Local Application of Melatonin on Dental Implant Osseointegration: Analysis of Removal Torque. Experimental Study on Rabbits

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**ABSTRACT**

Melatonin may interfere with the function of osteoclasts and inhibit bone resorption mainly due to its antioxidant properties and ability to detoxify free radicals. This inhibition can be enhanced by the reaction of indolamine in osseoclastogenesis and may have benefits in implantology.

**Aims:** To evaluate the mechanical and biological behavior of osseointegration using melatonin.

To compare the stability coefficient of implants at the time of placement (initial or mechanical ISQ) and after 60 days (final or biological ISQ).

To measure the disinsertion torque in both groups of rabbits, with and without melatonin application.

**Materials and Methods:** A total of 16 implants of the same brand and size were placed, with 9 of them receiving 3mg of melatonin powder prior to installation and 7 control implants without melatonin. Resonance frequency analysis (Osstell®) was used to measure initial and biological anchorage, as well as removal torque.

**Results:** The mean removal torque for melatonin-treated implants was 99.8 Ncm, and for control implants, the mean was 87.2 Ncm. The differences between the two groups were not statistically significant. However, there were significant differences in biological ISQ values.

**Conclusions:** The use of melatonin improved the response to osseointegration, particularly in the measurement of final or biological ISQ values.

**Keywords:** ISQ, Melatonin, Osseointegration, Removal torque.

I. INTRODUCTION

Dental implants have gained prestige as a successful treatment to restore aesthetics and functionality in patients with partial or total loss of their dental elements. Their osseointegration is fundamental for success and long-term stability [1], [2]. Osseointegration can be defined as the direct structural and functional connection between the ordered living bone and the surface of a load-bearing implant [3]. Several ongoing research projects seek to improve surface characteristics and find new biomimetic agents that expedite osteogenesis. A variety of substances have been used to enhance the peri-implant bone response, including growth factors [4] and morphogenetic proteins [5]. Recently, attention has focused on the osteogenic potential of hormones such as growth hormone and melatonin [6]-[9]. Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine derived from tryptophan, primarily secreted by the pineal gland. Melatonin has been implicated in the control of circadian rhythm, including sleep, actions that are mediated by the binding of the indoleamine to membrane receptors. Its secretion is related to the duration of darkness, with levels reaching their peak near mid-night and low levels during the day [10]. Due to its antioxidant properties and its ability to detoxify free radicals, melatonin can interfere with the function of osteoclasts and therefore inhibit bone resorption [11]. On the other hand, several in vitro studies have shown that melatonin is an important mediator in bone formation, promoting osteoblast differentiation [12]. In addition, melatonin has been reported to modulate calcium metabolism and prevent osteoporosis and hypocalcemia, probably by interacting with other regulatory factors such as parathyroid hormone, calcitonin, and prostaglandins [13]. Nakade et al. in 1999 demonstrated that micromolar concentrations of melatonin stimulate the proliferation and differentiation of human osteoblasts in vitro, as well as the synthesis of type I collagen and other bone matrix proteins, suggesting that melatonin may act as a potent bone formation stimulator [14]. In a recent case-control study, the effect of topical application of 1.2 mg of lyophilized melatonin powder was evaluated in premolar extraction sockets in Beagle dogs: at two weeks, the bone perimeter in contact with the melatonin-treated implants increased, bone density increased, and new bone formation, as well as inter-spiral bone, was enhanced compared to controls [15]. Tresguerres et al. in 2012 evaluated the effect of local application of melatonin in 10 female New Zealand
rabbits, five of which received local application of 3 mg of melatonin at the implant sites (rabbit tibia). Four weeks later, a statistically significant increase in trabecular bone-implant contact (BIC) was found in the melatonin group compared to the control group. Trabecular area density also significantly increased in the melatonin group [16]. These studies analyze the effectiveness of melatonin at a histomorphometric level.

