Evaluation of Effects of Topic Melatonin on Implant Surface at 5 and 8 Weeks in Beagle Dogs

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ABSTRACT

Purpose: The aim of this study was to evaluate the effect of the topical application of melatonin on osteointegration of dental implants in Beagle dogs 5 and 8 weeks after their insertion.

Materials and Methods: For subsequent insertion of dental implants, upper and lower premolars and molars were extracted from 12 Beagle dogs. Each mandible received cylindrical screw implants of 3.25 mm in diameter and 10 mm in length. The implants were randomly assigned to the mesial and distal sites on each side of the mandible. Prior to implanting, 1.2 mg lyophylized powder melatonin was applied to one bone hole at each side of the mandible. None was applied at the control sites. Eight histological sections per implant were obtained for histomorphometric studies.

Results: After 5- and 8-week treatment periods, melatonin significantly increased the inter-thread bone ($p < 0.05$) and new bone formation ($p < 0.05$) in comparison to control implants in both weeks. There were no significant increases in the bone-to-implant contact and peri-implant bone ($p > 0.05$).

Conclusion: Topical application of melatonin may act as a biomimetic agent in the placement of endo-osseous dental implants at 5 and 8 weeks after the implantation.

KEY WORDS: bone, bone formation, dental implants, melatonin

INTRODUCTION

Melatonin is an N-acetyl-5-metoxy-tryptamine. It is a hormone that is synthesized principally in the pineal gland and other organs, and in many ways, influences circadian and circannual rhythms as well as sleep.[1,2] It has been shown that melatonin is also capable of acting on the intracellular functions independently of the action it has on the receptors on the cellular membranes. These receptors are found on the cells of the central nervous system and on the cells of some peripheral organs.[3–5] Because of its excellent lipophylic properties, melatonin is able to reach the cellular organelles and enter into any cell. In the interior of the cells, melatonin is capable of binding to some cytosolic proteins like kinase C, calmodulin, and calreticulin.[6–8] It also probably binds to quinine-reductase-2.[9] This is why melatonin is not considered as a hormone in the classical sense and, as shown previously, it acts as an antioxidant and as an anti-inflammatory agent,[10] and it is in the buccal cavity where these functions are realized.[11]

For the synthesis of melatonin, the pinealocytes take tryptophan from blood and converts it into serotonin via enzymes that decarboxylates and hydrolyzes the serotonin.

During nocturnal periods, serotonin is transformed into N-acetyl-serotonin by the enzyme N-acetyl-transferase and, owing to the action of hydroxyindol-O-methyl-transferase, it later modifies the N-acetyl-serotonin into melatonin. All the enzymes mentioned are not found exclusively in the central nervous system. Recently, these enzymes have also been found in other organs like retina, thymus, spleen, B lymphocytes, ovaries and testicles, and in the small intestine.[12,13] All the extrapineal melatonin are used locally as

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an autocoid and do not enter into the general circulation but only act on the organs mentioned. Melatonin does not act in specific organs. It is capable of reaching all tissues and acts in subcellular compartments[14,15] like the nucleus and mitochondria.[3,16]

As well, as the functions described, melatonin is also a mediator in osseous formation and its stimulation.[17] Previous studies have shown that melatonin stimulates the synthesis and proliferation of collagen type 1 fibers in human osteoblasts in vitro.[18] It stimulates osseous sialoprotein, alkaline phosphatase, osteopontin, and osteocalcin in osseous cultures carried out in rats.[19] Melatonin acts on osteoclasts, multinuclear osseous cells involved in the reabsorption of osseous matrix in different forms, one of which is via the formation of free radicals. Thus, melatonin, being an antioxidant, is able to affect the process, detoxifying free radicals which are produced during osteoclastogenesis,[20] leading to an inhibition of reabsorption of the bone. On the other hand, melatonin is capable of influencing the Receptor Activator for Nuclear Factor κ B Ligand (RANKL) system, suppressing its activity[11,21] and favoring the formation of new bone.

