Dental Traumatology

Dental Traumatology 2008; **24**: 435–438; doi: 10.1111/j.1600-9657.2008.00608.x

Vascular endothelial growth factor (VEGF) response to dental trauma: a preliminary study in rats

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Accepted 2 March, 2007

Abstract –*Background*: Successful periodontal healing and pulpal revascularization after trauma may be age-related. After dental trauma, age-related impaired angiogenesis may play a role in the revascularization rate during the healing process. *Objectives*: The aim of the present preliminary *in vivo* study was to detect the presence of vascular endothelial growth factor (VEGF) in venous and whole blood from the alveolar socket immediately post-tooth extraction. *Materials and methods*: The study consisted of 16 Wistar rats divided into two groups: eight young (6 weeks) with incomplete root development and open apices, and eight adult (4 months) with complete root development. One first mandibular molar was extracted and whole blood from the alveolar socket collected immediately and at 5, 10 and 15 min post-extraction. Venous blood samples were collected immediately pre- and 24-h post-extraction. All samples were tested for VEGF. *Results*: Increased VEGF levels were found in the venous blood 24 h post-extraction, with a significant difference in the young rats ($P < 0.05$). The increased VEGF serum concentration in the young rats was significantly higher (12.2 ± 4.6 pg ml^{-1}, median 12.1) than that in the adult rats (7.6 ± 3.8 pg ml^{-1}, median 6.7) ($P = 0.037$). VEGF at the extraction site was not significantly different at 15 min post-extraction. At the extraction site, there was no significant difference of VEGF concentration between young and adult rats. *Conclusions*: Young rats had higher post-extraction serum VEGF levels, which may contribute to the healing process after dental trauma.

Successful periodontal healing and pulpal revascularization after trauma may be age-related. A key factor in the revascularization process is new blood vessel formation (angiogenesis). After tooth injury, e.g. luxation or avulsion with replantation, angiogenesis and revascularization are the desirable treatment results. With revascularization, the tissue in the canal space prevents penetration of infection, thus normal root development may continue. Pulp revascularization is mostly affected by the size of the apical foramen and the extra-oral dry time of the tooth (1–3). An important factor that may prevent revascularization is bacterial presence in the canal space. Bacteria induce an inflammatory environment, which does not allow for healing and revival of tissue in the canal (4–6).

Angiogenesis involves sprouting of new capillaries from existing vascular structures, a process triggered by endothelial cell migration and proliferation. These capillaries may be replaced by wider diameter conduits through the process of vascular remodelling, i.e., arteriogenesis (7, 8).

The regulation of angiogenesis and collateral vascular formation is a complex process that involves stimulators, inhibitors and modulators. Most angiogenic factors bind to specific receptors on the endothelial cells and induce basement membrane breakdown, endothelial cell migration and proliferation. In angiogenesis, several cytokines play important roles, but the vascular endothelial growth factor (VEGF) is considered to be vital (9). VEGF binds to receptors on the endothelial cells, which results in their growth, proliferation, and migration (10). Expression of both growth factors and their receptors is also up-regulated by hypoxia and ischaemia (11). VEGF immediately synthesizes after triggering with either hypoxia or ischaemia, as well as several other cytokines and growth factors. The increased VEGF levels induced by hypoxia make VEGF-driven angiogenesis a central response to low oxygen tension in tissues, and thus an appealing candidate in therapeutic angiogenesis (12, 13). Furthermore, it is possible that VEGF could promote human bone repair (14).

Angiogenic potential may be age-related. Angiogenesis, responsible for collateral blood vessel development in limb ischaemia, is impaired with aging. The mechanisms responsible include age-related endothelial dysfunction and reduced VEGF expression (15).

The objective of the present preliminary *in vivo* study was to detect the presence of VEGF in venous and whole

blood collected from the alveolar socket immediately post-extraction.

Materials and methods

The study was conducted according to the Animal Care and Use Committee, Tel-Aviv University, Faculty of Medicine. Two groups of animals, eight young (6 weeks, incomplete root development and open apices) and eight adult (4 months, complete root development) female Wistar rats were used.

