Growth hormone and Melatonin as Biomimetics (An Experimental Study)

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Abstract: Background: The foundation of implant success is osseointegration, a concentrated effort to provide bone in a faster and improved osseointegration process was an important research topic. **Aim:** The aim of the study is to evaluate the effect of local application of growth hormone and melatonin on osseointegration around immediate implants after 1, 2 and 3 months in dogs. **Material and Methods:** the 3rd mandibular premolar tooth was extracted bilaterally in six adult male dogs. Twelve implants were placed in their distal sockets**.** The test group received a mixture of growth hormone and melatonin before immediate implant incersion (right side), while in the control group the socket was left without any treatment before implant incersion (left side). Subgroups at 1, 2 and 3 months follow up periods were applied for both groups. After animal sacrifice, the bone blocks were subjected to histomorphometric assessments. **Results**: bone implant contact and bone area were statistically non significant in all test periods. In conclusion, Growth hormone and melatonin mixture did not affect bone implant contact and bone area of the new bone formed around immediate implant.
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1. Introduction:

An immediate dental implant is the implant that replaces an extracted tooth on the spot (immediately after extraction). It was first reported by Lazzara, in 1989. It has been determined that it is not always possible to prepare osteotomy without clinical voids or gaps surrounding the fixture. This leads to the next logical phase of implant therapy focused on placing dental implant in extraction socket immediately upon tooth removal, which become a very popular topic with reliable outcomes. The advantages of immediate implant are; Shortening the treatment time, marginal bone preservation, enhance soft tissue support, decrease the number of surgeries and the cost.[1-10]

Biomimetics is the investigation of addition of bioactive agents to titanium implant surfaces. A biomimetic agent is the material that has been designed to elicit specific cellular response that replicates the anatomy and function. Growth hormone GH and Melatonin are examples of biomimetics[11]. Their local application in a single large dose at surgery time gained great acceptance. Local GH accelerates bone remodeling process first by stimulating resorption and later by stimulating bone formation periostealy, trans-cortically and endostealy.[12] Melatonin stimulate bone formation by stimulating (osteoblastic differentiation and stimulate gene expression of certain bone proteins), and inhibiting osteoclastogenesis. Moreover, melatonin preserve functional integrity of certain antioxidant enzymes.[13-15] Accordingly, the combination between growth hormone and melatonin may influence the process of healing. Our study was directed toward the use of immediate implant in the lower jaw, without the use of guided tissue regeneration depending on the hypothesis that GH and Melatonin as biomimetics are accepted bone stimulators in such a situation.

Histomorphometric parameters such as the total bone area and the total bone perimeter, they are related to bone mass and structure. The assessment of bone structure is important due its relationship with bone strength .[1,13,16-18]

Molecular investigations have contributed to define cellular response to titanium as a biocompatible and advantageous as it showed relatively low inflammatory reaction within the adjacent cells. The biological tissue interact with the outermost atomic layers of the implant, it is logical to suppose that surface modification would serve to influence the cell response (bioactive).[19]

2.Material and Methods:

This study was performed at the Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Suez Canal University. Six adult healthy male mongrel dogs with average weight of 10-20 kg, comparable age (1- 2 years) with complete permanent dentition were included in this study. Animals were divided into 3 groups according to the

time of sacrifice (4, 8 and 12 weeks). Each of these groups includes two animals.

Premedication with intramuscular injection of Valpam® (AMOUN Pharmaceutical Industries co (APIC) El Salam city, Cairo, Egypt.) in a dose of 0.5mg / KG body weight. Induction anesthesia was carried out using IV administration of Sodium thiopental 2.5% solution (Egyptian International Pharmaceutical Industries co (EIPICO) 10th of Ramadan city, Egypt) 20-30mg/kg body weight. Maintenance anesthesia was achieved using IV thiopental sodium 20mg / kg body weight in dilution of 2.5% saline solution. Animals were subjected for extraction of the third premolar bilaterally. The crown was hemisectioned vertically with tungsten carbide fissure bur in a surgical hand piece under sterile saline irrigation into two halves. Elevators were used gently to extract roots without trauma to the alveolar bone.

An osteotomy preparation was created in the distal root socket to receive legacy direct Implant (ScrewPlant TM Molibu Hills Road Colobosas Hills California 91301 USA), using low speed drills extending 2-3mm bellow the apex of the socket. Successive drills of 2.2, 2.8 and 3.4 were used to prepare the socket for receiving 11.5mm length and 3.7 mm wide implant. This was based on the pilot study done to verify the ideal length and width for the distal premolar root.