MATERIALS AND METHODS

Animals

Twelve male beagle dogs (University of Córdoba, Córdoba, Spain), about 18 months old, were used in this study. The animals were kept in standard cages. They were fed a commercial diet for dogs. All experiments were performed according to Spanish Government Guidelines and European Community Guidelines for experimental animal care.

Implants

The implants used (Microdent® system mini micro, Implant Microdent® System, Santa Eulalia de Ronsana, Barcelona, Spain) had a diameter of 3.5 mm and included a 10-mm long intraosseous portion and a 1-mm high transmucosal collar. Endosseal portion had an acid-etched surface.

Surgical Procedures

During the first operation, upper and lower premolars and molars were removed. The animals were not given food for 12 hours before anesthesia to prevent vomiting. They were sedated by means of an intramuscular injection of 0.5–1 mg/kg body weight acepromazine maleate,

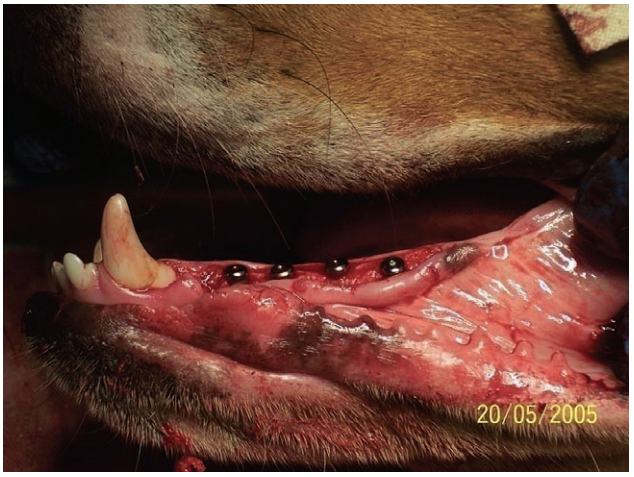

Figure 1 In situ implants placed in a beagle dog mandible (225 × 169 mm; 96 × 96 DPI).

and anaesthesia was induced by intravenous injection of 5–8 mg/kg body weight ketamine plus chlorbutol (5–8 mg/kg i.v.) and 0.05 mg/kg of atropine. The dogs were laid on their left side. Perioperatively, dexamethasone (2 mL i.m.) and amoxicillin (2 mL i.m.) were administrated immediately after surgery and subsequently for 7 days. The mucosa was rinsed with 0.2% chlorhexidine gluconate every day for 3 days. The animals were examined daily for a week after the operation for signs of wound dehiscence or infection and weekly thereafter to assess general health.

After a healing period of 2 months, implants were performed in a second operation. After a crestal incision, a full thickness mucoperiosteal flap was reflected. Sites were sequentially prepared to receive the implants in accordance with the protocol recommended by the manufacturer. A space of at least 5 mm was left between each implant for avoiding melatonin to affect other sockets. Each mandible received eight cylindrical screw implants of 3.25 mm in diameter and 10 mm in length.

Twelve dogs were divided into two groups (six animals per group) and, at 5 and 8 weeks, were sacrificed by inducing cardiac arrest by means of an intravenous injection of a 20% solution of pentobarbital (Dolethal, Vétoquinol, Buckingham, UK).

Two implants on each side (four in total) of the jaw were evaluated in this study: 24 implants at 5 weeks and 24 implants at 8 weeks. The remaining four implant sites were investigated for another study. The four implants were randomly assigned to the mesial and distal sites on each side of the mandible (Figure 1). Prior to implanting, a layer of 1.2 mg lyophylized powdered melatonin

TABLE 1 Histomorphometric Parameters in Dental Implants at 5 Weeks

Histomorphometric Parameters	Control Implants ($n = 12$)	Melatonin-Treated Implants ($n = 12$)
Bone-to-implant contact (% bone contact)	34.33 ± 2.35	38.74 ± 3.48
Total peri-implant area (%)	68.93 ± 3.97	70.02 ± 2.05
Inter-thread bone (%)	64.08 ± 8.68	72.56 ± 3.62*
Bone neoformation (%)	72.53 ± 4.54	80.48 ± 1.99*

*$p < .05$.

