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Physiological and histological studies of dental response of Luxate tooth with appliation of VEGF

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Abstract---Background During luxate injury many dental tissue will be involved including pulp, periodontal ligament, mucosa, hard dental tissue and alveolar bone with affecting the nerve and dental blood supplement. Growth factors may involved in healing process after replantation of tooth in its socket and the Vascular endothelial factor (VEGF) is a potent one that increase angiogenesis and accelerate repairing and healing of dental pulp and the supporting tissues. The study was designed to illustrate the effect of application of growth factor on healing process of luxate molar tooth of rat. Materials & Methods Twenty male Wistar rats with luxate lower right first molar teeth were included in this study. The control were injected with normal saline (0.5µl) around the apex of mesial root .Experimental group injected with VEGF (0.5µl). Histological assessment of physiological responses of (dental pulp, cementum, periodontal ligament (PDL) and alveolar bone in both control and experimental groups with Receptor activator of nuclear factor kappa-B ligand (RANKL) expression evaluation were recorded during the healing period in the examined tissue. Results: Control group showed rapture of (PDL), odontoblast disruption , hyalinization in dental soft tissue and necrosis reported in most of examined specimens ,while experimental group showed angiogenesis with fast healing. RANKL expression revealed a difference value in the percentage of positive cells for experimental group in comparison to control. Conclusion:

Vascular endothelial growth factor increased angiogenesis and affected RANKL expression that helps the healing process to be fast.

Keywords---physiological, histological studies, dental response, replantation tooth.

Introduction

Treatment of luxate tooth without its losing represent the important goal in dental branch to estimate the functional and esthetic objective problem ^{1,2}. Luxate injury affects dental tissues include (dental pulp, periodontal ligament(PDL), oral mucosa, gingiva and alveolar bone ³ and according to the types of the injuries the responses will be different ⁴. Five classification of luxation ⁵, one of them is lateral luxation that results in apical root displacement due to one of the buccal, lingual, mesial or distal direction of dislocation ^{6,7}. Many cellular events in dental tissue repair may regulated by growth factors ⁸. Vascular endothelial growth factor (VEGF) is reported as a potent angiogenic and vasculogenic factor that enhanced the proliferation of endothelial cell that facilitating healing process ^{9,10}.

Receptor activator of nuclear factor kappa-B ligand (RANKL) and VEGF are the two cytokines that have a chemotactic properties toward osteoclasts ¹¹. They include in differentiation of osteoclast and affect the activation of resorption ¹². Because there are few studies interesting in dental field show the effect of VEGF application with expression of RANKL, therefore, the current study was designed an experimental project for dental trauma in rats and estimate the used of VEGF with the expression of RANKL in healing of dental tissue.

Materials and Methods

Twenty 8-week-old male (Wistar) rats, weighted 220-240 g have been enrolled in this study. All experimental procedures were performed according to ethical approval from the ethical committee of the (Al-Mustaqbal University College) (license No: 069220) and under National Council's guide for the care of laboratory animals. The animals were kept in the animal department of Iraq National Center of Drug Control and Research under climate-controlled conditions. The animals received intramuscular (IM) injection of 100 mg/kg of Ketamin (Ketaset, Fort Dodge, Iowa, from USA) and 5 mg/kg Xylazin (Eurovet, Cuijk, from Holland) before tooth luxation process.

The luxation was executed by application of discoid carver instrument, dental tix that was inserted in proximal surface between the (lower right first) and (second molar), then a small force applied to the carver tip contact surfaces while the neighboring tooth was used as a dorsal tip contact point to create a luxating lever movements to get a slight mesial dislocation ¹³. The animals were randomly divided into two groups; control group (No.10) injected with (0.5µl) normal saline and experimental group (No.10) injected with (0.5µl) of VEGF. The injection was done for both study groups into the gingiva around the apex of mesial root and after (5 minutes) of luxation procedure. Determination of effective dose for VEGF depends on previous studies ^{14,15}.

