

Evaluation of Effects of Topical Melatonin Application on Osseointegration of Dental Implant: An Experimental Study

Serkan Dundar, DDS, PhD^{1*}
 Ferhan Yaman, DDS, PhD²
 Arif Saybak, DDS, PhD³
 M. Fatih Ozupek, DDS⁴
 Vesile Elif Toy, DDS, PhD⁵
 Mehmet Gul, DDS⁶
 İ. Hanifi Ozercan, MD⁷

The aim of the present study was to evaluate the effect of local melatonin application during surgery on bone implant connection (BIC) in rabbit tibiae. Six 0.8- to 1-year-old male New Zealand rabbits were divided into 3 groups: (1) a control group (CG) in which rabbits were not treated with additive materials and only implant integration was executed; (2) a melatonin dose 1 (MLT D-1) group in which rabbits were treated with 1.2 mg of melatonin locally before implant placement into the rabbits' tibiae; and (3) a melatonin dose 2 (MLT D-2) group in which rabbits were treated with 3 mg melatonin locally before implant placement into the rabbits' tibiae. Four weeks after the procedure, the rabbits were euthanized; their tibiae were dissected from muscles and soft tissues, fixed with formaldehyde, and later embedded in methacrylate. Histologic and histomorphometric analyses were then performed under light microscopy. Following this, BIC was detected histomorphometrically, and $P < .05$ was considered statistically significant. Results showed that the highest BIC percentage was detected in MLT D-2, with a mean value of $39.46\% \pm 0.78$, as compared with a mean value of $33.89\% \pm 0.92$ in group MLT D-1 and $27.42\% \pm 0.89$ in CG. Similarly, the mean BIC percentage of the MLT D-2 group was the highest among the three, with the mean BIC percentage of the MLT D-1 still registering as higher than CG. Within the limitations of this rabbit study, it appears that local melatonin application during implant surgery may improve BIC.

Key Words: bone implant connection, implant, local melatonin, rabbit, bone

INTRODUCTION

Osseointegration is defined as a direct connection between living bone tissues and a titanium implant without any connective tissues. Recently, dental implant supported prostheses have been widely preferred as a common treatment technique to partial or/and totally edentulous jaws.¹⁻⁴

Bone implant connection and load transmission in dental implant supported prosthesis is affected by the quality and quantity of the peri-implant bone tissues and the natural features of the surrounding bone/implant interface.³⁻⁷ Specifically, insufficient bone quantity and quality are the most frequent challenges for dental implant placement in maxillae and mandibles. In maxillary bone tissues, especially, the limited

bone amount is tied to inadequate bone quality, which constitutes a considerable problem for the dental implant-supported prosthesis.⁸ Insufficient bone quality and quantity can be seen frequently in elderly populations with atrophic and osteoporotic bones, and this situation stems from increased osteoclastic activity due to a reduction in osteoblastogenesis. In addition, local free radical concentrations in all body cells, including osteoblasts, can reduce osteoblastic activity in aged patients. This process could lead to a reduction in bone regeneration capacity.⁹⁻¹¹

Osteoblasts are bone formative cells, and osteoclasts are bone resorptive cells. When these cells work together physiologically, the formation and resorption processes of bone tissue is known as *bone remodeling*. The remodeling of bone tissues is regulated by the action of systemic hormones (estradiol, parathyroid, growth, and melatonin) and by the bone marrow and osteoid matrix-derived growth factors.^{9,12-14} Melatonin is secreted by the pineal gland and is a tryptophan-derived indolamine hormone. This hormone has well-known antioxidant capabilities and free radical scavenging abilities. Melatonin can also suppress osteoclastogenesis, thereby inhibiting bone tissue resorption.^{9,15-17} In addition, in vitro research has reported that melatonin can increase osteoblast proliferation and differentiation.^{9,15,16,18} Thus, we proposed that the local application of melatonin during surgical implant placement procedures would be an effective

¹ Department of Periodontology, Firat University, Elazig, Turkey.

² Department of Oral-Maxillofacial Surgery, Dicle University, Diyarbakir, Turkey.

³ Private practice, Adana, Turkey.

⁴ Department of Oral-Maxillofacial Surgery, Inonu University, Malatya, Turkey.

⁵ Department of Periodontology, Inonu University, Malatya, Turkey.

⁶ Department of Periodontology, Dicle University, Diyarbakir, Turkey.

⁷ Department of Medical Pathology, Firat University, Elazig, Turkey.