(Helsinn Advanced Synthesis SA, Biasca, Switzerland) was applied to one bone hole at each side of the mandible. None was applied at the control sites. Wound closure was carried out using single reabsorbable sutures (Dexon 3-0, Davis & Genk, Wayne, NJ, USA). Healing abutments were attached after implant placement. Two healing periods are evaluated in this paper, as the objective of the present study was the assessment of the response to the utilization of the melatonin.

Ground Sections

The implants were removed together with the surrounding bone and fixed in 10% neutral buffered formalin. The specimens were dehydrated in a graded series of alcohol, infiltrated, and embedded with Technovit 7200 VLC (Heraeus Kultzer, Dormagen, Germany). The samples were cut parallel (Exakt® Apparatebau, Norderstedt, Germany) to the longitudinal axis of the implant in an orovestibular direction and processed by the cutting-grinding method.[22] Eight sections were made per implant. Each section was ground down to the approximate thickness of 20 µm and stained using the Lévai–Laczkó staining technique.

Histomorphometric Measurements

For histomorphometric analysis, one calibrated masked examiner performed the histometric analysis using light microscopy and a PC-based image analysis system. Images magnified 40 were assessed digitally (DP12, Olympus, Nagano, Japan). Microimage 4.0 was used for image analysis (Media Cybernetics, Silver Spring, MD, USA). The analyses were all conducted by the same researcher, who was blind as to which group (experimental or control) each sample belonged.

- The bone-to-implant contact (BIC) was defined as the length of bone surface border that is in direct contact with the implant perimeter (100%) starting at the shoulder of the implant.
- The inter-thread bone density was defined as the area of bone inside the threads (100%) using the four most central threads in the vestibular section and four in the lingual section.
- The peri-implant bone density was determined as bone area/tissue area (100%). Surrounding the implant, using the central threads of the implants and up to a lateral distance of 1 mm from the threat, new bone and old bone were measured separately. It obtained eight squares with 1.2 mm^2 area. All images were calibrated using the software Microimage 4.0, and applying the Pythagorean theorem for distance calibration, which reports the number of pixels between two selected points: the scale bar was overimposed on each image by standard error of the mean (SEM). The linear remapping of the pixel values was used to calibrate the intensity of the images.
- The percentage of bone neoformation is defined as the area of bone which has formed after implant insertion. Newly formed bone is situated in the peri-implant area and between the implant threads.

Statistical Analysis

All data are expressed as the mean ± SEM. The *t*-test was employed to analyze differences among variables. Statistical analyses were carried out using the SPSS 11.0 computer program (SPSS, Chicago, IL, USA). The level of statistical significance was established at $p < .05$.

RESULTS

The results for the different histomorphometric parameters of osteointegration are presented in Tables 1 and 2. As regards the inter-thread bone parameter, it was observed that 5 and 8 weeks after surgery, melatonin had

TABLE 2 Histomorphometric Parameters in Dental Implants at 8 Weeks

Histomorphometric Parameters	Control Implants ($n = 12$)	Melatonin-Treated Implants ($n = 12$)
Bone-to-implant contact (% bone contact)	31.47 ± 3.09	34.29 ± 2.49
Total peri-implant area (%)	71.07 ± 1.61	71.51 ± 2.19
Inter-thread bone (%)	80.57 ± 2.28	82.49 ± 3.87*
Bone neoformation (%)	88.09 ± 1.38	90.71 ± 2.25*

*$p < .05$.

increased the inter-thread bone in a statistically significant manner ($p < .05$). In relation to the new bone formation, melatonin brought about a statistically significant increase in bone density around the implants ($p < .05$). In BIC parameter and peri-implant bone, melatonin-enhanced bone density is not statistically significant ($p > .05$). Figures 2 and 3 display a histological section of an untreated implant (5 and 8 weeks, respectively), which shows the small amount of new bone tissue in contact with the implant, and the large amount of vascular and conjunctive tissues and small quantity of bone that formed in the peri-implant area. In contrast, Figures 4 and 5, which present a histological section of a melatonin-treated implant (5 and 8 weeks), show that there is a greater amount of new bone tissue in contact with the implant, with an increment in bone growth, and scant vascular and conjunctive tissues formation in the peri-implant zone.

