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RESEARCH ARTICLE

Peculiarities of vascular endothelial growth factor of oral cavity in atopic condition VEGF of oral cavity in atopic condition

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ABSTRACT

Background and aims: Vascular endothelial growth factor (VEGF) is regarded as a potent stimulating factor for angiogenesis and vascular permeability and probably is connected with an inflammatory reaction. Our study aimed to determine the effect of VEGF in the inflammatory process in the oral mucosa of experimental animals in the modulation of atopic disease. *Materials and methods:* Atopic condition was simulated by the ovalbumin model. Obtained specimens of oral mucosa were examined histologically; immunohistochemistry was performed with detection VEGF, CD23, CD20. *Results:* Most pronounced changes with twice increased expression activity of VEGF has been detected in the affected areas of the lamina propria and were associated with perivascular inflammatory microinfiltration, but unexpected expression in the epithelial layer has been revealed surround of intraepithelial inflammatory cells mainly. Pronounced correlations have been detected as VEGF and CD23 ($r = 0.91$), VEGF and CD20 ($r = 0.87$), CD23 and CD20 ($r = 0.89$). *Discussion:* described the changes in the tissues of the oral mucosa could be served as a basis for the development of preventive measures in patients with atopic diseases.discussion *Conclusions:* Activation of VEGF is connected with accumulation of inflammatory infiltrate represented by B-lymphocytes, activated macrophages, eosinophils with a correlation in atopic process.

KEYWORDS

gingivitis, atopy, VEGF, oral cavity, experiment, CD23, CD20

INTRODUCTION

Nowadays, researches point out the need to consider the functional condition of oral health for the implementation and evaluation of public health dentistry interventions that it quite often depends on general condition with numerous possible comorbid diseases [1]. One of such diseases group connects with atopic pathology as bronchial asthma, allergic rhinitis, atopic dermatitis, which is progressively increasing mainly in children all over the world [2–4]. Atopic conditions are characterized by inherited peculiarity tendency to produce immunoglobulin E (IgE) antibodies in response to small amounts of common environmental proteins such as pollen, house dust mite, and food allergens [5].

There is a recent study that found a positive association between periodontal disease and severity of asthma [6] and studies which show that patients with severe periodontitis were less likely to have asthma with evidence of an inverse association was found when using asthma medication as outcome [7]. But generalized microcirculatory disturbance which is typical for atopic condition could be realized in periodontal injuries [8] and as result, periodontal problems are often observed in child age as consequences of different processes [9] and atopic conditions also [10, 11]. As result, research of the pathogenesis of atopic changes becomes is goal for allergists, internists, pediatricians, pathologists other specialists that realized in clinical and experimental investigation [12–14] searching of new methods of

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diagnostics and estimation severity [15, 16] which are directed for detection of pathogenetical links for creation of effective prophylactic and therapeutical measure.

One of potential key links in the development of atopic process could be vascular endothelial growth factor (VEGF) which is regarded as a potent stimulating factor for angiogenesis and vascular permeability [17]. VEGF has been initially described as vascular permeability factor (VPF) due to its ability to generate tissue edema [18]. It has been recognized as multifunctional angiogenic regulator which influent on epithelial cell, proliferation, blood vessel formation, and endothelial cell survival. Changed levels of VEGF have been detected in tissues and biologic samples from people with atopic conditions [19, 20].

Simultaneously, the role of VEGF in the pathogenesis of formation of atopic conditions and the effects or VEGF functions in the tissue have not been defined clear generally and in oral cavity particularly with research performing mainly with estimation of VEGF mainly in serum but not in tissue.

Our study aimed to determine the effect of vascular endothelial growth factor in the inflammatory process of soft tissues in the oral cavity of experimental animals in the modulation of atopic disease.

