Effects of Local Melatonin Application on Implant Osseointegration

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ABSTRACT

Purpose: The aim of this study was to assess the effect of local melatonin administration on bone osseointegration around implants in rabbit tibiae.

Material and Methods: Ten female, 6-month-old New Zealand rabbits were randomly divided into two groups: the experimental group, where five rabbits were treated with local application of melatonin (3 mg) to implant sites when placed into the rabbit tibia, and the control group, those who where without additive materials. Four weeks later, animals were sacrificed; tibiae were dissected from soft tissues and fixed in buffered formaldehyde, and then included in methacrylate. Histological sections were performed to be studied under light microscopy and analyzed morphometrically to evaluate the amount of bone to implant contact (BIC), trabecular area density, and cortical area density. One-way analysis of variance test was used for statistical evaluation. $p < .05$ was considered to be significant.

Results: Histological evaluation showed more trabecular reaction in the melatonin group. Morphometrical analysis showed a statistically significant increase in trabecular BIC in the melatonin group when compared with the control group (24.61% ± 2.87 vs 13.62% ± 1.44; $p < .01$). Cortical BIC was decreased in the melatonin group, without statistical significance (71.08 ± 3.63 vs 76.28 ± 2.57; $p = 0.31$). Trabecular area density was increased significantly in the melatonin group (8.68 ± 1.61 vs 4.02 ± 0.36; $p < .05$). Cortical area density was decreased significantly in the melatonin group (91.31 ± 1.6 vs 95.7 ± 0.5; $p < .05$).

Conclusion: Within the limitation of this animal study, local melatonin application at the time of implant placement might induce more trabecular bone at implant contact and higher trabecular area density.

KEY WORDS: BIC, bone, implants, local melatonin, rabbit

INTRODUCTION

Currently, the insufficient bone availability for prosthesis-guided implant placement is one of the most frequent challenges. In maxilla, the limited bone volume is related to poor bone quality that constitutes a challenge for the implant-supported rehabilitation. This poor bone quality is more evident in aged patients with atrophic and osteoporotic bones because of a reduction in the number and function of osteoprogenitor cells, an increase in osteoclastogenesis, and in local free radical concentrations in all types of cells, including osteoblasts. This could lead to a decrease in bone regenerative capability.[1,2]

Bone is a tissue submitted to a continuous remodeling process involving resorption of old bone by osteoclasts, and formation of new bone by osteoblasts. This process is regulated by growth factors produced in the bone marrow and osteoid matrix and by the action of

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systemic hormones, like estradiol, parathyroid hormone, growth hormone (GH), and, probably, melatonin.[3–7]

Melatonin is a tryptophan derived indolamine secreted by the pineal gland with well-known antioxidant properties[8–10] and free radical scavenging abilities.[11,12] Melatonin can also inhibit bone resorption by suppressing osteoclast activity.[13] Because melatonin was shown to also increase osteoblast proliferation and differentiation in vitro,[14,15] the authors thought that it could be a good agent to stimulate the peri-implant bone response during implant placement in rabbit tibiae.

The objectives of this study are, first, to observe whether local administration of melatonin during the implant insertion could induce differences in the histology of the peri-implant bone 4 weeks after surgery and second, to quantify the bone to implant contact (BIC) and bone area in the melatonin group versus the control group using a morphometrical analysis.

MATERIAL AND METHODS

This study included 10, 6-month-old female New Zealand rabbits weighing 3.5–4 kg. Standard laboratory chow and water ad libitum were available for rabbits. This study was approved by the local committee for the ethical use of animals of the Universidad Complutense of Madrid. The rabbits were randomly divided into two groups: the experimental group, treated with 3 mg of melatonin (Sigma, St. Louis, MO, USA) administered locally into the osteoctomy site in form of lyophilized powder into before implant placement and the control group (without treatment). In both groups, 20 implants from Zimmer Dental Implants (Tapered Screw-Vent, Zimmer Dental Inc., Carlsbad, CA, USA) of 8-mm length and 3.75-mm diameter were placed in the internal side of each tibia, i.e., one implant for each tibia and two implants for each animal. After intramuscular anesthesia with ketamine (Imalgene 1000, 0.75 mg/kg; Merial, Lyon, France) and xylazine (Rompun, 0.25 mg/kg; Bayer, Leverkusen, Germany), an incision was made on the internal side of the tibia, at the union of the diaphysis/proximal metaphysis. After detachment of the cutaneous-periosteal tissues, the bone bed was prepared for implant treatment following instructions of the implant manufacturer, using internal/external cooling Paragon drills. The implants were placed and achieved primary stability; then, the periosteal flap was sutured with Dexon sutures (Davis & Geck, Wayne, NJ, USA) and the skin with silk sutures. Oxytetracycline was administered orally to prevent postsurgical infection in both groups.[16] The surgical procedure was atraumatically performed by a single operator.