Histological evaluation

The rats were sacrificed by an overdose of carbon dioxide gas at 7 and 30 day post operatively. Sectioning of maxilla was collected, fixed in 10% neutral buffered formalin for (2-3)days at room temperature, and then demineralized in 10% formic acid for 5 days. By using of running water the specimens were washed thoroughly and dehydrated through ascending concentrations of ethanol. After dehydration, the specimens were embedded in paraffin blocks. Serial sections about (5- μ m) thickness were cut through the mesio-distal direction in molar area using the sliding microtome. The slides were stained with hematoxylin and eosin to evaluate physiological and biological responses and for general morphology of dental pulp tissue, cementum, periodontal ligament (PDL) and alveolar bone for both control and experimental groups.

Immunohistochemistry

Immuno-stained with (anti-RANKL antibody, Mouse anti-Rat Receptor Activator Of Nuclear Factor Kappa B Ligand (RANKL) Monoclonal Antibody (Clone C10)-BAB79694 (My BioSource Company) were done. Quantitative analysis of immunohistochemistry results was performed by counting the positive cell in each 100 cells in 5 fields at power x40 for different sections ¹⁶.

Statistical analysis

All records were entered into Excel spread sheets for evaluation with the Statistical package deal for Social studies (SPSS) (Chicago, IL, united states of America). By using one-way ANOVA test with multiple comparisons of LSD the data was analysed.

Results

Histological findings

Histological alterations observed mostly in control group for the whole periods (PDL rupture, odontoblast disruption, hyalinization of dental soft tissue and necrosis, while angiogenesis was observed in most of experimental group, table(1) Microscopical views for the mesial root of lower right 1st molar of control group at 7days post-operatively showed odontoclast presents in resorbed dentin area with presences of disrupted of odontoblast cells. Macrophage also presented in pulp tissue. Osteoclast occupies the pay resorbed bone area was recorded with presence of ruptured periodontal ligament (PDL). Control at 30 days period shows necrosis in periodontal ligament (PDL) with obvious resorption in dentin, cementum of mesial root. Pulp also shows internal dentin resorption with presence of hyalinized blood vessels, figure(1)

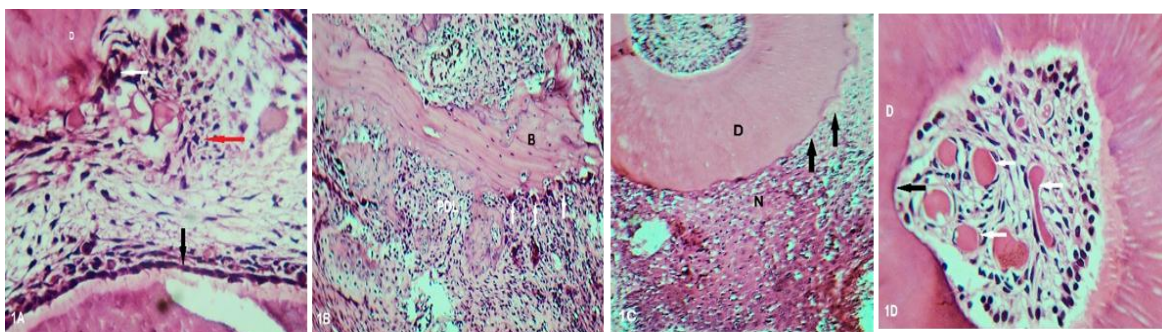
Experimental group at 7days post-operatively shows that dental pulp illustrates new blood vessels with proliferated endothelial cells filled the histological views. Odontoblast arranged well without any disruption at pulp surface are recognized. Osteoclast, cementoclast are present near the bone tissue and cementum. Histological features for the experimental at (30 days) period shows active

angiogenesis in pulp and in periodontal ligament (PDL) with obvious normal cementum and dentin. Pulp shows well-arranged odontoblast on its surface with presence of hyalinized blood vessels, figure(2)

