In vivo immunohistochemical investigation of the effect of the topical application of growth hormone on the osseointegration of cpTi implant

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ABSTRACT

Background: Dental implants are a suitable option for the replacement of some or all missing teeth. The successful insertion of a biocompatible material into living tissue with little to no evidence of rejection has revolutionized medicine and dentistry. An increase in bone response was observed with local administration of growth hormone around dental implants. Growth hormone may act as a bone stimulant in the placement of endosseous dental implants and enhances osseointegration. The aim of the study was to evaluate immunohistochemically the effect of the topical application of growth hormone on the osseointegration of cpTi implant.

Materials and Methods: Eighty titanium screw implants were inserted in the tibia of the forty adult rabbits. Growth hormone was applied on experimental implants. Immunohistochemical tests were performed on the implants of both control and experimental groups for (3 days1, 2, and 6 weeks) healing intervals.

Results: Titanium implants coated with growth hormone revealed an early bone formation, minerlization and maturation in comparison to control. Immunohistochemical findings revealed positive expression for VEGF in experimental implant in comparison to control one.

Conclusion: Topical application of growth hormone may act as a bone stimulant in the placement of endosseous dental implants and enhances osseointegration.

Key words: Growth hormone, dental implant, biochemical bone marker. (J Bagh Coll Dentistry 2014; 26(2): 58-63).

الخلاصة

المقدمة: إن الغرسة السنية هي الطريقة المثلى لتعويض الأسنان المفقودة. إن الغرس الناجح للمواد المطابقة للجسم في داخل الأنسجة الحية مع عدم حدوث رفض لهذه المواد قد احدث ثورة في الطب عامة وطب الأسنان خاصة وقد لوحظ زيادة في استجابة العظم عند إضافة هرمون النمو حول الغرسة السنية. إن هرمون النمو ربما يعمل كمحفز للعظم في عملية استبدال الغرسان السنية وكذلك يحفز تكامل التعظم. الهداف الدراسة التقييم النسيجي والنسيجي المناعي الكيميائي لتأثير الاستخدام الموضعي لهرمون النمو على الاندماج العظمي باستخدام غرسة التيتانيوم.

المواد والعمل: تم إدخال ثمانون غرسة من التيتانيوم في عظمة القصبة (الساق) لأربعين أرنب ناضج. تم إضافة هرمون النمو لغرسات التجربة. استخدمت فحوصات نسيجية مناعية كيميائية اجريت لكافة الغرسات ولكلا المجموعتين التحكم والتجربة ، ولكل مراحل الالتئام (3 ايام ، 1 ، 2 ، 6 اسابيع).

النتائج: غرسة التيتانيوم المغطاة بهرمون النمو أظهرت تمام وتوج التكوين العظمي في مراحل مبكرة مقارنة بمجموعة التحكم أعطت النتائج النسيجية المناعية الكيميائية إظهارا ايجابيا عاليا لهرمونات النمو وخصوصا لعامل النمو الوعائي البطاني (VEGF) في غرسات التجربة بالمقارنة لغرسات التحكم.

الاستنتاج: هذه الدراسة تخلص إلى الاستنتاج بان الاستخدام الموضعي لهرمون النمو يعمل كمحفز للعظم في عملية غرسات الأسنان ويحفز عملية تكامل التعظم.

كلمات مفتاحيه:- هرمون النمو ، الغرسة السنية ، علامات العظم الكيميائية.

INTRODUCTION

Dental implants provide a unique treatment modality for the replacement lost dentition. This is accomplished by the insertion of relatively inert material (a biomaterial) into the soft and hard tissue of the jaws; thereby providing support and retention for dental prostheses, there have to be effective biological adaptability between the implant material and the tissues of the jaws [1]. Despite the ongoing improvement in implant characteristics, bone intrinsic potential for regeneration may be stimulated with adjuvant therapies to standard surgical procedures, as it is important to achieve the best possible implant osseointegration into the adjacent bone and to ensure therefore long-term implant stability. For this purpose various pharmacological, biological or biophysical modalities have been developed, such as bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins [2].

Growth hormone (GH) belongs to the group of growth factors. These substances have been proposed to improve and accelerate osseous healing using topical applications [3]. Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate [4].

Vascular endothelial growth factor (VEGF), which is secreted by many cells including osteoblasts and osteoblast-like cells, may be intimately related to bone development and fracture healing because both intramembranous and endochondral ossifications are associated with capillary development [5].