* Corresponding author, e-mail: dtserkandundar@gmail.com, sdundar@firat.edu.tr

DOI: 10.1563/aaid-joi-D-16-00048

treatment technique for dental implant osseointegration. As such, the aim of this study was to evaluate the histomorphometric effects of local melatonin application on the bone implant connection (BIC) during surgical implant placements in rabbit tibiae.

MATERIALS AND METHODS

The experimental design and study protocol were approved by the Local Experimental Animal Ethics Committee at the University of Firat, Elazig, Turkey. In total, six 0.8- to 1-year-old male New Zealand rabbits were used. Their average body weights were 3000 to 3200 g on the first day of the experimental protocol. The animals were kept in temperature-controlled cages, exposed to a 12/12-h light/dark cycle, and had ad libitum access to food and water during the experimental period.

First, the rabbits were divided randomly into 3 groups, as listed below:

- Control group (CG) ($n = 2$): No treatment was applied, and dental implants were simply inserted in rabbit tibiae.
- Melatonin dose 1 (MLT D-1) group ($n = 2$): 1.2 mg lyophilized powder melatonin (Sigma-Aldrich, St Louis, Mo) was administered locally into the dental implant socket before implant placement in rabbit tibiae.^{16,18}
- Melatonin dose 2 (MLT D-2) group ($n = 2$): 3 mg lyophilized powder melatonin (Sigma-Aldrich) was administered locally into the dental implant socket before implant placement in rabbit tibiae.⁹

General anesthesia was administered using ketamine hydrochloride 35 mg/kg and 5 mg/kg xylazine intramuscularly. Surgical operations were performed under sterile conditions. Following general anesthesia but prior to the surgical application, the tibial skin was washed with povidone iodine and the surgical area was shaved. In each case, the skin incision was made over the tibial crest. We used a periosteal elevator to lift the flap and periosteum to reach the tibial bone. The tibial skin was then sutured with 4/0 polyglactin resorbable sutures. Penicillin and an analgesic were injected intramuscularly in all animals for 3 days after the operation.

In all 3 groups, a total of 24 sandblasted large acid-etched surface dental implants (Es-Dent, Gulmaksan Izmir, Turkey) measuring 6 mm in length and 3 mm in diameter were integrated in the metaphyseal part of the tibiae. Two implants for each tibia and 4 implants for each animal were integrated. All the surgical procedures were performed by the same researcher atraumatically.

Four weeks after the surgical implant placement, the rabbits were euthanized. The rabbit tibiae were then dissected from muscles and soft tissues and fixed in a 10% formaldehyde solution. The specimens were embedded into 2-hydroxyethyl-metacrylate resin, allowing us to cut undecalcified bone and titanium with the Exakt Microtome (Leica Biosystems, Nussloch, Germany). For the histomorphometric evaluation, each section was ground with the Exakt grinder (Leica), and a 50- μ m thickness section was obtained for the light microscope analysis (Olympus, Tokyo, Japan). Toluidine blue stains were

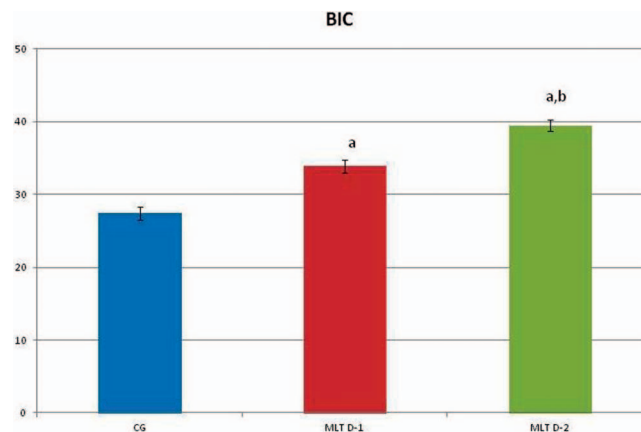


FIGURE 1. Comparison percentage of bone implant connection. (a) Statistically significant difference was detected compared with control group (CG). (b) Statistically significant difference was detected compared with melatonin dose-1 group (MLT D-1).

used for the histomorphometric analysis. After this procedure, a histomorphometrical analysis was performed to quantify the bone tissue response in peri-implant bone. This was done by the faculty at Firat University Department of Medical Pathology, Elazig, Turkey. The histomorphometrical analysis of osseointegration was performed with an image analyzer (Olympus); BIC was recorded as a proportion of the total implant surface length (from the coronal margin to the most apical point of the implant) in direct contact with the bone.⁹

Statistical analysis

SPSS software was used for statistical analysis. Mean values \pm a standard error equal to the mean of each group were calculated for all analyzed data. The differences among groups were tested with a one-way ANOVA for parameters that showed a normal distribution. To detect the group causing the differences, Tukey's HSD test was used, and P -values $< .05$ were sufficient to indicate statistical significance.

