$TcO$_4$ in the thyroid, including active membrane transport of the Na$^+/K^+$ATPase system and a mechanical entrainment of the ion $^{99m}$TcO$_4^-$ into the thyrocyte, instead of iodine (I$^-$) as both have a similar tetrahedral shape (Saha 2010).

When the results are compared with that of manual acupuncture (Senna-Fernandes et al. 2009), it is clear that we cannot have definitive clear-cut results on other organs, although no significant increase of the uptake of the radiopharmaceutical was found in the stomach, pancreas and spleen. Perhaps it is because that the procedures used in manual acupuncture have induced a certain amount of injury current in the meridian system so that the impedance change influences the uptake of Na$^{99m}$TcO$_4$ on other organs. Moreover, it is relevant to consider that the penetration of a needle in the body can be different in comparison to the laser, as the penetration of this physical agent depends on the wavelengths (Whittaker 2004; Round et al. 2013). This fact could justify some different findings when the stimulation is with a needle (manual or electric stimulus) or with a laser beam.

Clearly, LA has not been able to do that in our experiment. Consequently, it is imperative to study if the feeling of numbness, prickling, flowing, or heaviness around the acupoint (a sensation such as heaviness, an electrical current, or a ‘Deqi’ sensation) is necessary.

The action mechanism related to the stimulation of the ST.36 in LA in the thyroid in not fully understood, such as the influence of the stomach meridian that passes close to the thyroid (figure 2). Nevertheless, this effect in the thyroid could justify the application of this acupoint because of its homeostatic action in endocrine and metabolic diseases, as suggested by Stux and Pomeranz (1998). Another suggestion is to understand the influence of the response of the central nervous system due to the stimulation of the Suzanli. Yin et al. (2003) have described an increase of glycometabolism in the hypothalamus, head of the caudate nucleus, temporal lobe, brain stem, and sinistral cerebellum, and postcentral gyrus while puncturing at acupoint ST.36 on the right leg. Notice that the hypothalamus and head of the caudate nucleus are vegetative nerve centers.

Considering to be important, but with no statistical significance effect in the stomach, pancreas and spleen; the alterations in the uptake in these organs could be justified with the concept

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control GROUP</th>
<th>Experimental GROUP</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>7.04 ± 1.22</td>
<td>9.59 ± 1.91*</td>
<td>0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.43 ± 0.83</td>
<td>6.66 ± 3.29</td>
<td>0.18</td>
</tr>
<tr>
<td>Intestine</td>
<td>6.66 ± 3.29</td>
<td>4.43 ± 0.83</td>
<td>0.19</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.48 ± 0.44</td>
<td>1.56 ± 0.38</td>
<td>0.40</td>
</tr>
<tr>
<td>Liver</td>
<td>1.50 ± 0.23</td>
<td>1.67 ± 0.24</td>
<td>0.12</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.12 ± 0.09</td>
<td>0.27 ± 0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>Brain</td>
<td>0.11 ± 0.04</td>
<td>0.12 ± 0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>Bone</td>
<td>0.41 ± 0.14</td>
<td>0.44 ± 0.13</td>
<td>0.30</td>
</tr>
<tr>
<td>Lung</td>
<td>1.87 ± 0.26</td>
<td>1.72 ± 0.41</td>
<td>0.17</td>
</tr>
<tr>
<td>Heart</td>
<td>0.88 ± 0.19</td>
<td>1.00 ± 0.51</td>
<td>0.37</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.55 ± 0.32</td>
<td>0.94 ± 0.26</td>
<td>0.07</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.32 ± 0.08</td>
<td>0.33 ± 0.08</td>
<td>0.86</td>
</tr>
</tbody>
</table>

$p$-Value determined in the statistical analysis.

*p<0.05 in radiotracer uptake in the thyroid.
of the meridian system and the interconnections with visceral organs (Zan-Fu in TCM). Moreover, stomach, pancreas and spleen are in the element ‘Earth’ (Five Elements Theory) (Stux and Pomeranz 1998; Souza et al. 2014).

In conclusion, although further investigations are needed to clarify the mechanism by which the LA acts, it is possible to conclude that the stimulation of ST.36 leads to biological phenomena that interfere in the metabolism of the thyroid, leading, at last, to the increase of the uptake of the radiopharmaceutical in the Na\(^{99m}\)TeO\(_4\) in this gland. This finding might be relevant in the procedures used to control the function of the thyroid. Moreover, it is also interesting to investigate if LA combined with vibration of the body will be able to improve its efficacy.

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