MATERIALS AND METHODS

Materials

- Commercially pure titanium (cpTi) rods 3.5mm diameter,
- Growth hormone(Somatotropine 10mg/1.5ml MOH/Iraq).

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- Vascular Endothelial Growth Factor Antibody (VEGF) from Abcam company USA (ab28775)
- Mouse specific HRP/DAB detection IHC kit
- (Abcam company England (ab80436).
- Hydrogen peroxide Block.
- Protein Block.
- Biotinylated goat anti-mouse IgG.
- Streptavidin peroxidase.
- DAB chromogen.

Methods

Forty adult New Zealand white rabbits aged (10-12 months) were used in this study. They were divided into two groups for (3 days, 1, 2, and 6weeks) healing intervals, 10 animals for each period. Atraumatic surgical technique was performed to prepare one hole in each tibia, the Rt side considered as experimental group (with topical application of growth hormone) and Lt side as control group. Animal were scarified after 3 days, 1, 2 and 6 weeks.

Immunohistochemical staining procedure for detection of VEGF:

All tissue specimens, samples and controls, were fixed in 10% neutral formalin and processed in a routine paraffin blocks. Each formalin-fixed paraffin-embedded specimen had serial sections were prepared as follows: 5µm thickness sections were mounted on clean glass slides for routine Haematoxylin and Eosin staining (H&E), from each block of the studied sample and the control group for histo-pathological re-examination. Other 4 sections of 4µm thickness were mounted on positively charged microscopic slides to obtain a greater tissue adherence for immunohistochemistry. The procedure of the IHC assay adapted by this study was carried out in accordance with the manufacturer instructions (Abcam UK).

RESULTS

Immunohistochemical finding of the of 3 days control rabbit show positive expression of VEGF in marrow tissue and fat cells embedded in ground substance (Figure 1).

Immunohistochemical localization of VEGF is indicated by the brown color of marrow tissue as positive DAB stain. The view shows positive staining of fat cells embedded in ground substance of the marrow tissue (experimental group) (Figure2)

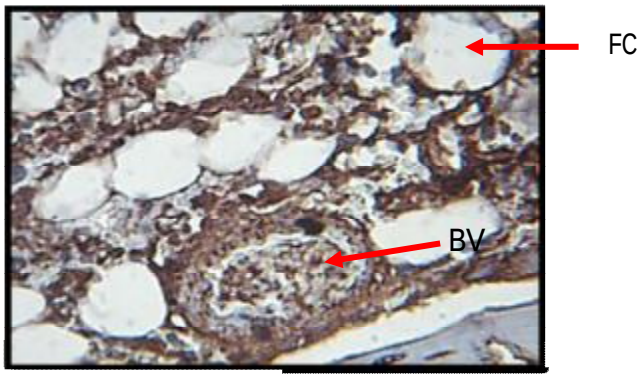

Figure 1: Microphotograph view shows positive expression for VEGF in ground substance, B.V., bone marrow and fat cell. DAB stain with counter stain hematoxylin.

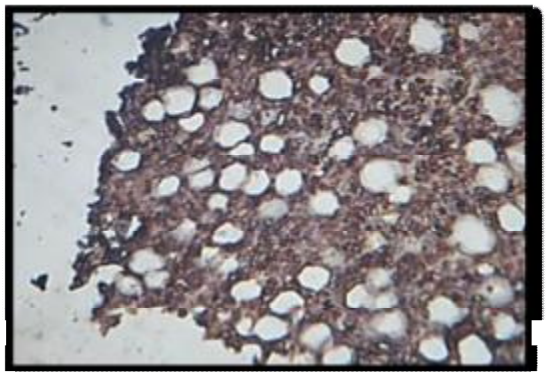

Figure 2: View of implant site coated with GH in rabbit tibia shows positive DAB stain for VEGF include only bone marrow tissue. DAB stain with counter stain hematoxylin X200.

Microscopic evaluation of the bone section related to uncoated implant after one week of implantation, shows positive immunohist-ochemical stain for VEGF localization, in osteoid tissue (Figure 3).