MATERIALS AND METHODS

We performed experimental investigation as described early [12] for study of the morpho-functional state of tissues of the oral mucosa in atopic disease that allows to eliminate the influence of somatic pathology and social factors. For modulation of atopic changed we used Ovalbumin as it had been suggested in the previously proposed and widely used scheme [21–23]. Intraperitoneal injection of ovalbumin and aluminum hydroxide were performed for modeling of experimental atopic process in young animals (rabbits, males, aging three-month) during first 3 days of the experiment. The twice lower dose of ovalbumin was instilled intranasally under local anesthesia five days later (Day 8) with repeated intranasal administration of ovalbumin through on the 16th, 17th, 20th, and 21st day of the experiment. Doses of used medicine were determined according to animal body weight. We formed two groups with 8 animals each – intact animals and group of animals with simulated atopy. The specimens of soft tissues of the oral cavity (alveolar mucosa attached gingival and buccal mucosa from area to lateral side of incisors margins) were stained with hematoxylin and eosin (H&E), according to van Gieson after the routine proceeding. Immunohistochemical examination (IHC) was performed by indirect immunoperoxidase reaction [24] with monoclonal antibodies (mAb) to VEGF, CD23 (detected on mature B cells, activated macrophages, eosinophils, has affinity with IgE [25]), CD20 (receptor located on the surface of all B-lymphocytes [26]). All used mAbs are manufactured by Thermo scientific. The reaction was visualized using a set of UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen (Thermo scientific). All microspecimens were performed in the Department of Pathological Anatomy of the Kharkiv Medical Academy of Postgraduate Education (head of the Department I.I. Yakovtsova).

The slides were studied with the microscope "Olympus BX-41" and followed interpretation by "Olympus DP-soft version 3.2," which was used for definition of the intensity of immunohistochemical reactions, morphometric study. The intensity of IHC was analysed by detecting of the optical density of the relevant morphological structures in conventional unit (conv. un.). Evaluation of VEGF expression was performed using a quantitative scale also. Staining was categorized as either positive or negative, whereas the intensity of staining was not considered. The intensity was considered to be score zero when there were no stained cells; a score 1 (+) when the stain was weak; a score 2 (++) when the stain was moderate; and a score 3 (+++) when the intensity of stained cells was strong. Morphometric studies (CD23, CD20) was performed by superimposing a grid with square cell (side 10^{-4} m) and detection of cellular density for inflammatory elements including immunopositive stained cells with CD23, CD20. All values are expressed as means, standard deviation (SD) and standard error of the mean (SEM) for statistical analysis. Statistical comparison was performed using Mann–Whitney test for statistical analysis. Spearman's rank correlation coefficient (r) was counted for measure of the strength of relationship between paired data [27]. The accepted level of significance was $P \leq 0.05$.

The procedure was done strictly in adherence with the Helsinki Declaration, European Convention for the protection of vertebrate animals (18.03.1986), European Economic Society Council Directive on the Protection of Vertebrate Animals (24.11.1986) after approval from the Regional Ethical Review Board at Kharkiv National Medical University (protocol 8, 4.11.2015).

RESULTS

Redness of visible mucosa, edematous changes, hemorrhages surround overfilled blood vessels, isolate erosive defects had been revealed on examination of the oral mucosa group of animals with modeling atopic process in comparison group.

Initial histological investigation of obtained microspecimens shows that atopic modeling process is implemented by complex of pathological changes in oral mucosa. Squamous epithelium is characterized by uneven thickness with the presence of intraepithelial lymphocytes, eosinophils, focal erosive lesions (Fig. 1a and b), signs of proliferation in the basal cellular layer, moderate development of papillomatous changes; inflammatory infiltration is expressed in the lamina propria. Simultaneously there are areas with infiltration by inflammatory both in the lamina propria and epithelium of the oral cavity (Fig. 1a and b); there are isolated mucosal erosions.

