Assessment of LLLT systemic effects on thyroid hormones function after dental titanium implant installation: An experimental rabbit model*

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ABSTRACT

This study aimed to assess the systemic effect of LLLT on thyroid gland functioning and consequently on calcium regulation through Triiodothyronine (T₃) and Thyroxine (T₄) measurements in rabbits' serum. A total of thirty two New Zealand male rabbits were randomly distributed in four groups with eight animals each: control group C (nonirradiated animals), group EI (5 J/cm² per session), group EII (10 J/cm² per session) and group EIII (20 J/cm² per session). All animals underwent lower left incisor extraction followed by immediate insertion of an osseointegrated implant, providing an equality of initial clinical condition between the groups. The experimental groups were irradiated with aluminium gallium arsenide diode laser (GaAlAs, λ = 830 nm, 50 mW, CW), during 13 days at each 48 hours, totalizing 7 sessions. Laboratorial T₃ and T₄ measurements were done in four distinct moments (before surgical procedure, immediately after surgical procedure, after the first LLLT session and after the last LLLT session) in all animals. The results obtained showed statistically significant differences in Triiodothyronine values between the groups throughout the experiment. It was concluded that the LLLT, in the protocol of irradiation used in this study, promoted a significantly alteration on rabbits’ serum hormonal levels.

Keywords: Thyroid Gland; Laser Therapy; Low Level; Thyroid Hormones; Dental Implant

1. INTRODUCTION

The devices of laser radiation are widely used by health care professionals, mostly for therapeutic uses and complementary diagnosis. Especially in Dentistry, the use of laser with different wavelengths and in distinct uses allowed the application of this technology in most diverse clinical procedures [1-3].

The clinical use of LLLT (low level laser therapy) is grounded on its capacity of promoting stimulating effects, at cellular level, on the biochemical and molecular processes that occur during the intrinsic mechanisms of tissue repair. Among the therapeutic effects, it can mention the increase of fibroblast proliferation, epithelial proliferation and collagen synthesis, which promotes the acceleration of the wound healing process; the functional neural recovery after injury; the hormone function regulation; the increased potential for remodeling and bone repair; reducing inflammation and edema; immune system regulation; modulation and relief of pain symptoms; besides post-operative analgesia [4-11].

Related literature presents several studies in vivo and in vitro which report the LLLT beneficial effects on repair process in animal models and tissue culture [10, 12-14]. According to some authors [15], the irradiation dose is the most important parameter in laser therapy and, even so, there is still no definitive protocol for its use in different clinical situations, which makes these parameters a matter of literature discussion [16,17-25] (Table 1).
Table 1. Therapeutical lower level laser (LLLT) protocols used in previous studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Laser type</th>
<th>Animal model</th>
<th>Sample</th>
<th>Wave-length (nm)</th>
<th>Power (mW)</th>
<th>Dose/Session (J/cm²)</th>
<th>Total dose (J/cm²)</th>
<th>Total of sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinheiro et al.</td>
<td>2003</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>14</td>
<td>830</td>
<td>10</td>
<td>86</td>
<td>602</td>
<td>7</td>
</tr>
<tr>
<td>Khadra et al.</td>
<td>2004</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>12</td>
<td>830</td>
<td>150</td>
<td>27</td>
<td>270</td>
<td>10</td>
</tr>
<tr>
<td>Lopes et al.</td>
<td>2005</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>14</td>
<td>830</td>
<td>10</td>
<td>86</td>
<td>602</td>
<td>7</td>
</tr>
<tr>
<td>Jakse et al.</td>
<td>2007</td>
<td>Infrared</td>
<td>Sheep</td>
<td>12</td>
<td>680</td>
<td>75</td>
<td>4</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>2007</td>
<td>Infrared</td>
<td>Rat</td>
<td>20</td>
<td>830</td>
<td>96</td>
<td>6.75</td>
<td>40.32</td>
<td>7</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>2007</td>
<td>Infrared</td>
<td>Rat</td>
<td>20</td>
<td>830</td>
<td>96</td>
<td>6.75</td>
<td>40.32</td>
<td>7</td>
</tr>
<tr>
<td>Lopes et al.</td>
<td>2007</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>14</td>
<td>830</td>
<td>10</td>
<td>86</td>
<td>602</td>
<td>7</td>
</tr>
<tr>
<td>Pereira et al.</td>
<td>2009</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>12</td>
<td>780</td>
<td>70</td>
<td>52.5</td>
<td>367.5</td>
<td>7</td>
</tr>
<tr>
<td>Campanha et al.</td>
<td>2010</td>
<td>Infrared</td>
<td>Rabbit</td>
<td>30</td>
<td>830</td>
<td>10</td>
<td>86</td>
<td>602</td>
<td>7</td>
</tr>
<tr>
<td>Maluf et al.</td>
<td>2010</td>
<td>Infrared</td>
<td>Rat</td>
<td>24</td>
<td>795</td>
<td>120</td>
<td>8</td>
<td>48</td>
<td>6</td>
</tr>
</tbody>
</table>