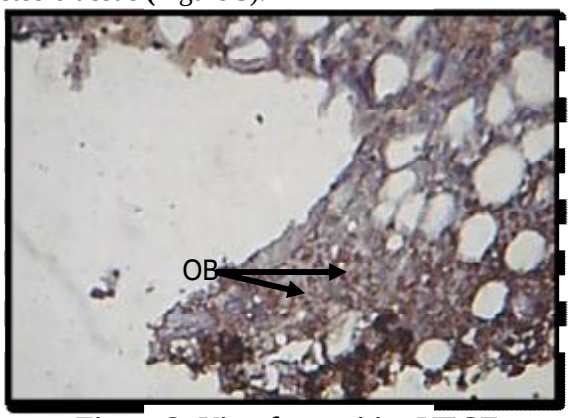

Figure 3: View for positive VEGF expression in osteoid tissue of one week duration, see positive osteoblast (OB). DAB stain with hematoxylin counter stain, X200.

A view of positive immunohistochemical localization of VEGF in osteoid tissue and osteoblast cells in experimental rabbit after one week (Figure 4).

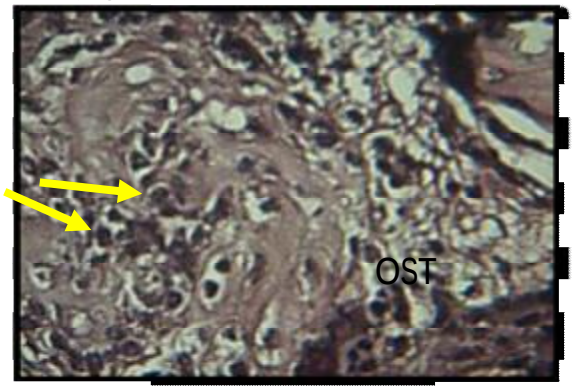

Figure 4: View for positive DAB immunohisto- chemical stain of osteoid tissue (OST), and osteoblasts (arrows) DAB stain with counter stain hematoxylin, X400.

Two weeks after implantation in control rabbits, positively stained osteoblasts, and immature bone formation is detected after 2 weeks of implantation. Microphotograph view of bone section in rabbit tibia shows formation of bone trabeculae with numerous scattered osteocytes and areas of marrow tissue of different sizes showing positive stain are seen (Figure 5).

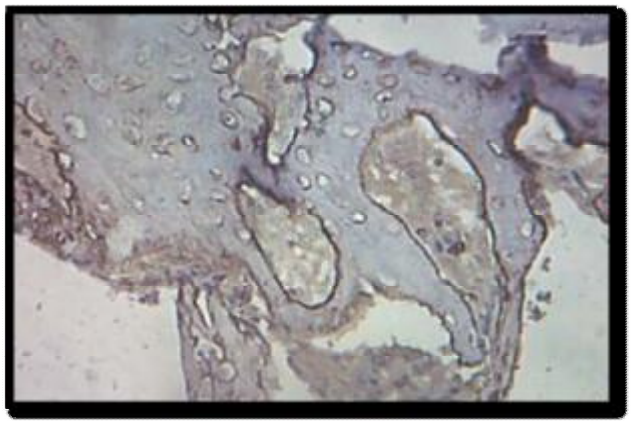

Figure 5: View of positive DAB stain in marrow tissue. DAB stain with counter stain hematoxylin, X200.

Experimental group show View of immature bone trabeculae with numerous irregularly arranged osteocytes that are positively stained. Other view of implant site shows osteoid tissue, bone trabeculae in which osteocytes are embedded, osteoblasts, are positively stained (Figure 6).

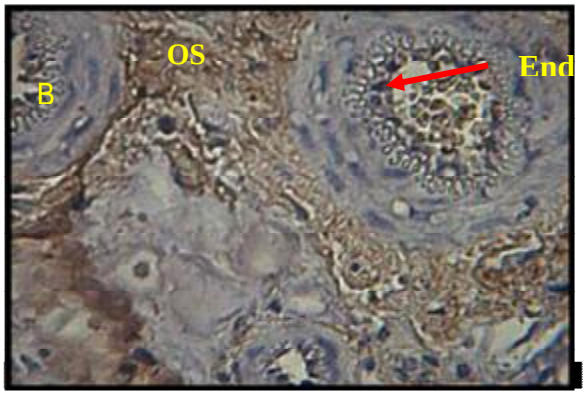

Figure 6: Positive staining of osteoid tissue and blood vessel endothelium(End). DAB stain with counter stain hematoxylin, X400.