Histologically epithelium is uneven with presence of just two-three cells thickness area which accordance erosive

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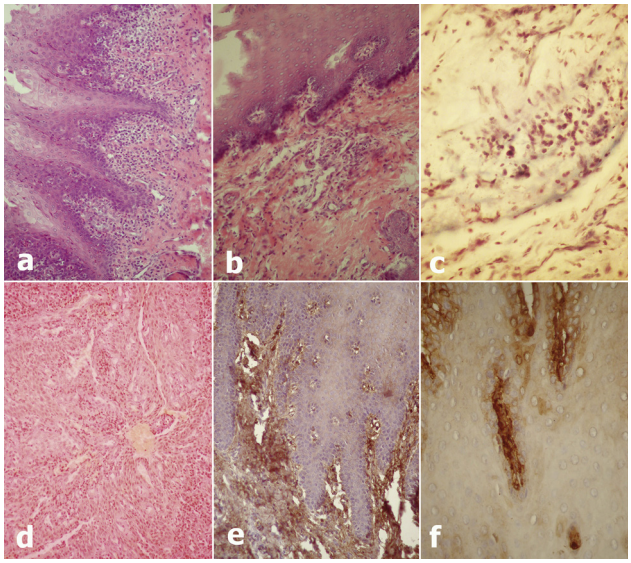

Figure 1. Uneven thickness of squamous epithelium with the presence of intraepithelial lymphocytes, eosinophils, accumulation of inflammatory cells under the basal membrane mainly, appearence of eosinophils in the epithelial layer, formation of erosion, H&E stain, magnification ×200 (a); new formed vessels of microcirculatory bed with large endothelial and adventitial cells, presence of perivascular inflammatory infiltrates with eosinophils and their diffuse distribution in the lamina propria, proliferation of basal layer cells, initial formation of acanthotic strands, swelling of connective tissue fibers, H&E stain, magnification ×400 (b); distribution o mature B-cells, activated macrophages, eosinophils wich has affinity with IgE, immunohistochemical study of CD23, magnification ×400 (c); diffuse distribution of B-lymphocytes, immunohistochemical study of CD20, magnification ×200 (d); increased expression of VEGF with most pronounced subepithelial zone with marking of new formed vessels of microcirculatory bed in lamina propria, immunohistochemical study of VEGF, magnification ×200 (e); intensive staining of peribasal area with appearance of receptors prior to vascular endothelial growth factor in epithelium, appearance of receptors surround of inflammatory cells located intraepithelially, immunohistochemical study of VEGF, magnification ×400 (f)

changes mainly (Fig. 1a and b). Degenerative processes in epithelial cells are pronounced with often pycnosis phenomenon. As an approach to basal membrane cells are increased in volume by both the nuclei and the cytoplasm size. The shape of the cells is changed from elongate to oval with simultaneously changing the orientation of the epithelial cells and the almost vertical position in the basal membrane (Fig. 1b). The nuclei of the basal epithelial cells are well defined, oval, uniform, hyperchromatic; cytoplasm is moderately basophilic. Isolate and grouped intraepithelial lymphocytes, eosinophils have been revealed (Fig. 1a and b). The basement membrane is uneven with uneven thickness. Acantotic strips of lamina propria are started to be formed (Fig. 1b).

The superficial papillary layer of the lamina propria consists of loose connective tissue which is represented mainly elastic fibers. Reticular layer is located deeper and is represented by rough connective tissue fibers. Perivascular inflammatory infiltrates, diffuse distribution of inflammatory cell as B-lymphocytes, activated macrophages, eosinophils which has affinity with IgE (CD23, CD20) (Fig. 1c and d), swelling of connective tissue fibers have been revealed in the lamina propria. Microcirculation is characterized by uneven blood supplying with presence of dilated vessels predominantly and appearance of newly formed vessels of microcirculatory bed (Fig. 1b).

VEGF immunopositive tissue is intensive and has been detected diffusely but there are more intensive are primarily in perivascular space of the microvasculature and under basal area in lamina propria (Fig. 1e). There is appearance of stained VEGF receptors in epithelium that was not observed in control group. Most pronounced staining in epithelium has been expressed surround of inflammatory cells located intraepithelially (Fig. 1f).

Determination of the dyestuff accumulation indicates that the activity level is significantly differ VEGF staining of control group that was confirmed by optical density estimation (Table 1) and using of quantitative scale. So, according to quantitative scale VEGF staining was estimated as score 1 (+) in 6 animals and as score 2 (++) in 2 animals in control group and as score 2 (++) in 5 animals and score 3 (+++) in 3 animals with atopy modeling.