The laser biomodulation effects promotion on the site of its application may equally occur on tissues at a distance from the irradiation point [26]. So, when a LLLT is performed, distant organs from the application site may be affected, being these effects are called “systemics effects”. Literature suggests that LLLT may reflect on endocrine functions, from possible laser effects on secretory glands [27], among them, the thyroid gland, which secretes important metabolism regulating hormones—Triiodothyronine and Thyroxine [28].

Previous studies about infrared laser irradiation on the thyroid gland showed temporary hyperactivity in some follicles and increased mitotic activity of follicular cells [29,30] and, therefore, alterations in hormone levels of Triiodothyronine (T3) and Thyroxine (T4) [31].

The present study aimed to assess the systemic effect of LLLT on thyroid gland functioning, by measuring the amount of Triiodothyronine (T3) and Thyroxine (T4) present in rabbits’ serum after laser application as an auxiliary therapy on osseointegration of immediate implants placed in fresh extraction sockets.

2. MATERIALS AND METHODS

2.1. Animals

Thirty-two New Zealand male rabbits (Oryctolagus cuniculus) were used, weighing 3 - 4 kg and aged 3 months. They were distributed randomly in four different groups of 8 rabbits, being three groups assigned as experimentals (EI, EII and EIII) and one as control (C-nonnirradiated animals). The animals were fed with solid diet (Purina®) and water ad libitum during all the experiment. They were kept in a climate-controlled environment, under normal conditions of light, humidity and temperature. Rabbits from the experimental groups (EI, EII and EIII) and control group (C) underwent lower left incisor surgical extraction and immediate placement of an osseointegrated implant, providing an equality of initial clinical condition between the four groups (Table 2).

2.2. Surgical Procedure

The animals were anesthetized by intramuscular injection of ketamine hydrochloride (Dopalen®, Vetbrands Animal Health, São Paulo, SP), at a dose of 40 mg/kg and xylazine hydrochloride (Anasedan®, Vetbrands Animal Health, São Paulo, SP), at 3 mg/kg. Antisepsis was performed in the area of the lower left incisor with chlorhexidine gluconate 2% (FGM dental products), infiltration of 0.5 mL of 2% lidocaine with 1:100,000 epi-nephrine for a local vasoconstriction (Figure 1A) and, soon after, the incisor extraction with the aid of a pediatric forceps No. 5 (Edlo S/A, Canoas, Brazil) (Figure 1B). After that, the implant socket was prepared with sequentially sized drills (under copious irrigation with physiological saline) according to the sequence recommended by the manufacturer allowing the insertion of a nanoparticle-treated-surface osseointegrated implant (3.25ø × 11.5 mm, Implant NanoTite®, Biomet3i, Florida, USA) on that prepared socket (Figures 1C and D). The wound was closed with 4-0 nylon monofilament (Ethicon®, Johnson & Johnson) (Figure 1E). At the end of the procedure was performed trichotomy and marking with a dermographic pen (Codman®, Johnson & Johnson, New Jersey, EUA) on the area corresponding to the long axis of the implant, in order to facilitate the identification of the site of laser irradiation (Figure 1F). Immediately after surgical procedure and 24 hours after this, the animals received analgesic therapy with Tramadol® (IM at a dose of 5 mg/kg); as well as antimicrobial therapy with Enrofloxacin® (IM, at a dose of 5 mg/kg, once a day during 3 days).
Table 2. Study parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C group</td>
<td>EI group</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Surgical extraction</td>
<td>Left mand. incisor</td>
<td>Left mand. incisor</td>
</tr>
<tr>
<td>Dental implant insertion</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Light source type</td>
<td>-</td>
<td>Laser (AlGaAs)</td>
</tr>
<tr>
<td>Average power (mW)</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>-</td>
<td>830</td>
</tr>
<tr>
<td>Pulse parameters</td>
<td>-</td>
<td>CW</td>
</tr>
<tr>
<td>Irradiation points</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>No. applications per point</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Energy per point (J/cm²)</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Total energy density (J/cm²)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Irradiation time/point (s)</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>Total dose (J/cm²)</td>
<td>0</td>
<td>35</td>
</tr>
</tbody>
</table>