The right side was treated by a mixture of 4IU (1.6mg) **Growth hormone** (Somatropin® 4 IU SEDICO Pharmaceutical Industries co, 6thoctober city. EGYPT) in a vial form and 3mg **Melatonin (**VIVA-MAX3® product of AMOUN Pharmaceutical industries co (APIC) El Salam city, Cairo, Egypt) in a powder form before implant insertion while the left side was left as a control. Melatonin tablet was crushed in a sterile dappen dish until powder form was established; GH powder was injected with 1mml of sterile water supplied by the manufacturer, gentile injection was important to allow all powder to dissolve without remnants. Both components were mixed and put in a plastic syringe for easy application in the wound site. Implant insertion followed by simple rotation movements using the insertion tool to achieve maximum stabilization. The ratchet was then used until submerged position reached. Cover screws were placed, releasing incisions followed for tension free wound closure with continuous sutures using 4.0 vicryl sutures.

Postoperative antibiotic therapy (flumox®) 500 mg/2ml IM every 24 hours (Egyptian International Pharmaceutical Industries co (EIPICO) 10th of Ramadan city, Egypt) and analgesics (Cataflam®) 75mg/2ml IM (NOVARTIS PHARMA Pharmaceutical industries co, Cairo, Egypt) was administrated once a day for 3 days. Plaque control with chlorohexidine mouth lavage was used. Clinical follow up for each animal regarding any complications was registered (visual signs of inflammation, mobility and dehiscence). Animal sacrifice was done after 4, 8 and 12 weeks postoperatively. Sacrifice was performed by rapid IV injection of 1mg 5% thiopental sodium. None of the implants in this study were loaded. The mandibles were dissected, removed, cleaned, sectioned and fixed in neutral formalin 10% for further investigations.

Histomorphometric study:

The samples were prepared and analyzed at the histopathology Unit, Oral Pathology department, Faculty of Oral and Dental Medicine, Cairo University. The bone blocks were dehydrated in ascending graded alcohol series for 10 hours and embedded in a low viscosity embedding medium (acrylic resin). After acrylic resin polymerization, sections were made through the longitudinal axis of the implants. The embedded tissue was cut into thin ground sections with low speed diamond wheel using tap water lubrication. The sections were sanded on an abrasive paper under tap water to obtain a uniform surface finish. The ground sections emphasize BIC % which was measured by the ratio between the linear measurement of the middle threads (line contacting the implant threads) and the linear measurement of the bone contacting the middle threads (line contacting bone between threads), 100 % means complete contact. Moreover, bone area % was measured by the ratio between bone in the inter-thread area (bone traced by the tracer) and the inter thread space (space traced) in the middle ⅓ by Leica software analysis (Figs. 1a & 1b). Paired Student's t-Test was used to compare the mean % values of bone implant contact between control group and experimental groups. A *p*-value $p < 0.05$ was considered significant.

3. Results:

Histomorphometric analysis:

Stereomicroscope was used to assess the degree of osseointegration as regard bone implant contact and bone area.

Bone implant contact (tables 1, 2)

Assessment of osseointegration was evaluated by calculating the ratio between linear contact of new bone to implant threads and the metal line of the middle ⅓ of implant, along the follow up periods at the mesial and distal surfaces of each implant for control and experimental groups. Equal ratios were demonstrated between control and test implants in all test periods. The control and test groups were compared separately during the follow up periods. A non-significant difference was recorded between all periods (tables 1 & 2).

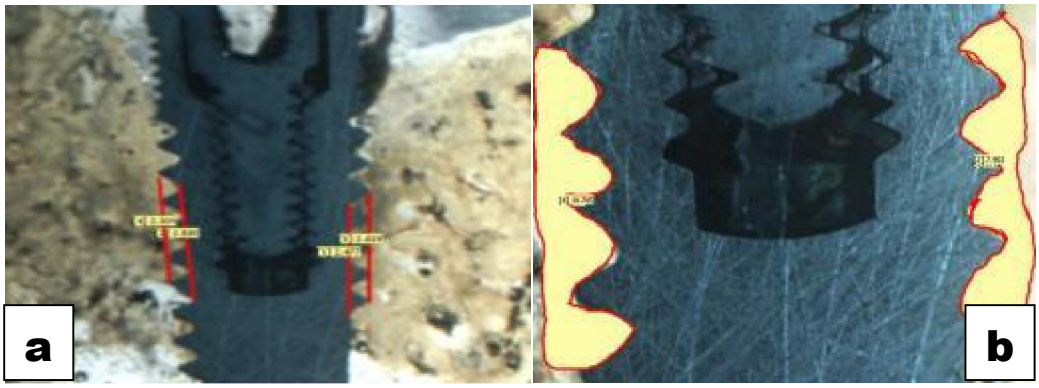

Fig.1a: A photomicrograph showing bone implant contact measurements (x10). Fig.1b: A histomorphometric photograph showing bone area measurements (x30)

Table (1): Comparison between the means of bone implant contact in control and experimental groups

Follow up periods	Groups		*P* value
	Experimental	Control	
	Mean ± SD	Mean ± SD	
1M	1 ± 0.23	1 ± 0.11	0.773
2M	0.89 ± 0.12	0.89 ± 0.27	0.149
3M	0.99 ± 0.04	0.98 ± 0.1	0.749