Another way to study the capacity for osseointegration is with removal torque (RTV) as an indirect measure of BIC and osseointegration [17], [18]. For its measurement, devices are used that express their results in Newton Centimeters (Ncm) as a unit of force. Regarding the values of removal torque considered as successful osseointegration, Sullivan et al. [17] recommended an RTV threshold of 20 Ncm for clinical tests of osseointegration, while the study by Simeone et al. [19] considered that the appropriate value of RTV should be 30 Ncm.

Another procedure used to analyse osseointegration is proposed by Meredith et al. [20], who propose the use of Resonance Frequency Analysis (RFA). This is a non-invasive clinical procedure for measuring the stability of the implant once placed in the bone (primary stability) and also allows monitoring the stability of the implant during the healing period and over time. It is based on a bending test where a transducer (Ostell® - Integration Diagnosis AB, Gothenburg, Sweden) applies an extremely small bending force to the implant-bone interface, quantifying the readings on a centesimal scale called the Implant Stability Quotient (ISQ), which transforms the unit of measure KHZ into ISQ values, measured on a scale of 1 to 100. High values on this scale indicate high stability, while low values indicate low stability [21].

In a study conducted by Balleti et al. [22], which included 45 implants after 1 year of loading, successful integration was shown, with ISQ levels ranging from 57 to 82 with an average of 69, similar to the study conducted by Degidi et al. [23] where failed implants showed an average ISQ value of 46, while successfully osseointegrated implants had ISQ values around 60.

ISQ values could vary depending on the different positions of the transducer, which is why they recommend standardizing the measurements [24].

Removal torque (RTV) and resonance frequency analysis (RFA) tests are considered reliable methods for studying the behavior of osseointegration, establishing values to consider the bone-implant connection by quantifying its integration. However, no research has been conducted placing melatonin locally and evaluating its effectiveness through ISQ and removal torque. In our work, the quality of osseointegration will be evaluated using removal torque and resonance analysis.

II. MATERIALS AND METHODS

A prospective experimental study was conducted on 7 female rabbits of the New Zealand and French Hybrid variety, one year of age, weighing between 4 to 4.5 kg. Dental implants were placed in the femurs of the rabbits, and the implant stability quotient (ISQ) was measured during surgery and again 60 days after insertion along with the insertion torque.

The protocol used was approved by the Ethics and Animal Welfare Committee of the Faculty of Agricultural Sciences - Catholic University of Córdoba. The research was conducted at the Veterinary Clinic of the Faculty of Agricultural Sciences of the Catholic University of Córdoba. The rabbits were transferred 7 days before the surgeries to allow for adaptation to a new environment, thus reducing emotional stress. (Fig. 1).

Fig.1. Animal adaptation.

A. Surgical protocol

Anesthetic medication was administered as follows: Xylazine (0.7 mg/kg) and ketamine (25 mg/kg) intramuscularly (total dose divided into thirds for maintenance during surgery). Analgesic medication was Tramadol (3 mg/kg every 12 hours) subcutaneously and Meloxicam (0.1 mg/kg) intramuscularly every 24 hours. Pre-surgical antimicrobial therapy was administered using Enrofloxacin (5 mg/kg) intramuscularly. Anesthetic monitoring was performed using electrocardiography, pulse oximetry, and non-invasive blood pressure monitoring. All measured parameters were maintained within normal ranges during the anesthetic procedure (Fig. 2).

The surgeries were carried out in the operating rooms of the Veterinary Clinic of the Faculty of Agricultural Sciences of the UCC. General surgical procedures on the rabbits were performed by veterinarians from the institution, while the implant placement procedure in the femurs was carried out by dentists from the Oral Implantology Specialization Program at the Faculty of Medicine of the UCC and the Dental Circle of Córdoba.

Initially, the area to be intervened was disinfected with 10% Iodopovidone and shaved. Subsequently, the incision was made using a Bard Parker scalpel handle with a number 12 blade, and the surrounding tissue was dissected (Fig. 3, Fig. 4a).
Afterward, incision was started using a Bard Parker scalpel handle with a number 12 blade, and the planes were dissected until access to the bone tissue was obtained (Fig. 4b and c). Trepanation was performed using an Anthogyr contra-angle with a 20:1 reduction, powered by an Onirium physiodispenser, using the corresponding drills for the implant system following the sequence indicated by the manufacturer (Fig. 4d, e, and f).