Figure 1. Surgical procedure: A) Infiltration with 0.5mL lidocaine 2% with epinephrine 1:100.000; B) Extraction of left mandibular incisor through pediatric forceps no. 5; C) Insertion of an osseointegrated implant 3.25ø × 11.5 mm (NanoTite®) into the fresh extraction socket; D) Occlusal view after implant insertion; E) Suture with mononylon 4-0; F) Long axis of the implant demarcated in order to guide the application of LLLT.

2.3. Laser Irradiation

Laser therapy was performed with a GaAlAs infrared diode laser [16-25], wavelength of 830 nm [17,19-24], in continuous emission mode and 50 mW power (Thera Lase®—DMC Equipament, São Carlos, Brazil) in animals of the experimental groups (EI, EII and EIII) immediately after surgery. Irradiation was performed in intervals of 48 hours, totalizing 7 application/sessions in a period of 13 days. The doses per session were fractionated on 2 points—one medial and one lateral marking the long axis of the implant (Figure 1F). The laser probe was oriented perpendicularly to the underlying jaw, not overlapping implants in order to avoid reflection on the
titanium surface. The nonirradiated animals (control group C) were subjected to a simulation of irradiation with the laser device unpowered, going through the same routine of irradiated animals.

The animals belonging to group EI received a total dose of 35 J/cm²—2.5 J/cm² per point, totaling 5 J/cm² per session, applied in 51 seconds in each point (time adjustment was performed by the laser unit after determining the other parameters). Animals from group EII received a total dose of 70 J/cm²—5 J/cm² per point, totaling 10 J/cm² per session, applied in 101 seconds in each point. Animals from group EIII received a total dose of 140 J/cm², being 20 J/cm² per session, applied in 201 seconds (Figure 2A and Table 2).

2.4. Laboratory Tests

The Triiodothyronine (T₃) and Thyroxine (T₄) measurements were performed in all animals from the four groups in four distinct moments: Time 1—72 hours before dental extraction/implant placement, Time 2—immediately after surgery, Time 3—72 hours after the first LLLT session, and Time 4—72 hours after the last LLLT session. In order to perform these tests 3 mL of blood was collected by venipuncture of the jugular vein (Figure 2B). The blood collected was placed in appropriate Vacutainer™ tubes (BD-Vacutainer®, Pediatric Systems, Becton & Dickinson Co. Franklin Lakes, NJ, USA), without anticoagulant and sent under refrigeration, to the Veterinary Clinical Chemistry Laboratory, Universidade Federal do Rio Grande do Sul (LacVet-UFRGS, Porto Alegre, Brazil) in order to test hormone levels of Triiodothyronine (T₃) measured in ng/dL and Thyroxine (T₄) measured in µg/dL present in serum of rabbits (Table 3).