The animals were sacrificed 4 weeks after surgery. The tibiae were dissected from soft tissues and fixed in 10% buffered pH 7 formaldehyde. Then they were embedded into 2-hydroxyetylmetacrylate resin, according to Donath and Breuner's method,[17] so as allowing to cut simultaneously undecalcified bone and titanium with the Exaktâ microtome (Exakt Apparatebau, Norderstedt, Germany). Each section was ground with the Exakt® grinder (Exakt Apparatebau) until a final thickness of 50–80 mm was obtained for the study under light microscope (Leica, Wetzlar, Germany). Histological analysis was made using the Masson and Toluidine blue stains.[18] A morphometrical study was subsequently performed to quantify the bone response around the implants. These procedures were performed at the Department of Anatomy and Embriology in the Medical School from the University of Alcalá de Henares, Madrid.

The morphometrical study to quantify the newly formed bone around the implants was performed with a MIP-4 imaging analyzer (Digital Image System, Barcelona, Spain). The parameters calculated were BIC, cortical area density, and trabecular area density. BIC was defined as the length of bone surface border in direct contact with the implant perimeter (×100%).[19] BIC has been measured at the cortical zone in contact with the implant (cortical level) and at the medullar zone in contact with the implant (trabecular level) following a previously described method.[16] Cortical area density was defined as the bone area in the cortical level with respect to the total bone area (×100%). Trabecular area density was defined as the bone area in the trabecular level with respect to the total bone area (×100%).

The BIC and the areas mean values ± standard error of mean of each group were calculated. The data of the different groups were statistically tested by one-way analysis of variance. Results and statistical analysis were elaborated with the SPSS® software (Version 13.0.0, SPSS Inc., Chicago, IL, USA). $p < .05$ was considered to be significant.

RESULTS

Histological Results

Changes in cortical bone could be seen in the melatonin group showing a more remodeled bone with respect to

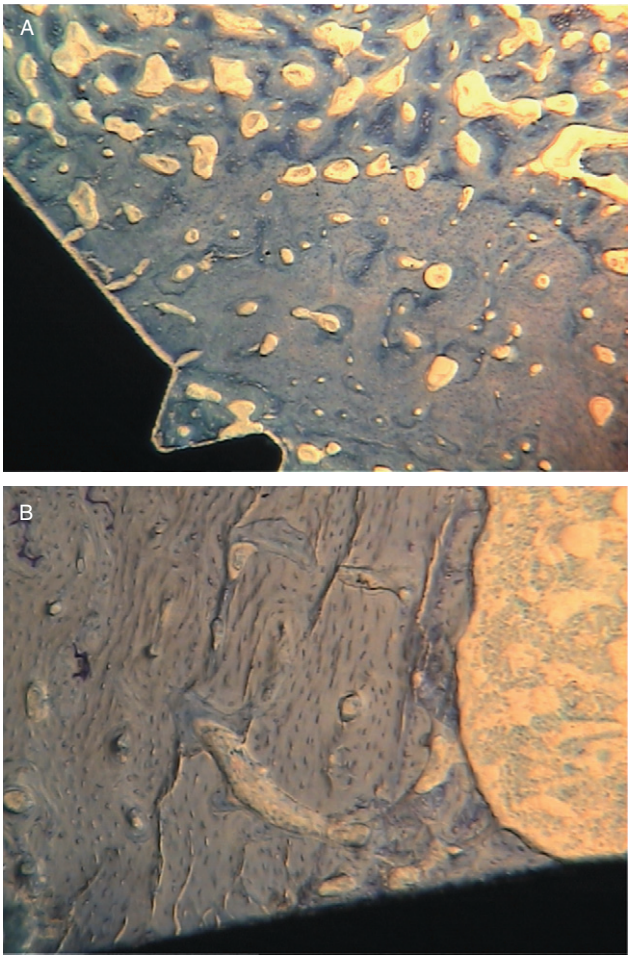

Figure 1 Toluidine blue sections (×30). Cortical reaction was more remodeled in the melatonin group (A) as compared with control (B). Adjacent to normal osteons into the old cortical, some areas containing bone marrow can be seen in melatonin group ([A] on the upper part), but not in control group (B).