A total of 16 implants of the same brand and size were placed. In nine of these surgical sites, 3mg of melatonin powder (Droguería Saporiti S.A.C.I.F.I.A, Buenos Aires, Argentina) were placed before implant installation. The powder was brought to the surgical site using a bone curette, and before being placed, it was aspirated to ensure that the powder was completely inside the surgical site (Fig. 4g, h). Almost simultaneously, the implant was positioned and screwed in (Fig. 4i).

To determine the percentage of alveolar occupancy with melatonin, an experimental determination was performed by carrying out the same milling procedure in an acrylic phantom and placing 3mg of melatonin inside. Measurements were taken, and the percentage was estimated to be 21±5% of the total volume of the cavity (Fig. 5). In the other surgical sites, no treatment was performed before implant placement, using the same drilling protocol (sequence indicated by the manufacturer), which was used as controls.

All implants used were 3.3 mm in diameter and 7mm in length from the Tree-Oss (Rapid) brand.

**Fig. 2.** Vital symptoms monitoring and surgical field preparation.

**Fig. 3.** Disinfection and shaving of surgical area.

**Fig. 4 (a).** Surgical field.

**Fig. 4 (b).** Incision.

**Fig. 4 (c).** Exposed Femur.
Fig. 4(d). Drilling sequence.

Fig. 4(e). Drilling sequence: 2 mm Drill.

Fig. 4(f). 2.5 mm Drill.

Fig. 4(g). Aspirate from the surgical bed, previous to the placement of 3 mg of melatonin.

Fig. 4(h). Placement of 3mg melatonin.

Fig. 4(i). Tree Oss Implant.
After implant placement, resonance frequency analysis was performed to measure the initial stability quotient (ISQ) using the Osstell ISQ device (Osstell, Integration Diagnosis AB, Gothenburg, Sweden) and SmartPeg Type 1 (Integration Diagnostics, Gothenburg, Sweden) (Fig. 6a, b). In this study, the most recent version of the Osstell ISQ device was used. The stability measurement of the implant is performed together with a wireless SmartPeg device (Integration Diagnostics AB, Gothenburg, Sweden) screwed onto the implant [26]. The measurements were carried out according to the manufacturer's instructions, which state that after screwing the SmartPeg device onto the implant, a measurement should be taken with the sensor oriented parallel to the maxillary line and another oriented perpendicular to that line. These measurements will be horizontal or perpendicular to the SmartPeg [25]. It is important to respect these instructions, as taking the vertical ISQ measurement could differ significantly from the horizontal measurement (the recommended position by the manufacturer) and increase the probability that the vertical ISQ value is higher than the horizontal ISQ value [24].

The flaps were then repositioned and sutured separately, with Reverdin suture used in the muscle, Surget suture used in the subcutaneous layer, and continuous horizontal U-stitch with 3-0 monofilament nylon suture used in the skin (Fig. 7a, b) (Fig. 8a, b).
After the surgical procedure was completed, the animals recovered from anesthesia in an appropriate room and were monitored until they were able to maintain sternal recumbence. They were isolated in a recovery room for monitoring and wound care for 7 days, then identified and housed under appropriate environmental conditions with other rabbits with balanced nutrition and ad libitum water access for 60 days.

As postoperative medication, analgesia was provided with meloxicam (0.1 mg/kg; q24 h) intramuscularly for 3-5 days. Postoperative antibiotic therapy was maintained with enrofloxacin (5 mg/kg; q12 h) for 7 days.

B. Measurement of removal torque and final ISQ

The animals were euthanized 60 days after the surgical intervention, following appropriate protocols and standards, by anesthetizing the animals with 2 mg/kg of 2% Xylazine with 20 mg/kg of 10% Ketamine IM. Once the animals were anesthetized, they were administered sodium pentobarbital 40% and phenytoin 5% (1ml of Euthanyle for every 10 kg of weight).

The implants were exposed by a second dissection surgery along the plane until the joints were located and the femur was removed. The soft tissue was carefully removed until a clear zone was obtained for the measurements (Fig. 9).