RESULTS

The histomorphometric results of osseointegration in the 3 groups are presented in Figure 1. As can be seen in Figure 2 (a through c), the highest BIC percentage was detected in the MLT D-2 group, with a mean value of $39.59\% \pm 1.2$, compared with $33.89\% \pm 1.8$ in the MLT D-1 group and $27.42\% \pm 2.2$ in CG (Figure 1). The mean BIC percentage of the MLT D-2 group was the highest among the three groups, and the mean BIC percentage in MLT D-1 was determined to be higher than that of CG (Figure 1).

DISCUSSION

Various studies have reported that melatonin stimulates the osteogenic activity of bone tissue.^{15,16,21–23} However, the effects of local melatonin application during the surgical phase on increasing dental implant osseointegration has rarely been studied.^{9,16,18,24} The histomorphometrical analysis reported in

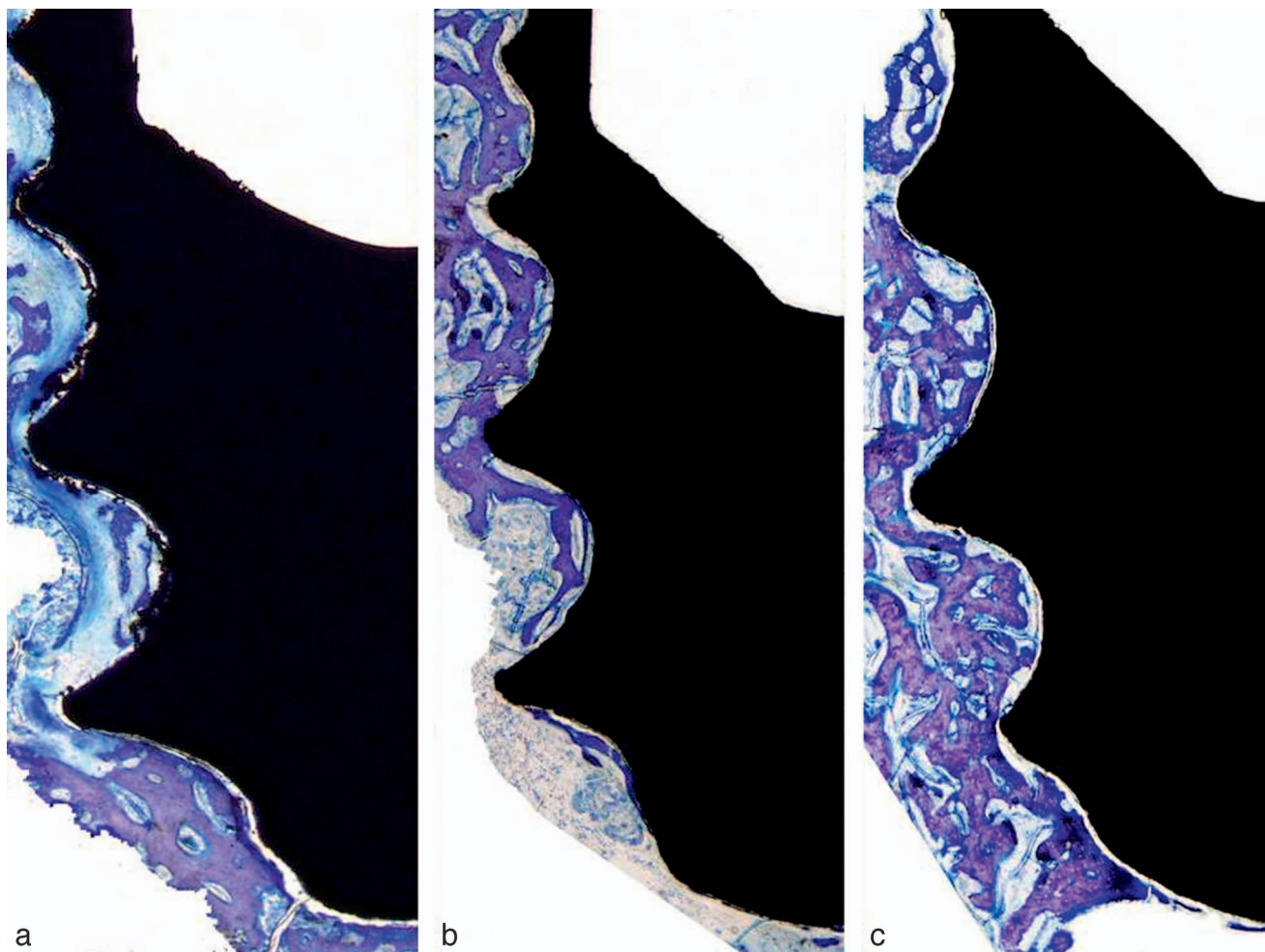


FIGURE 2. (a) Histologic view of control group (CG-2X). (b) Histologic view of melatonin dose-1 group (MLT D-1-2X). (c) Histologic view of melatonin dose-2 group (MLT D-2-2X).