Six weeks after implantation Microphotograph view in control rabbit tibia of 6weeks duration, shows large number of positively stained osteocytes that are irregularly scattered in calcified bone trabeculae (Figure 7).

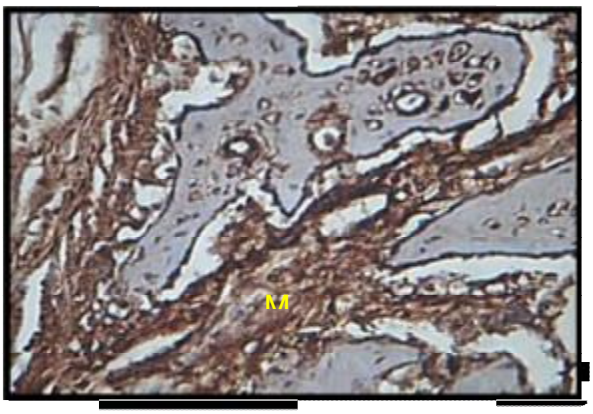

Figure 7: View of implant site, shows positive staining of osteocytes (OC), and marrow tissue. (MT).DAB stain with counter stain hemat-oxylin,X200.

Microphotograph view of bone section in experimental rabbit tibia, in thread area of 6 weeks duration, shows mature bone with numerous osteocytes surrounding haversian canal inside bone trabeculae which are positively stained (Figure 8).

The results of the present study show that there was an obvious decrease in BMSC score mean values of positively stained cells for VEGF, during the 3 days, 1 and 2 weeks healing intervals concerning control group, while concerning the experimental group a slight increase in VEGF score at 6 weeks period, whereas the mean values of scores of VEGF showed slight increase during 3days 1 and 2 weeks periods, as shown in (Figure 9).

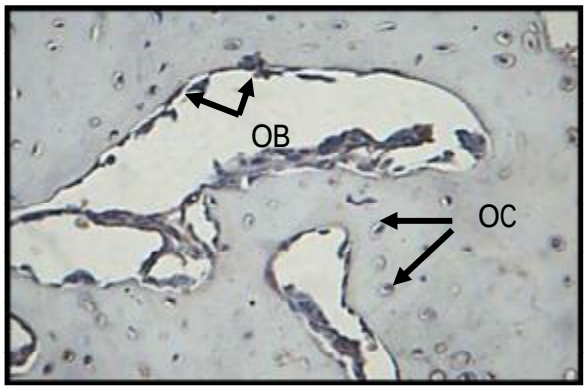

Figure 8: Immunohistochemical localization of VEGF, observed in osteoblasts (OB) at periphery of bone matrix, and in osteocytes (OC). DAB stain with counter stain hematoxylin, X200

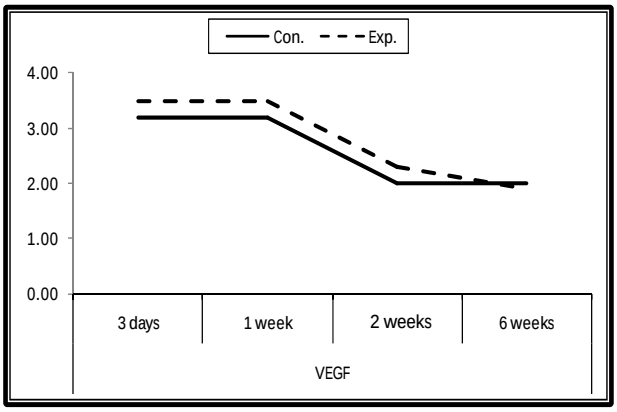

Figure 9: Line Chart of BMSC Parameter's means of score values distributed according to different S.O.V. at VEGF Marker

Table 1 presents the conventional statistics estimators for studying and analyzing the studied (BMSC) parameter, due to different sources of variations in compact form (i.e. in general), as well as Figure 10 illustrated graphically marginal mean values of the studied parameter which were distributed among different sources of variations in compact form.

Table 1: Summary statistics of the bone cells outcomes according to different sources of variation (S.O.V.) in compact form.