Finally, the removal torque of the implants was measured. For this, an implant extractor from the brand B&W was used, which is screwed inside the implant in the opposite direction of the implant thread. The femurs of the specimens were stabilized on a table by holding them at the ends. The digital precision torque meter Mark-10 (Mark-10 Corp., USA, Digital Torque Gauge Series TTT/ Model No: MTT03-50/ Serial No: 1234567/ Version 1.0 ©) was attached to the implant extractor, and the implants were removed by reverse rotation, obtaining the peak value of removal torque expressed in Ncm (Fig. 11).

The implants were exposed by a second dissection surgery along the plane until the joints were located and the femur was removed. The soft tissue was carefully removed until a clear zone was obtained for the measurements (Fig. 9).

B. Measurement of removal torque and final ISQ

The animals were euthanized 60 days after the surgical intervention, following appropriate protocols and standards, by anesthetizing the animals with 2 mg/kg of 2% Xylazine with 20 mg/kg of 10% Ketamine IM. Once the animals were anesthetized, they were administered sodium pentobarbital 40% and phenytoin 5% (1ml of Euthanyle for every 10 kg of weight).

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The final stability coefficient of the implants was measured through resonance frequency analysis, in the same way as in the first surgery (Fig. 10).

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III. RESULTS

A. Implant Torque

Table 1 shows the central tendency and dispersion values of insertion and removal torque (mean and standard deviation) according to group and the significance (p-value) of the contrast between groups according to stage. Fig. 11 shows the schematic distributions of torque values recorded according to group and stage, where the similarity in the distributions corresponding to the initial stage is appreciated, and an apparent advantage for the melatonin group in the removal stage with slightly higher values in general.

<table>
<thead>
<tr>
<th>TABLE I: TORQUE ACCORDING TO GROUP AND STAGE: COUNT OF CASES (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion Torque (Ncm)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Melatonin (n=9)</td>
</tr>
<tr>
<td>Control(n=7)</td>
</tr>
<tr>
<td>Mean ± standard deviation and significance of the contrast test (mann-whitney; p-value.</td>
</tr>
</tbody>
</table>

Fig. 10. ISQ Measurement.

Fig. 11. Distribution of torque values according to group and stage.
The groups were compared considering each stage separately. In the insertion stage, the differences were not significant (Mann-Whitney test: \( p = 0.76 \)). The differences in the removal stage, which was performed 60 days after implant placement, were also not significant (Mann-Whitney: \( p = 0.41 \)). Although there were no statistically significant differences, the melatonin group showed a minimum torque of 80 Ncm, in contrast to the control group that recorded a minimum of 50 Ncm and another value of 61.5 Ncm.

Since they are related samples and to minimize the intrasubject effect, the differences in torque between stages registered in each of the implants were evaluated (Table II and Fig. 12), and these differences were not significant (Mann-Whitney: \( p = 0.41 \)). In view of this result and considering only the removal stage, it is suggested to accept the null hypothesis: "the addition of melatonin would not have a significant effect on implant torque values”.

### TABLE II: DIFFERENCES IN TORQUE BY GROUP: CASE COUNT (N)

<table>
<thead>
<tr>
<th>Group</th>
<th>Torque difference between stages (Ncm)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin (n=9)</td>
<td>77.6 ± 22.7</td>
<td>0.41</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>63.9 ± 23.9</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± standard deviation and significance of the contrast test (mann-whitney; p-value).

Fig. 12. Distributions of torque difference values between stages by group.

### B. Implant Stability

Table III shows the central tendency and dispersion values (mean and standard deviation) of implant stability (ISQ) according to group and stage, and Fig. 13 shows the distributions of ISQ values corresponding to each subgroup. In both stages, the stability of the experimental group’s implants was slightly higher than that of the control group.