this study has indicated an increase in osteogenesis in peri-implant bone tissues with local melatonin application during surgical integration in 2 experimental groups. Specifically, we found that melatonin increased bone area in peri-implant bone tissues. Therefore, we concluded that the local administration of melatonin during a surgical implant integration procedure could directly induce osteoblastogenesis. Moreover, in the MLT D-2 group, the histomorphometric BIC parameters detected were highly comparable with those of the MLT D-1 group.^{9,15,16,18}

The osteoblastic properties of melatonin have been shown in several other studies, and our findings were in line with what has been reported previously.^{21,22} For instance, the results of our research were in agreement with the Koyama et al.²⁵ experimental animal study in which the authors investigated the effects of systemic melatonin application on bone tissues in mice. Koyama et al.²⁵ stated that pharmacological doses of melatonin were able to increase bone tissue mass, bone tissue mineral density, and trabecular bone volume. The authors reported that this situation obviously stemmed from an anti-osteoclastic effect of the melatonin hormone.

Moreover, Satomura et al.²¹ demonstrated the systemic

osteogenic effects of melatonin administration intraperitoneally in their experimental study. Specifically, they reported an increase in the volume of the newly formed cortical bone tissues of mouse femora. In the current study, as in these previous examples, we used melatonin locally and found that the osteogenic effects of the melatonin hormone could be explained by its inductive effect on osteoblastic cells.^{9,14-16,18,21,22} These results were similar to those of Cutando et al.,¹⁶ who applied 1.2 mg of lyophilized melatonin locally into the mandibular implant sockets of dogs before implant placement; they reported an increase in the osseointegration of the implant. Takechi et al.²⁴ produced similar results in their experimental research. In particular, they reported that systemic melatonin used with local Fibroblast Growth Factor-2 resulted in an increase in peri-implant bone tissue formation in rats.

Likewise, Munoz et al.¹⁸ conducted a study in which, prior to implantation, 4 IU of recombinant human growth hormone and 1.2 mg of lyophilized powdered melatonin were applied to implant sockets on each side of the dog mandible. They reported that melatonin and growth hormone synergistically enhanced new peri-implant bone formation in early stages of the tissue healing process.¹⁸ In a similar study, Teresguerras et

al.⁹ stated that a 3-mg local melatonin application during surgical implant integration might increase trabecular BIC as well as trabecular bone area density. In clinical research, Elgammal et al.²³ has described results comparable to previous experimental studies. For instance, they indicated that local application of melatonin at the osteotomy site prior to the implant integration is associated with good implant stability and minimal bone resorption.

CONCLUSION

On the whole, our results confirmed the findings of these studies. In fact, morphometrical outcomes in our study suggested the presence of increased BIC in the melatonin groups when compared with the controls, in a dose-dependent manner. Within the limitations of this study, therefore, it can be concluded that local melatonin administration at the osteotomy site during surgical implant insertion may stimulate more BIC. However, more investigation is required regarding the increasing of BIC between peri-implant bone tissue and dental implant surfaces.

ABBREVIATIONS

BIC: bone implant connection
CG: control group
MLT D-1: melatonin dose-1
MLT D-2: melatonin dose-2

NOTE

This study was presented as an oral presentation at the 22nd International Scientific Turkish Dental Association Congress, May 19–21, 2016, Izmir, Turkey.

ACKNOWLEDGMENTS

The authors wish to extend their gratitude to Es-Dent for providing the dental implants. The authors also thank Dr Cem Gurgan, Hasan Ekeer (Erciyes University, Faculty of Dentistry), and Dr Selcuk Ilhan (Firat University) for his helpful support on histomorphometric analysis.