2.5. Statistical Analysis

The statistical analysis was performed using software SPSS®, version 17.0 (Statistical Package for Social Science, Chicago, USA) and software SAS®, version 8.0 (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) and a 5% (p ≤ 0.05) maximum significance level was set. The laboratory values obtained for each animal/group were compared through repeated measures

![Figure 2. A) Application of LLLT. B) 3 mL of blood collection by venipuncture of the jugular vein.](image)

Table 3. Statistical analysis: hormone levels of T₃ and T₄ measurement. Baseline levels expressed as mean ± standard deviations. Means followed by different uppercase superscript letters across the same row denote significant differences. Means followed by different lowercase superscript letters across the same column denote significant differences on repeated measures analysis of variance (ANOVA) with Tukey’s multiple comparisons test, for a significance level of 5% (p ≤ 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>Time 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>143.07 ± 17.42&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>168.98 ± 23.19&lt;sup&gt;ABa&lt;/sup&gt;</td>
<td>181.57 ± 23.63&lt;sup&gt;AB-ab&lt;/sup&gt;</td>
<td>198.64 ± 52.62&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triiodothyronine T₃</td>
<td>144.32 ± 13.34&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>175.64 ± 25.15&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>179.23 ± 24.07&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>163.02 ± 22.85&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>C group—0 J/cm²</td>
<td>163.42 ± 26.86&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>193.90 ± 28.62&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>165.68 ± 43.81&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>223.86 ± 40.26&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>EI group—35 J/cm²</td>
<td>166.72 ± 39.68&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>182.80 ± 19.05&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>185.94 ± 38.77&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>196.96 ± 52.35&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>EII group—70 J/cm²</td>
<td>2.30 ± 0.56&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.03 ± 0.56&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>2.69 ± 0.66&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.18 ± 0.65&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>EIII group—140 J/cm²</td>
<td>2.19 ± 0.73&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.05 ± 0.79&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.29 ± 0.92&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.07 ± 0.47&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thyroxine T₄</td>
<td>2.40 ± 0.83&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>2.73 ± 0.85&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>2.75 ± 0.79&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.12 ± 0.84&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>C group—0 J/cm²</td>
<td>2.75 ± 0.82&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.29 ± 0.93&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.39 ± 1.20&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.37 ± 0.71&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
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</table>
analysis of variances (ANOVA), complemented by Tukey’s post-hoc multiple comparisons test.

3. RESULTS

This study attempted to assess the systemic effect of LLLT in thyroid gland function through measurement of serum levels of Triiodothyronine (T₃) and Thyroxine (T₄) in blood samples serially collected. Tests were carried out on collected samples in four different moments throughout the experiment, allowing this way the comparison between the groups. Features of each one of the samples are expressed as absolute frequencies (nominal variables), averages and standard deviations (Figures 3 and 4). Level of significance was set in 5% (p < 0.05) for all analyses.

Evaluating separately the values found for each one of the studied laboratory tests, it’s observed that Triiodothyronine (T₃) countings presented significant variations when taking into consideration the low level laser therapy (LLLT), but throughout the experiment (Table 3).

4. DISCUSSION

A rabbit model was chosen, as in some previous investigations [17-22], by the easiness of animal handling, surgical preparation and mostly by the animal proper size which enabled the insertion of standard implants (the same as used in humans) besides the collection of necessary volume blood for laboratory tests, unlikely from rat or mouse [16,23,24], that due to their small size, it would be difficult to perform the proposed methodology. Regarding the placement of the implant in the alveolar area of the recently extracted incisor, it was chosen this area over the tibia in order to be nearest to the human clinical situation. Moreover, this method allowed more reliability, since the alveolar mandibular bone suffer a different load of the rabbits’ tibia [32]. Likewise, accordingly with recent literature, we used the LLLT infrared spectrum [16-25], 830 nm wavelength [17,19-25], 50 mW power, 2 points laser application close to dental implants (without overlaying) on the animal lower jaws. It was performed a total of seven laser irradiation sessions [17,19,21,22] with every 48 hours intervals and using different doses for each experimental group. The control group (sham group) did not receive any dose of LLLT, only the simulation of this was the method chosen in order to evaluate the laser systemic effects, not being possible such assessment in split-mouth studies [3] or a subject-as-own-control design [18].

LLLT has been shown to be effective and beneficial in several dental treatments. However, due to the wide use of laser by professionals and the lack of a well-defined protocol to the different kinds of treatment, the effects of LLLT on anatomical structures and their clinical applications have been studied by researchers; among the numerous indications of LLLT reported in the literature, it can be included the use in wounds healing in skin tissue [7,10,13,26,33], use in post-extraction sockets [1,4,6], regeneration of nerve tissue [9,26] and use in perimplant tissue repair after implant placement [3,16-25].
In order to obtain an initial clinical condition for using laser in this experiment, it was performed the mandibular left incisor extraction and the insertion of an osseointegrated implant with the same surgical technique and by the same operator. Likewise, the choice of the anterior region of the mandible was based on the fact that when laser is applied on this location it could lead to indirect exposure of the thyroid gland, since that the application of laser in a well-defined area can affect deeper areas, presenting both a local and systemic effect [26,33,34].

Some authors reported that after thyroid gland laser irradiation, some morphological and functional changes occur [29,31,35]. These findings stimulated this research, since thyroid is located next to the mandibular region being potentially irradiated in many dental procedures. However, it could be verified that in this research format, LLLT altered the T₃ as a result of the treatment. Therefore, it’s observed that there was LLLT influence on the thyroid hormone levels action, as well as the elapsed time throughout the experiment.

Thus, the possibility of LLLT systemic effects [26,33] with distance site application on photobiomodulation action, the sample was divided into separate distinct control and experiment groups. In disagreement with some works that used in the same animal one side as control and the other as experiment [18,25] and in humans, split-mouth studies [3], it was verified that these works suggested that the experimental side was similar to the control side when LLLT was used. It is not impossible that such results were influenced by the laser systemic action.

In this study, the diode laser GaAlAs (λ = 830 nm) was used by its capacity of tissue penetration. Infrared lasers have higher penetration into subcutaneous tissues due to poor absorption by water or skin pigments [1,2].

The thyroid gland function is usually assessed by measuring basal serum concentration of its hormones. The radioimmunoassay (RIA) is one of the methods used for hormone assays that have been used by researchers to measure the amount of Triiodothyronine (T₃) and Thyroxine (T₄) circulating in blood serum [12,35]. RIA is based on the observation of the reaction between antibodies and soluble antigen, forming a precipitate of antigen-antibody or an insoluble aggregate. Among its advantages are high sensitivity on antigens detection in small concentrations and convenience when lots of samples are tested together, reducing the amount of manual operations to be performed, facilitating the process of separation and reducing the chances of error [36].

The results of this study demonstrated significant differences in hormone levels of the thyroid gland after LLLT application, differing from Lerma et al. [35] findings, who analyzing the HeNe laser effects on Wistar rats thyroid glands, did not find any concrete evidence of alteration in thyroid hormones serum levels. The same way, Fronza et al. [34], demonstrated that in rabbits there is no significant difference between the control and experimental groups before and after irradiation by LLLT on blood values of T₃ and T₄, indicating that laser did not affect thyroid function on circulating serum hormone levels in rabbit model. Our results, however, confirm the findings of Azevedo et al. [12], who performed the application of LLLT (λ = 780 nm) on Swiss mice thyroid glands. Authors observed significant differences in T₃ and T₄ hormone levels from the first day of laser application and seventh day after the last application.

In order to verify the individual variability of the studied specimen, the same animal was subjected to measurements of T₃ and T₄ with and without laser therapy in distinct moments. Such methodology made possible the comparison of obtained values before, during and after administration of the LLLT protocol in each study group.

Also relevant is the fact that different protocols used in LLLT made difficult the interpretation of the effect of laser on thyroid gland function, as well as the comparison of results of other variables among the studies. We agree with Basford [2], Kolávorá et al. [37], Belkin and Schwartz [38] and Schindl et al. [39], which indicate the need for more well-defined protocols in the methodology of works in order to obtain more reliable comparisons, thus providing the correct conduction of studies that verify the real performance of laser on tissues, organs and systems.

New researches varying the animal’s number, laser dosages and power settings, as well as the irradiation period, must be performed in order to better understand the effects of LLLT on this gland functioning.

5. CONCLUSION

In spite of having significantly alteration on Triiodothyronine (T₃) hormone levels, the LLLT did not permanently affect the rabbit’s thyroid gland function, in the irradiation protocol used in this study. However, this work showed irradiation distance action by altering the gland secretion. For these reason, it must be considered a special care on closely secretory glands LLLT application.

6. ACKNOWLEDGEMENTS

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