DISCUSSION

A variety of substances have been used to enhance peri-implant bone response: growth factors,[23] morphogenetic proteins,[24] and, more recently, hormones, such as growth hormone and melatonin.[25] Links between melatonin and bone metabolism have been documented in many studies.[18,19,21,26] In these investigations, melatonin acted on the bone as a local growth factor, with paracrine effects on nearby cells.[27,28] Melatonin has been found to be a significant modulator of the metabolism of calcium, and prevents osteoporosis and hypocalcemia

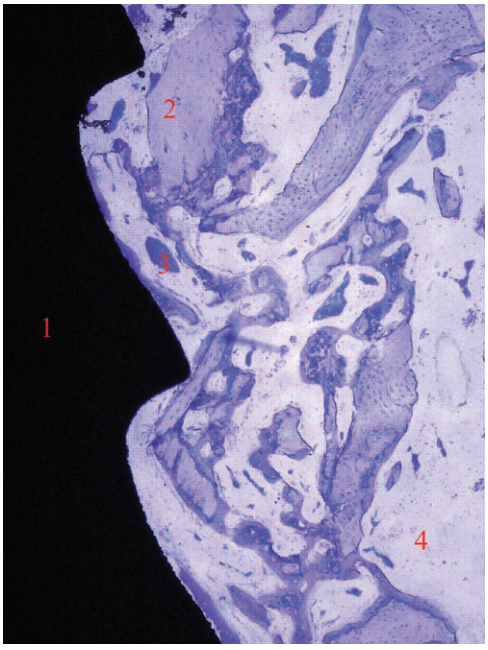

Figure 2 Histological image of control implant at 5 weeks. "1," implant section; "2," mature bone; "3," new bone; and "4," conjunctive and vascular tissue (480 × 639 mm; 72 × 72 DPI).

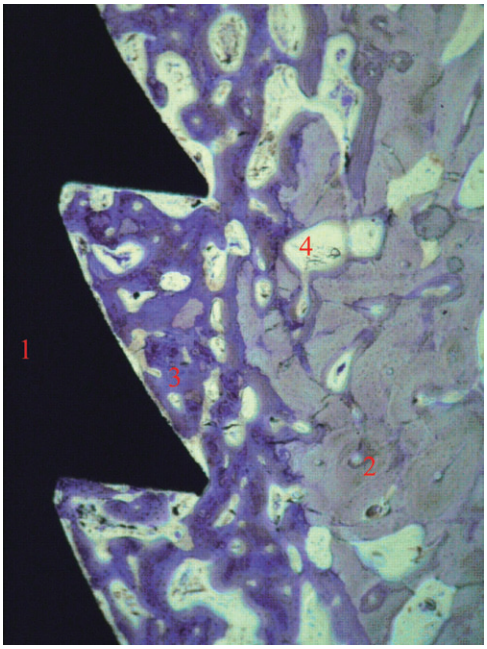

Figure 3 Histological image of melatonin-treated implant at 5 weeks. "1," implant section; "2," mature bone; "3," new bone; and "4," conjunctive and vascular tissue (480 × 639 mm; 72 × 72 DPI).

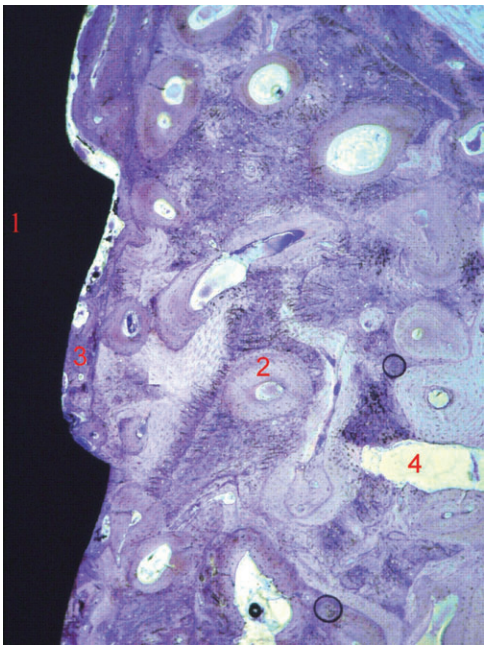

Figure 4 Histological image of control implant at 8 weeks. "1," implant section; "2," mature bone; "3," new bone; and "4," conjunctive and vascular tissue (360 × 479 mm; 96 × 96 DPI).