### TABLE III: ISQ ACCORDING TO GROUP AND STAGE: COUNT OF CASES (N)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mechanical ISQ</th>
<th>P</th>
<th>Biological ISQ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin (n=9)</td>
<td>49.2 ± 2.0</td>
<td>0.17</td>
<td>53.4 ± 2.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>44.7 ± 6.9</td>
<td></td>
<td>50.5 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± standard deviation and significance of the contrast test (mann-whitney; p-value).

The groups were contrasted in both stages using non-parametric Mann-Whitney tests, and the differences in the mechanical stability of the implants were not significant (initial stage: \( p = 0.17 \)), while the differences in biological ISQ values were significant (final stage: \( p = 0.02 \)). According to this result, it would be appropriate to accept the alternative hypothesis: that "the addition of melatonin would have a significant effect on implant stability values”. Therefore, it would be more convenient to evaluate the differences in ISQ stability between stages registered in each of the implants (Table IV and Fig. 14), since this way the intrasubject effect can be minimized.

The groups were contrasted by evaluating the increase in ISQ experienced in each case, and the differences were not significant (Mann-Whitney: \( p = 0.76 \)). Therefore, it is suggested to accept the null hypothesis: "the addition of melatonin would not have a significant effect on implant stability”.

### TABLE IV: ISQ DIFFERENCES ACCORDING TO GROUP: CASE COUNT (N)

<table>
<thead>
<tr>
<th>Group</th>
<th>ISQ DIFFERENCE BETWEEN STAGES</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELATONIN (N=9)</td>
<td>4.2 ± 2.2</td>
<td>0.76</td>
</tr>
<tr>
<td>CONTROL (N=7)</td>
<td>5.8 ± 5.3</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± standard deviation and significance of the contrast test (mann-whitney; p-value).

The scatter plot in Fig. 15a shows the stability and torque distributions corresponding to each implant, while Fig. 15b includes the centroids of each subgroup matching their respective mean values, which are also shown in the figure. Considering the projections on the vertical axis (ISQ), the difference between groups is somewhat greater in the initial stage and of lesser amplitude in the final stage. Regarding torque (horizontal axis), the situation is inverse; in the initial stage, the differences between groups are minimal and with little dispersion, but in the removal stage, both the differences between groups and the dispersions are significant.

### IV. DISCUSSION

Numerous studies in the literature [15], [16], [25]-[28] have demonstrated that melatonin increases all parameters of osseointegration, such as BIC and peri-implant bone.
studies obtained their results through histomorphometric studies, in contrast to our work, in which we evaluated osseointegration through removal torque and resonance frequency analysis (ISQ), obtaining a positive correlation between both analyses. No study was found in the literature using melatonin with this mode of administration, evaluating its efficacy through removal torque, as in our case. In implants placed in beds treated with melatonin, values of 80-133 Ncm were obtained, with a mean of 99.8 Ncm, while in control implants, values obtained were 50-118.5 Ncm, with a mean of 87.2 Ncm. A slight advantage of the melatonin group over the control group was evidenced, although the differences between the two studied groups were not statistically significant.

On the other hand, in the present study, initial ISQ values (measured immediately after implant placement) were also compared between the two surfaces, showing similar values in both groups, with no statistically significant differences (initial stage; p=0.17) [24]. At 60 days, before removal, final or biological ISQ was also measured, and when comparing the values, the differences were statistically significant (p=0.02). Elgammal et al. [29] obtained similar results to our study. They used a melatonin gel prepared by mixing melatonin powder with propylene glycol (1.2 mg/ml) to act as a vehicle. One month after implant placement, a significantly greater stability (Periotest) was observed in the study group (p=0.01). In subsequent follow-up periods, there was no statistically significant difference in measurements between the two groups. Additionally, this study establishes that there is no constant ratio between ISQ values and RTV, but there are some correlations.

All these studies show that melatonin seems to have a positive effect on dental implant osseointegration, but several points remain to be addressed. One of the most important is that there is no consensus on the most effective delivery method, route of administration, and dose of melatonin.

V. CONCLUSION

The use of melatonin would improve the healing response of bone around implants, with its effect being clearer in measuring the final or biological ISQ values for osseointegration.

More exhaustive studies are needed to expand the therapeutic possibilities of melatonin in dentistry.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES