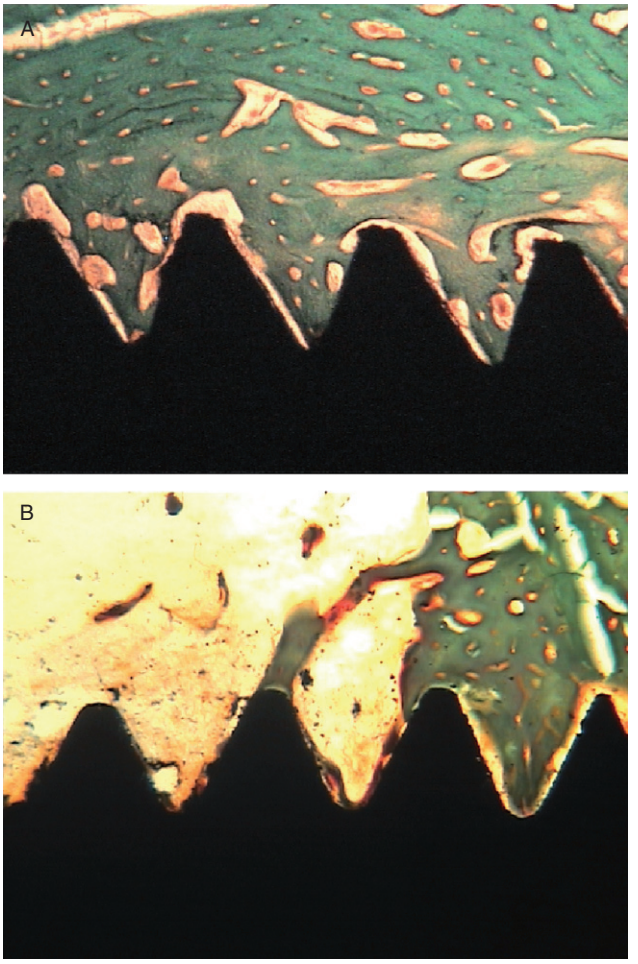

Figure 2 Masson sections (×30). Trabecular reaction in melatonin group (A) enhanced when compared with control group (B).

the control group. Adjacent to normal osteons in the old cortical, some areas containing bone marrow could be seen producing new osteoid tissue because of the presence of new fronts of osteoblasts (Figure 1A). This was markedly different when compared with the control group, in which only normal osteons could be seen along the cortical bone without the presence of bone marrow sites (Figure 1B).

In the medullar zone, more trabecular tissue could be seen in contact with implants of the melatonin group (Figure 2A) with respect to the control group (Figure 2B).

Morphometrical Evaluation

Cortical BIC directly measured by the MIP-4 was decreased in the melatonin group, without statistical significance (76.28% ± 2.57 vs 71.08% ± 3.63) ($p = .31$) (Figure 3). Trabecular BIC was significantly increased in the melatonin group, as compared with the control (13.62% ± 1.44 vs 24.61% ± 2.87) ($p = .0074$) (Figure 4).

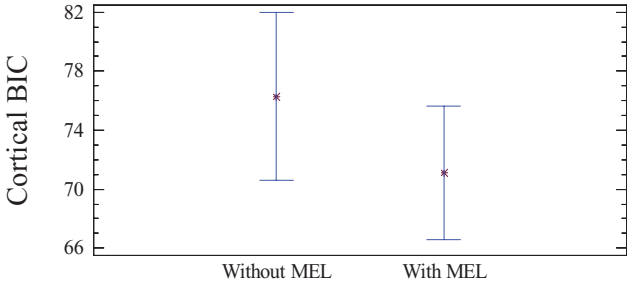

Figure 3 Cortical bone to implant contact (BIC) significantly increased in melatonin compared with control groups. MEL = melatonin.

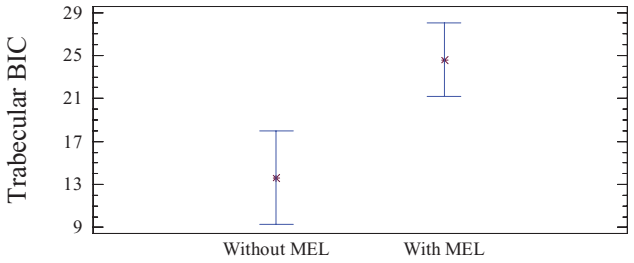

Figure 4 Trabecular bone to implant contact (BIC) significantly increased in melatonin compared with control groups. MEL = melatonin.