in certain cases, probably because of its interaction with other bone regulatory factors, such as parathormone, calcitonin, or prostaglandins.[29,30] One important action of melatonin is the formation of bone cells. In this regard, several studies have shown that melatonin stimulates the proliferation and differentiation of human osteoblasts in vitro, as well as the synthesis of type I collagen and other proteins of the bone matrix.[18,19,31] Melatonin stimulates differentiation in preosteoblast lines; thus, melatonin-treated cells matured into osteoblasts after a period of 12 days, in comparison to 21 days for the preosteoblasts of the control group.[19]

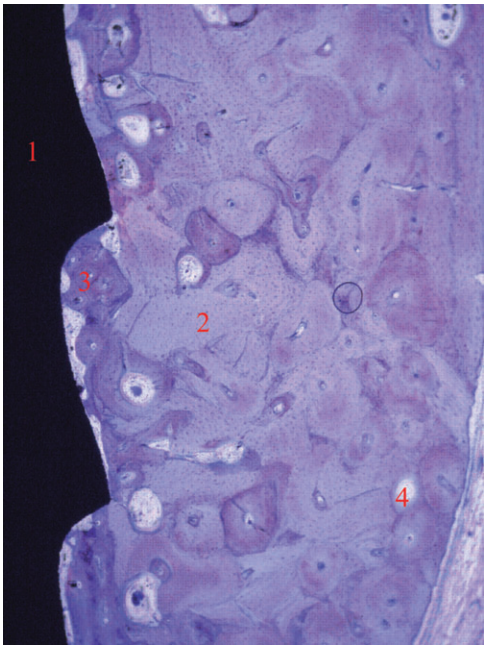

Figure 5 Histological image of melatonin-treated implant at 8 weeks. "1," implant section; "2," mature bone; "3," new bone; and "4," conjunctive and vascular tissue (480 × 639 mm; 72 × 72 DPI).

Our results show that BIC and total peri-implant bone area at 5 and 8 weeks are higher in melatonin-treated implant than in controls, according to previous studies by Cutando and colleagues at 2 weeks,[32] but not in a significant manner; this demonstrates that melatonin has a maximum activity over those parameters at 2 weeks. From our point of view, and based on other studies,[32] reference to BIC signifies a direct action of melatonin on the osteoblasts, which induces a higher rate of maturity of preosteoblasts to osteoblasts both in quantity and velocity, with a higher rate of production of the osseous matrix and its corresponding calcification. Also, other studies[31] show that melatonin could favor bone formation via other routes, as is the case of stimulation of certain genes which control the presence of determined proteins at the osteoid matrix. Accordingly, it has been shown that melatonin is capable, after a period of 5 and 9 days, of stimulating the presence of osteocalcin, sialoprotein, and alkaline phosphatase.

Our findings show a greater percentage of inter-thread bone and new bone formation around the implants treated with topical melatonin in a significant manner, which are in line with previously published findings at 2 weeks. Support for this relationship is to be found in the fact that the genes of a large portion of bone matrix (bone sialoprotein, alkaline phosphatase, osteocalcin, and osteonectin) contain the sequence of bases (RGGTCA) necessary for the nuclear receptor of melatonin to bind with its promoting zone. Moreover, it may be that this increase in the formation of bone tissue is also mediated by the membrane receptors for the indolamine, as treatment with luzindole or pertussis toxin reduces bone sialoprotein and alkaline phosphatase expression.[19,31]

Inter-thread bone is an intermediate situation of new bone in contact with the implant by stimulation of osteoblasts and the old bone in the total peri-implant area by osteoclast inhibition, where grinding has eliminated bone, and melatonin continues its activity passing

on from the second to the fifth week and to the eighth week with clear statistical significance. This is the same situation which occurs with reference to neoformation osseous. In certain ways, this supports the same action which occurs at the inter-thread bone level either by remodeling the existing bone or increasing the production of osteoid matrix by osteoblasts.