*Values considered significant when the *p* value ≤ 0.05

Table (2): Comparison between the bone implant contact means along the study periods in the control and experimental groups each alone

Groups	Control			Experimental		
Study periods	1-2	1-3	2-3	1-2	1-3	2-3
P-value	0.465	0.144	0.465	0.144	0.715	0.273

*Values considered significant when the *p* value ≤ 0.05

Bone area

Assessment of osseointegration was evaluated by calculating the ratio between the area occupied by new bone and the total inter-thread area, in the middle ⅓ of the implant along the follow up periods at the mesial and distal surfaces of each implant. Demonstrating higher bone area at the test group in the first and second months, while in the 3rd month they were equal the difference was statistically non significant (Table 3). The control and test groups were compared separately during the follow up periods. A non significant difference was recorded between all periods (Table 4).

Table (3): Comparison between the means of bone area in control and experimental groups.

Study periods	Groups		*P*- value
	Exp.	Cont.	
	Mean ± SD	Mean ± SD	
1M	0.667 ± 0.09	0.57 ± 0.28	0.773
2M	0.59 ± 0.217	0.7 ± 0.11	0.564
3M	0.7 ± 0.045	0.69 ± 0.06	0.376

*Values considered significant when the *p* value ≤ 0.05

Table (4): Comparison between the means of bone area along the study periods in the control and experimental groups each alone.

Groups	Control			Exp.		
Study periods	1-2	1-3	2-3	1-2	1-3	2-3
P-value	0.456	1	0.273	0.456	0.581	0.456

*Values considered significant when the *p* value ≤ 0.05

4. Discussion:

It is challenging to explore the concept of a single large dose of growth hormone (GH) and melatonin in fresh extraction socket. Such information will be a key to developing biomimetic materials. Growth hormone and Melatonin are examples of biomimetics. Animals treated with GH showed increased bone formation without bone mineral density BMD in short term treatment while untreated animals showed more BMD and bone area than GH group. GH locally applied in the mandible of rat stimulate local bone formation[12,20]. Growth hormone (GH) could exert an impulse effect in the first hours of the process of osseointegration by accelerating the recruitment of preosteoblasts. Concerning the GH and its binding peptide that had a network shape that favors osteoconduction, favors cells to settle down and tissue to mineralize. Seventy two Suggestions of synergy between different stimulating agents is a working concept as GH itself cannot keep a long term effect. Hence the trial of synergy between melatonin and GH in this study.

Melatonin stimulates gene expression of certain bone proteins (osteopontain, osteocalcin and alkaline phosphatase). The relation between melatonin and calcium metabolism was noticed. The relation between melatonin, growth hormone and bone has been studied on delayed implant type which demonstrated synergy between them[21]. They act as bone stimulators by increasing osteoblastic lineage differentiation and proliferation, matrix production and potentiating mineral deposition. Bone healing around immediate implants involves bundle bone that possesses a high remodeling capacity. New research protocols suggesting mandatory initial stability, five walls defect was mandatory to maintain a firm blood clot which is an indicator for immediate implant (adequate bone room), to enhance the primary stability, immediate implant should be stabilized using the surrounding socket wall and bone beyond the root apex at least 3mm.[22-23]On the otherhand, the sample preparation was challenging in analysis of the bone–implant interface. It was technically difficult to prepare specimens of intact titanium bone interface. In the present study, the middle ⅓ area of implant was recommended for histological evaluation as the coronal ⅓ may result in errors attributed to the downward proliferation of epithelium, while apical ⅓ was conducted to perforations of the upper part of mandibular canal.[24]

The results demonstrated the negative effect of single dose of growth hormone and melatonin mixture on bone implant contact (BIC) and bone area. This may be attributed to the circulating half life of melatonin and growth hormone (23 and 20 min respectively). After 5 weeks the remodeling process around dental implant in a dog mandible was healed. The process of bone modeling and remodeling appears to be more influenced by the dimension of the socket rather than the implant surface modification, as bone remodeling was more pronounced at narrower sockets, when implants adapted more intimately to the socket walls. Many authors documented the link between melatonin and bone metabolism, stated that melatonin acted on bone as a local growth factor with paracrine effect on neighboring cells. Single large dose of growth hormone and melatonin did not affect BIC as equal results between test and control demonstrated a biocompatible mixture that did not inhibit the contact. While higher results of bone area in the first and second months may be attributed to carbonate substitution in the hydroxyl apatite lattice that increase the surface area (from needle like to plate like), taking into consideration that the increase is not significant. Suggestion of repeated local applications and continous slow release may be choice for further researches.[25-27]In conclusion; a single large dose of growth hormone and melatonin mixture at immediate implant site insertion did not significantly increase the bone implant contact and bone area.

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