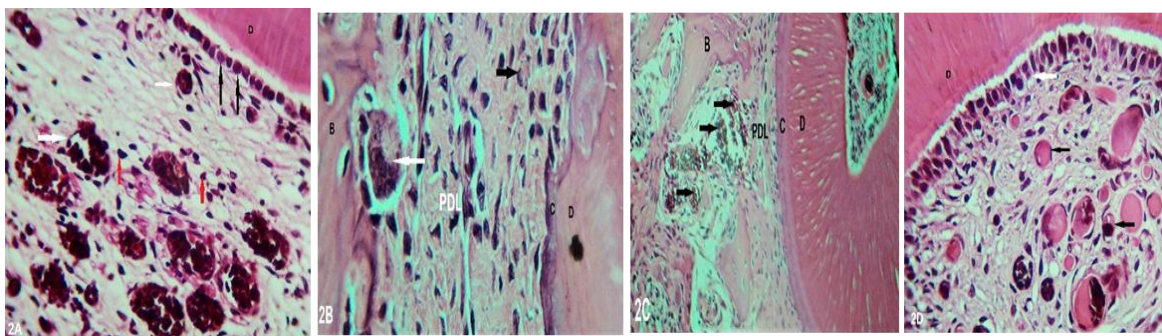
Table(1) Histological alterations observed in examined specimens in whole periods

Groups	Specimens No.	Histological alteration				
		RPDL Necrosis	Hyaline odontoblast	Angiogenesis	Disrupted	
Control	10	7*	7*	1*	6*	5*
Experimental	10	2*	4*	8*	2*	0

*Asterisk indicates presence of the histological event; RPDL: rupture of the PDL



Figure(1) Microscopical views for control group (with NS injection), mesial root of lower right 1st molar at 7 & 30 days post-operatively. **A:** Odontoclast (white arrow) occupies the resorbed area in dentin (D), with disrupted odontoblast (black arrow) and macrophage (red arrow) presented too. H&E X20. **B:** Resorbed bone (B) with osteoclast (white arrows), rupture of periodontal ligament (PDL), H&E X4. **C:** View for control at 30 days period shows necrosis (N) in periodontal ligament with obvious resorbed in apical third of root (arrows) in cementum and dentin (D), H&E X10. **D:** View for pulp shows internal resorbed dentin (black arrow) with hyalinized blood vessels (white arrows), H&E X10.

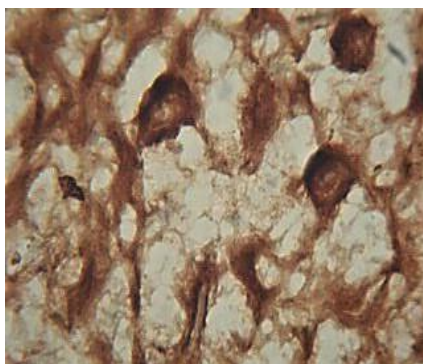


Figure(2) Microscopical views for experimental group (with VEGF injection), mesial root of lower right 1st molar at 7 & 30 days post-operatively. **A:** New blood

vessel(white arrow) with scattered endothelial cells(red arrows),odontoblast well arranged(black arrows), dentin(D),H&E X10. B: Resorbed bone with osteoclast(white arrow) ,cementoclast (arrow), periodontal ligament (PDL),H&E X20. C: View for experimental at 30 days period shows angiogenesis (black arrows) in periodontal ligament (PDL) with obvious normal cementum(C) and dentin(D),H&E X4. D: View for pulp shows well odontoblast (white arrow) with hyalinized blood vessels (black arrows),H&E X10.