Melatonin also acts by inhibiting the action of osteoclasts. This is a direct action lasting a very short time, as there is no remodelation of bone but only the existence of inter-thread bone and total peri-implant bone area. For this reason, melatonin continues acting on that part of the bone that has suffered aggressive placement of the implant, necessitating remodelation by osseous matrix production which requires 5 to 8 weeks. Moreover, osteoclasts secrete another enzyme, acid phosphatase, that is resistant to tartrate, which has a binucleate center with an active iron which reacts with hydrogen peroxide and, via the Fenton reaction, produces the hydroxyl radical (OH).

It appears that melatonin acts at the level of the osteoclast lacuna, because of its antioxidant properties and its ability to neutralize reactive species, thereby inhibiting bone resorption.[26,27] After implant placement, despite the care with which the surgical procedure is performed, bone necrosis often occurs around the implant and there is an inflammatory reaction as a direct consequence of surgery.[33,34]

This inhibition of bone resorption may be reinforced by another reaction induced by melatonin on the osteoclastogenesis process. According to some authors, the application of indoleamine at concentrations ranging from 5 to 500 lm lowers, in a dose-dependent manner, the expression of mRNA from the RANK and increases levels of both osteoprogeterin (OPG) as well as of mRNA from the OPG in preosteoblast cell lines MC3T3-E1.[21] This indicates that melatonin may bring about a reduction in bone resorption and an increase in bone mass because of its repression of osteoclast activation by means of RANK.[26] These actions of melatonin on bone tissue are of interest, as it may be possible to apply melatonin during endo-osseous dental implant surgery as a biomimetic agent.[35] As a result, the process of healing may be more precise, initial conditions of receptor tissues may be enhanced, the period of osteointegration and settling of the implant may be reduced, and, therefore, the quality of life of the patient may be improved.

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REFERENCES

1. Vanecek J. Melatonin binding sites. J Neurochem 1988; 51:1436–1440.
2. Cajochen C, Krauchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. J Neuroendocrinol 2003; 15:432–437.
3. Acuna-Castroviejo D, Pablos MI, Menendez-Pelaez A, Reiter RJ. Melatonin receptors in purified cell nuclei of liver. Res Commun Chem Pathol Pharmacol 1993; 82:253–256.
4. Hirose T, Smith RJ, Jetten AM. ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. Biochem Biophys Res Commun 1994; 205:1976–1983.
5. Carlberg C, Hooft van Huijsduijnen R, Staple JK, DeLamarter JF, Becker-André M. RZRs, a new family of retinoid-related orphan receptors that function as both monomers and homodimers. Mol Endocrinol 1994; 8:757–770.
6. Anton-Tay F, Ramirez G, Martinez I, Benitez-King G. In vitro stimulation of protein kinase C by melatonin. Neurochem Res 1998; 23:601–606.
7. Huerto-Delgadillo L, Antón-Tay F, Benítez-King G. Effects of melatonin on microtubule assembly depend on hormone concentration: role of melatonin as a calmodulin antagonist. J Pineal Res 1994; 17:55–62.
8. Macías M, Escames G, Leon J, et al. Calreticulin-melatonin. An unexpected relationship. Eur J Biochem 2003; 270:832–840.
9. Tan DX, Manchester LC, Terron MP, Flores LJ, Tamura H, Reiter RJ. Melatonin as a naturally occurring co-substrate of quinone reductase-2, the putative MT(3) melatonin membrane receptor: hypothesis and significance. J Pineal Res 2007; 43:317–320.
10. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derives: a never ending interaction of melatonin with reactive oxygen and reactive nitrogen species? J Pineal Res 2007; 42:28–42.
11. Cutando A, Gómez-Moreno G, Arana C, Acuña-Castroviejo D, Reiter R. Melatonin: potential functions in the oral cavity. J Periodontol 2007; 78:1094–1102.