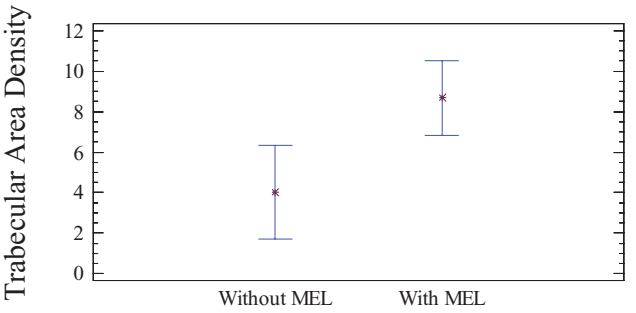

Figure 6 Trabecular area density significantly increased in melatonin compared with control groups. MEL = melatonin.

Cortical area density was significantly decreased in the melatonin group (91.31 ± 1.61 vs 95.7 ± 0.5) ($p = .043$), while trabecular area density was significantly increased in the same group when compared with controls (8.68 ± 1.61 vs 4.02 ± 0.36) ($p = .031$) (Figures 5 and 6).

DISCUSSION

Melatonin has been recently reported to stimulate osteoblasts proliferation and differentiation.[14,15] However, the role of melatonin administration in enhancing osteogenesis around titanium implants has been rarely studied.[20,21]

In this study, the histological analysis of the peri-implant bone has shown an increase in trabecular reaction with local melatonin administration. These new trabeculae originated from the endosteum in contact with the implant. Thus, it seems that local administration of melatonin during implant placement could directly stimulate osteoblasts from the endosteum. This was in agreement with the findings shown by Nakade et al.[15] Moreover, these results were found to be similar to those obtained by the authors using local administration of GH during surgical procedure.[16] GHs were able to enhance trabeculae from the endosteum and periosteum in contact to implant, even though it appeared more irregular and disorganized when compared with the actual data using melatonin in the present study.

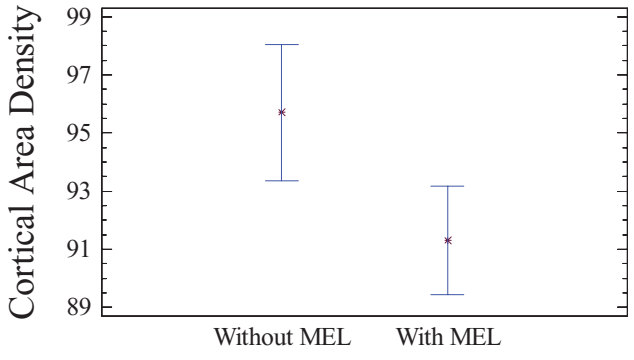

Figure 5 Cortical area density significantly decreased in melatonin compared with control groups. MEL = melatonin.

The results of the present study were also in agreement with those that studied the effects of systemic melatonin administration on bone. Koyama et al.[13] observed that pharmacological doses of melatonin were able to increase bone mass, bone mineral density, and trabecular volume in mice. This was apparently because of an inhibition of bone resorption. In addition, Satomura et al.[22] recently described the osteogenic effects of intraperitoneal melatonin administration. An increase in the volume of the newly formed cortical bone of mouse femora was observed.[22]

Histological changes in the cortical bone of the melatonin group had been observed in this study. This might be due to the relatively high local dosage of melatonin used (3 mg). At low magnification some "empty zones" could be observed in the cortical bone leading to an irregular pattern. Using higher magnification, these empty zones were identified as new fronts of bone formation in which bone marrow and osteoblasts synthesizing osteoid tissue could be seen. On the other hand, no histological changes could be observed in the cortical bone of the control group.

The morphometrical analysis showed a statistically significant increase in trabecular bone to implant contact in the melatonin group when compared with the control group ($p < .01$). These results could be explained by a direct stimulatory effect of melatonin on endosteal osteoblasts proliferation and differentiation, as previously documented by Roth[14] and Satomura.[22] These results were also in agreement with those of Cutando et al.,[20] where a statistically significant increase in the

BIC was found. This was applied on dogs 2 weeks after local application of melatonin into the mandibular ostectomy site prior to implant placement. Similar results were reported by Takechi et al.,[21] where systemic melatonin plus local Fibroblast Growth Factor 2 (FGF-2) were applied and resulted in an increase in bone formation around implants in rats.

Morphometrical results suggested the presence of less cortical bone to implant contact in the melatonin group when compared with the control group, although without statistical significance. The evidence of irregularities in cortical bone that were measured by the MIP-4 as empty lacunae might explain these results. However, new fronts of bone formation were demonstrated.

CONCLUSIONS

Considering the limitations of the present pilot study, it can be concluded that local melatonin administered in the osteoctomy site at the time of implant placement may induce more trabecular bone to implant contact and more trabecular area density.

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