REFERENCES

1. Buser D, Mericske-Stern R, Bernard JP, et al. Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. *Clin Oral Implants Res.* 1997;8:161–172.
2. Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg Hand Surg.* 1969;3:81–100.
3. Guven S, Beydemir K, Dundar S, Eratilla V. Evaluation of stress distributions in peri-implant and periodontal bone tissues in 3- and 5-unit tooth and implant-supported fixed zirconia restorations by finite elements analysis. *Eur J Dent.* 2015;9:329–339.
4. Dundar S, Topkaya T, Solmaz MY, et al. Finite element analysis of

the stress distributions in peri-implant bone in modified and standard threaded dental implants. *Biotechnol Biotechnol Equip.* 2016;30:127–133.

5. Petrie CS, Williams JL. Comparative evaluation of implant designs: influence of diameter, length, and taper on strains in the alveolar crest. A three-dimensional finite-element analysis. *Clin Oral Implants Res.* 2005;16:486–494.
6. Bozkaya D, Muftu S, Muftu A. Evaluation of load transfer characteristics of five different implants in compact bone at different load levels by finite elements analysis. *J Prosthet Dent.* 2004;92:523–530.
7. Ledermann PD, Schenk RK, Buser D. Long-lasting osseointegration of immediately loaded, bar-connected TPS screws after 12 years of function: a histologic case report of a 95-year-old patient. *Int J Periodontics Restorative Dent.* 1998;18:552–563.
8. Eltas A, Dundar S, Eltas SD, Altun O, Yolcu U, Saybak A. Accidental displacement of dental implants into both maxillary sinuses during surgery. *J Oral Implantol.* 2015;41:601–603.
9. Tresguerres IF, Clemente C, Blanco L, Khraisat A, Tamimi F, Tresguerres JA. Effects of local melatonin application on implant osseointegration. *Clin Implant Dent Relat Res.* 2012;14:395–399.
10. Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone.* 2003;33:919–926.
11. Cao JJ, Wronski TJ, Iwaniec U, et al. Aging increases stromal/osteoblastic cell-induced osteoclastogenesis and alters the osteoclast precursor pool in the mouse. *J Bone Miner Res.* 2005;20:1659–1668.
12. Kumar AA, Saravanan K, Kohila K, Kumar SS. Biomarkers in orthodontic tooth movement. *J Pharm Bioallied Sci.* 2015;7(suppl 2):S325–S330.
13. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop.* 2006;129:469.e1–469.e32.
14. Nakade O, Koyama H, Arijji H, Yajima A, Kaku T. Melatonin stimulates proliferation and type I collagen synthesis in human bone cells in vitro. *J Pineal Res.* 1999;27:106–110.
15. Lopez-Martinez F, Olivares Ponce PN, Guerra Rodriguez M, Martinez Pedraza R. Melatonin: bone metabolism in oral cavity. *Int J Dent.* 2012;2012:628406.
16. Cutando A, Gomez-Moreno G, Arana C, et al. Melatonin stimulates osteointegration of dental implants. *J Pineal Res.* 2008;45:174–179.
17. Witt-Enderby PA, Radio NM, Doctor JS, Davis VL. Therapeutic treatments potentially mediated by melatonin receptors: potential clinical uses in the prevention of osteoporosis, cancer and as an adjuvant therapy. *J Pineal Res.* 2006;41:297–305.
18. Munoz F, Lopez-Pena M, Mino N, Gomez-Moreno G, Guardia J, Cutando A. Topical application of melatonin and growth hormone accelerates bone healing around dental implants in dogs. *Clin Implant Dent Relat Res.* 2012;14:226–235.
19. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol.* 1982;11:318–326.
20. Wang D, Kunzel A, Golubovic V, et al. Accuracy of peri-implant bone thickness and validity of assessing bone augmentation material using cone beam computed tomography. *Clin Oral Investig.* 2013;17:1601–1609.
21. Satomura K, Tobiume S, Tokuyama R, et al. Melatonin at pharmacological doses enhances human osteoblastic differentiation in vitro and promotes mouse cortical bone formation in vivo. *J Pineal Res.* 2007;42:231–239.
22. Roth JA, Kim BG, Lin WL, Cho MI. Melatonin promotes osteoblast differentiation and bone formation. *J Biol Chem.* 1999;274:22041–22047.
23. Elgammal MY, Salem AS, Anees MM, Tawfik MA. Clinical and radiographic evaluation of immediate loaded dental implants with local application of melatonin: a preliminary randomized controlled clinical trial. *J Oral Implantol.* 2016;42:119–125.
24. Takechi M, Tatehara S, Satomura K, Fujisawa K, Nagayama M. Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study. *J Mater Sci Mater Med.* 2008;19:2949–2952.
25. Koyama H, Nakade O, Takada Y, Kaku T, Lau KH. Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. *J Bone Miner Res.* 2002;17:1219–1229.