Parameters	Groups	Mean	S.E.	95% Confidence Interval	
				Lower Bound	Upper Bound
Groups	Control	16.22	0.47	15.27	17.17
	Experiment	19.66	0.47	18.71	20.60
Periods	3 d.	31.81	0.67	30.47	33.15
	1 wk.	24.44	0.67	23.10	25.78
	2 wk.	8.50	0.67	7.16	9.84
	6 wk.	7.00	0.67	5.66	8.34
Markers	TGF	16.81	0.47	15.86	17.76
	VEGF	19.06	0.47	18.11	20.01

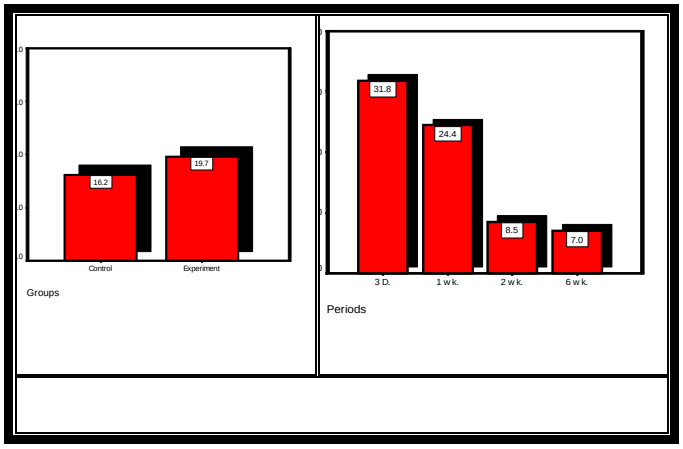

Figure 10: Bar charts of BMSC parameter's mean values distributed according to different S.O.V. in compact form

R-Square coefficient indicated that the studied sources of variation are interpreted about 85.8% for the actual variations of the " bone cells parameter outcomes" changes, the assignable factor (the bone cells) had reported a highly significant effectiveness at P<0.01.In addition to that, results showed that a highly significant difference are accounted at P<0.01 due to the effective of positively stained bone cells (OB,OC,OSCL),(Table2).

Table 2: Multiple Comparisons by (LSD Method) among all pairs of different bone cells types for (BMSC) Parameter effectiveness in compact form

(I) group	(J) group	Mean Diff.	Sig.(*)	C.S
OB	OC	-0.225	0.008	HS
	OSCL	0.988	0.000	HS
OC	OSCL	1.213	0.000	HS

(*) HS: Highly Sig. at P< 0.01; S: Sig. at P< 0.05; NS: Non Sig. at P> 0.05

DISCUSSION

Growth factors released during the inflammatory phase have the potential of attracting undifferentiated mesenchymal stem cells to the injured site; these factors are released in the injured sites by cells involved in tissue healing [6].

Expression of growth factors, such as TGF and VEGF suggest that these may be of importance to the bone healing process [7].

The VEGF system has been investigated for several years, but a standardized interpretation of the immunohistochemistry (IHC) staining of the ligands and receptors has not yet been validated) 8).

In the present results, VEGF was positively expressed in both control and experimental groups and in different intervals periods and in different levels according to osteoblast activity in osteoid formation. At 3 days after implantation shows bone marrow tissue with stromal cells as a large number of active progenitor cells.

In one week after implantation VEGF shows positive expression in experimental implants. VEGF is expressed in the osteoid tissue which indicated rapid woven bone formation and deposition of osteoid matrix include osteoblast and extracellular matrix this finding in agreement with Mora et al [9].

Jung et al [10] found that, VEGF was expressed more strongly within the vascular endothelial cells of the extracellular matrix, in agreement with our results, where positive localization of VEGF by endothelial cells, was detected at 2 weeks.

In 2 weeks after implantation VEGF shows positive expression by osteoblast cells that are located at the periphery of calcified bone tissue and in osteocyte cell located within trabacule of active bone. VEGF is known to promote bone turnover by stimulating chemotaxis and activity of osteoclasts and osteoblasts, cartilage remodeling and enchondralossification and thus should help to remodel the bonesubstitute into native bone [11].

At 6 weeks duration, negative immune-histochemical localization for VEGF marker was detected in bone, but positive expression was shown by osteoblast lining its surface. At six weeks period the control implant reported immature bone with implant surface while experimental implant show mature bone [12].

We conclude that VEGF, growth factor may contribute to the progression of osseo-integration by increasing angiogenesis, thus leading to the formation of new blood vessels from the pre-existing vasculature. Immunohistochemical findings revealed positive localization of VEGF, by BMSC and BC with higher detected scores of mean values in experimental group (with GH, indicating that the cellular mechanisms involved improved osseointegration throughout healing intervals).

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