Immunohistochemical of RANKL expression

In current study the Receptor activator of nuclear factor- κ B ligand- RANKL is recorded to be expressed by osteoclast near the resorbed bone and also expressed by macrophage in dental pulp tissue for both examined groups with an intense brown stain .Statistic analysis revealed a significant difference in percentage (%) of positive cells at 7 days for experimental group in comparison to control and recorded also a non significant difference value between the examined groups at 30 day period. On other hand, the expression was found to be decreased with period and showed a significant difference value for both groups (control & experimental) at the period 7 day in comparison to 30 day, figure (3),tables (2,3)



Figure(3) Immunohistochemical view for positive RANKL expression for macrophage in pulp,DAB stain X20

Table (2) Positive RANKL at different periods measured in different groups

Groups	Days	Sample No.	Mean	S.D.	S.E.	95% C. I. for Mean		Min.	Max.
						L.b.	U.b.		
Control	Day 7	5	2.75	0.96	0.48	1.23	4.27	2	3.5
	Day 30	5	2	0.82	0.41	2.70	5.30	1.4	2.5
Experimental	Day 7	5	3.75	0.58	0.29	0.58	2.42	3	4.5
	Day 30	5	2	0.58	0.29	1.58	3.42	1.5	2.9

Table(3) LSD after ANOVA test for RANKL distributed among different groups

periods LSD		Positive RANKL cells No. In groups		
			Sig.(*)	C.S.
	7 day Control	Experimental	0.000	HS
	30 day Control	Experimental	0.97	NS
Periods	No. of cells at 7 ; 30 Day	Control	0.010	S
	No. Of cells at 7 ; 30 Day	Experimental	0.011	S

Discussion

The present results show physiological and histological changes that observed in control group for the whole periods included PDL tearing , disrupted odontoblast, hyalinization of blood vessels and necrosis of dental tissue due to luxation trauma while experimental group recorded a reverse results included well arranged odontoblast, reestablishment of periodontal attachment (PDL) , less hyalinization in soft tissues (pulp and periodontal tissues) and with no evidence for necrosis in all dental tissue. These results could be explained according to many facts:

- ❖ Application of VEGF might enhanced differentiation of stem cell in dental pulp, periodontal ligament with activation of angiogenesis¹⁷, these events may help to subside the negative response and increase the positive one and increase the regeneration and repair in short period^{18,19} . Several evidences have revealed that local application of cytokines can affect the regeneration of dental tissues^{20,21}.
- ❖ Angiogenesis is an important factor to recover the damaged dental tissues and helps in healing process²². Although periodontal ligament and pulp are communicated areas with rich vascularized field and have one supplement source including the apical vessels that protects against frequent inflammatory insults¹⁷ . Many researches recorded that there is a correlation between the presence of inflammation and the reduction of the number of blood vessels and VEGF expression levels^{24,25}. In current study application of exogenous VEGF enhanced formation of new blood vessels with proliferation of endothelial cells and resulted in reduction of inflammation reaction as it reported in experimental group in comparison to control which showed necrosis stroke. Moreover, VEGF may share other growth factors and provide minerals that helps in mineralization of ground matrix in bone. VEGF shows to be involved in differentiation of osteoblasts and osteoclasts²⁶.

Immunohistochemical evaluation RANKL expression by osteoclast cell and macrophage cell reported a significant difference value for experimental in comparison to control, these results may related to that RANKL may play a role in bone and dentin resorption²⁷. Our findings coincide with a study which found that RANKL was expressed in inflammatory cells²⁸. Moreover, RANKL was found as a member of the TNF cytokine that may involved in bone resorption^{29,30}. The present study is the first study that illustrates RANKL expression by macrophage in dental pulp tissue.

The study found an increment in RANKL expression by dental cells in experimental group with VEGF application showing that both cytokines play roles during healing process. The present finding is in agreement with Henriksen et al³¹ finding who showed that RANKL and VEGF have a chemotactic properties toward osteoclast cell.

Conclusion

The study concludes that VEGF may cause modulation and enhancement of dental repair and can be used to treat luxate tooth as one of therapeutic strategies.

Conflict of Interest: No conflict of interest

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