12. Siu AW, Maldonado M, Sanchez-Hidalgo M, Tan DX, Reiter RJ. Protective effects of melatonin in experimental free radical-related ocular diseases. J Pineal Res 2006; 40:101–109.
13. Brzozowski T, Konturek PC, Zwirska-Korczala K, et al. Importance of the pineal gland, endogenous prostaglandins and sensory nerves in the gastroprotective actions of central and peripheral melatonin against stress-induced damage. J Pineal Res 2005; 39:375–385.
14. Acuña-Castroviejo D, Escames G, Macías M, et al. Cell protective role of melatonin in the brain. J Pineal Res 1995; 19:57–63.
15. Tan DX, Manchester LC, Reiter RJ, et al. Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochim Biophys Acta 1999; 1472:206–214.
16. Acuña CD, Escames G, Carazo A, León J, Khaldy H, Reiter RJ. Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. Curr Top Med Chem 2002; 2:133–151.
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. Nakade O, Koyama H, Ariji 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.
19. Roth JA, Kim BG, Lin WL, Cho MI. Melatonin promotes osteoblast differentiation and bone formation. J Biol Chem 1999; 274:22041–22047.
20. Maldonado MD, Murillo-Cabezas F, Terron MP, et al. The potential of melatonin in reducing morbidity-mortality after craniocerebral trauma. J Pineal Res 2007; 42:1–11.
21. 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.
22. 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 Med 1982; 11:318–326.
23. Lynch SE, Buser D, Hernandez RA, et al. Effects of the platelet-derived growth factor/insulin-like growth factor-I combination on bone regeneration around titanium dental implants. Results of a pilot study in beagle dogs. J Periodontol 1991; 62:710–716.
24. Cook JJ, Haynes KM, Werther GA. Mitogenic effects of growth hormone in cultured human fibroblasts. Evidence for action via local insulin-like growth factor I production. J Clin Invest 1988; 81:206–212.
25. Tresguerres IF, Clemente C, Donado M, et al. Local administration of growth hormone enhances periimplant bone reaction in an osteoporotic rabbit model. Clin Oral Implants Res 2002; 13:631–636.
26. Cardinali DP, Ladizesky MG, Boggio V, Cutrera RA, Mautalen C. Melatonin effects on bone: experimental facts and clinical perspectives. J Pineal Res 2003; 34:81–87.
27. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent endogenous hydroxyl radical scavenger. Endocrine J 1993; 1:57–60.
28. Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM. Evidence for melatonin synthesis in mouse and human bone marrow cells. J Pineal Res 2000; 28:193–202.
29. Hakanson DO, Penny R, Bergstrom WH. Calcemic responses to photic and pharmacologic manipulation of serum melatonin. Pediatr Res 1987; 22:414–416.
30. Ostrowska Z, Kos-Kudla B, Nowak M, et al. The relationship between bone metabolism, melatonin and other hormones in sham-operated and pinealectomized rats. Endocr Regul 2003; 37:211–224.
31. Radio NM, Doctor JS, Witt-Enderby PA. Melatonin enhances alkaline phosphatase activity in differentiating human adult mesenchymal stem cells grown in osteogenic medium via MT2 melatonin receptors and the MEK/ERK (1/2) signaling cascade. J Pineal Res 2006; 40:332–342.
32. Cutando A, Gómez-Moreno G, Arana C, et al. Melatonin stimulates osteointegration of dental implants. J Pineal Res 2008; 45:174–179.
33. Mitri FF, Yoshimoto M, Allegrini Junior S, et al. Histological findings in titanium implants coated with calcium phosphate ceramics installed in rabbit's tibias. Ann Anat 2005; 187:93–98.
34. Lee MC, Yoshino F, Shoji H, et al. Characterization by electron spin resonance spectroscopy of reactive oxygen species generated by titanium dioxide and hydrogen peroxide. J Dent Res 2005; 84:178–182.
35. Simon Z, Watson PA. Biomimetic dental implants – new ways to enhance osseointegration. J Can Dent Assoc 2002; 68:286–288.