Animals were anesthetized during all experimental procedures, using 75 mg kg^{-1} of ketamine and 10 mg kg^{-1} of xylazine, administered by intraperitoneal injection.

Experimental procedures

The first right mandibular molar was extracted from each rat, using elevators and forceps as needed. Teeth were extracted with minimal trauma to the socket. Whole blood from the alveolar socket was collected immediately and at 5, 10 and 15 min post-extraction. Samples from the extraction site were collected using paper filter strips (Periopaper – gingival fluid collection strips; ProFlow, Amityville, NY, USA). Each strip was held in the socket for 30 s, then covered with aluminum foil, placed in an eppendorf tube and stored at −20°C. This method has been used to collect and analyze small volumes of biological fluids (16).

Venous blood was collected pre-treatment and 24 h post-extraction. Subsequent to clotting and centrifugation, serum was separated and stored at −20°C. After thawing, a paper filter strip was inserted into the tube for 30 s, similar to the method used to collect the extraction site samples.

Each paper strip was inserted into an individual test tube containing 200-µl of distilled water. Tubes were allowed to stand at room temperature for 30 min, while shaking every 5 min to facilitate extraction of the sample from the filter papers. Aliquots of the extracted sample were diluted two-fold with the Calibrator Diluent from the kit (Quantikine® Mouse VEGF, R&D Systems, Minneapolis, MN, USA) and used in the ELISA assay, performed according to the manufacturer's instruction.

This assay uses a quantitative sandwich enzyme immunoassay technique. Affinity purified polyclonal antibody specific for mouse VEGF was pre-coated onto a microplate. Standards, controls and samples were pipetted into the wells and any mouse VEGF present in the tested sample was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse VEGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The intensity of the colour measured was in proportion to the amount of mouse VEGF bound in the initial step. Sample values were read from the standard curve set to 450 nm with a wavelength correction between 540 and 570 nm, and measurement units expressed as pg ml^{-1}. All samples were assayed simultaneously. Data were collected and analyzed by the Mann–Whitney test for increasing group variables.

Results

Serum VEGF

An increase in venous blood VEGF levels was found post-extraction in both the groups. A significantly higher VEGF serum concentration was found in the young group (12.2 ± 4.6, median 12.1) whe compared with the adult group (7.6 ± 3.8, median 6.7), ($P = 0.037$) (Fig. 1).

Local VEGF

VEGF at the extraction site did not show a significant difference in post-extraction. No significant differences were found between VEGF concentration at the extraction sites between both the groups (Fig. 2).

Systemic vs local VEGF concentration

There was no difference at baseline when systemic vs local VEGF concentrations were compared. However, when the concentration was compared after extraction, the young group exhibited a higher rise in VEGF ($P = 0.004$) (Table 1).

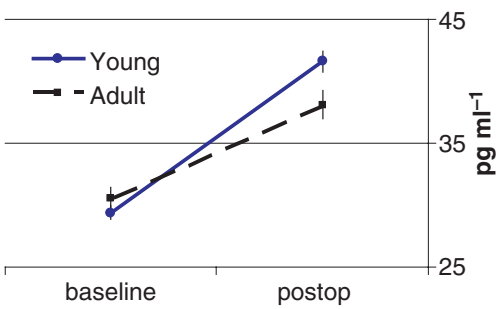

Fig. 1. Mean serum VEGF levels in the young and adult rats; preoperative (baseline) and 24 h post-extraction (postop).

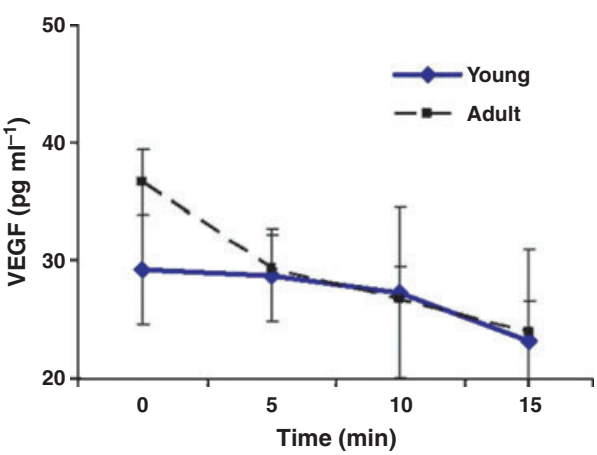

Fig. 2. Mean local VEGF levels in the young and adult rats at different time intervals post-extraction.

Table 1. Comparison between mean VEGF levels at baseline and at 15 min per 24 h: serum vs local

	Group			
	Young		Adult	
Level	Serum	Local	Serum	Local
Baseline	29.4 ± 4.8*	29.3 ± 4.6	30.5 ± 2.9	36.7 ± 11.2
15 min		23.2 ± 7.7		23.9 ± 12.1
24 h	41.6 ± 2.1*		38.1 ± 2.3	

*P = 0.004.

Discussion

The present study showed that there was a difference in VEGF serum concentrations between young and adult rats after use of a trigger, e.g. molar extraction. This could potentially result in a difference in the angiogenic and periodontal post-traumatic healing response, and may partially explain the clinical difference observed after tooth trauma between children and adults.

Revascularization, the re-establishment of blood supply to a tissue that has turned ischaemic or necrotic due to injury that disrupted its blood vessels, includes formation of new blood vessels by direct migration of endothelial cells, their proliferation and new lumen formation. After trauma to the periodontal ligament and dental pulp, revascularization is a desirable outcome (17–19).

Blood supply to the dental pulp passes from the periodontium through the apical foramen and lateral canals. As a result of dental trauma, these vessels and small capillaries in the periodontium can become injured after subjected to stretching and crushing.

Angiogenesis, including arterogenesis and vasculogenesis processes, initiates the formation of new vessels in the presence of low oxygen tension. This includes vasculogenesis, the *in situ* formation of new blood vessels from circulating bone marrow-derived endothelial progenitor cells, which differentiate into endothelial cells and fuse into luminal structures (7, 8). Previously, vasculogenesis was assumed to occur only during embryological development, but increasing evidence shows that neo-vascularization in adult tissues also involves both angiogenesis and vasculogenesis processes (20). A key factor in angiogenesis is VEGF (21) because it binds to receptors on endothelial cells, resulting in their growth, proliferation and migration (10).

In the present preliminary study, the higher concentration of serum VEGF in the young group may contribute to the revascularization process after tooth trauma. However, other factors probably affect this process. Pulp revascularization after trauma is possible only when the ischaemic/necrotic tissue remains sterile, since invading infection triggers an immunologic response and ceases normal healing. The probability of revascularization occurrence is affected by the size of the apical foramen and the apico-coronal length of the pulp (stage of root development); presence of infection and effect of systemic and topically applied antibiotics; length of extra-alveolar time; and conditions of extra-alveolar storage of the tooth (dry vs different types of wet media) (1, 2, 22).

No significant elevation in VEGF concentration was found in the adult group, which can be explained by either the small sample collected, or could be one of the factors that prevents revascularization in adults (1). Angiogenesis is usually impaired with age (15). The potential mechanisms in which aging can affect angiogenesis are diverse. Angiogenesis includes activation, migration and proliferation of endothelial cells (23). The responsible mechanisms that explain this phenomenon include age-related endothelial dysfunction and reduced VEGF expression (15). This reduction could also play a role in the failure of periodontal and pulpal revascularization after tooth trauma in adults.

The present study failed to show an early local increase in VEGF concentration at the extraction site after the first 15 min post-extraction. This could be attributed to the fact that the coagulation process starts immediately after extraction and intricate blood collection. Alternatively, the VEGF response may require more time than allowed before collecting the alveolar samples in the present study. As this was only a preliminary study with a small number of animals, further research is required to elucidate the VEGF response to dental trauma and its effect on the revascularization process. Moreover, further research that includes other factors that could be involved in these processes is warranted as different growth factors play a role in periodontal and pulpal healing after trauma (24).

Acknowledgements

The authors would like to thank Ms Rita Lazar for editorial assistance and Ms Margarita Filatov for Laboratory assistance.

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