Comparing of the results for the peroxidase reaction identified significant differences between two groups for VEGF, CD20, CD23. So, more pronounced rate has been revealed in the group of rabbits with the modeling of atopy, that is confirmed by morphometry almost twice growth for VEGF. In this case there are areas as with diffuse and with focal immunopositive amplification of stained tissues. The presence of local zones of increased immunoreactivity led us to assume that such changes could be the result of the activation of inflammatory cells. The fact of most active VEGF localization surround of the inflammatory cells in epithelial layer and in the lamina propria could be used as evidence of such immunomodulatory interactions.

Most pronounced activity of VEGF has been detected in the affected areas of the lamina propria and was associated with perivascular inflammatory microinfiltration and level of immunoreactivity intensity was associated with the quantitative and qualitative composition of the cellular infiltration of tissues in atopy. Study of cellular density of cells with immunoreactivity mAb for CD23 was interesting as it is protein, which, as stated above, is detected in mature B-cells, activated macrophages, and eosinophils, and that is

Table 1. Morphometric data of activity of immunohistochemical study

Groups	Intact animals	Animals with simulated atopy
VEGF staining, conv. un.	0.471 ± 0.057	0.813 ± 0.131*
CD20 (cell in square with side 10^{-4} m)	3.03 ± 0.14	29.4 ± 1.28*
CD23 (cell in square with side 10^{-4} m)	2.39 ± 0.12	21.3 ± 0.52*

*$P < 0.05$ compared to the intact animals.

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important for the study of atopic process CD23 has affinity to IgE. It is found that such elements in the group of intact animals are found as single cells in the lamina propria. In the group of animals with simulated atopy their distribution differs both qualitatively and quantitatively. First, almost all intraepithelial inflammatory cells were positive for CD23. Majority of the cellular elements were also proved immunopositive to CD23 in the lamina propria where the density of cells with CD23 nuclear staining was significantly higher than in intact animals.

Studying of CD20 distribution (co-receptor located on the surface of B-lymphocytes) revealed qualitatively similar changes to the study of CD23 detection (appearance of the immunopositive cells in the epithelium and a sharp increase in the number of lamina propria) with membrane substance localization. Comparison of cell density of CD20 intact animals and animals with atopy showed significant growth also (Table 1). Comparing of the IHC results for eNOs, iNOs, CD23, CD20 revealed the most pronounced correlation between CD23 and CD20 ($r = 0.89$), VEGF and CD23 ($r = 0.91$), VEGF and CD20 ($r = 0.87$).

DISCUSSION

Increased activity of vascular endothelial growth factor in oral mucosa in atopic process was noted in our work and it is combined with previously published data about VEGF increasing in the skin in atopic dermatitis [19], that is interesting from point of view for angiogenesis. The growth of new blood vessels from previously existing blood vessels occurs physiologically in healing wounds, with inflammatory diseases, and in tumor growth [28, 29]. Lymphangiogenesis can be activated by inflammation and tumor metastases. The family of VEGF and angiopoietins are important for angiogenesis and lymphangiogenesis. The angiogenic process is closely regulated by VEGF, angiopoietins, and endogenous inhibitors. VEGF and angiopoietins have the effect of activating specific receptors present in the blood and lymphatic endothelial cells. There is now convincing evidence that immune cells are the main source of angiogenic and lymphogenic factors. Angiogenin should be remained in that concerning [30] as its important interaction with VEGF in atopic processes [31]. Chronic inflammatory diseases of the skin, such as psoriasis and atopic dermatitis, are characterized by altered angiogenesis, lymphangiogenesis, or both. However, there are still no clinical trials that show that canonical strategies that target VEGF receptors can modulate inflammatory diseases.

It is known, according to the literature, that VEGF is among the differentially expressed genes that present the greatest number of interactions with other genes in the presence of an atopic process [32]. It is known that VEGF is one of the major mediators of angiogenesis, stimulation of fibroblasts and tissue remodeling under allergic conditions. It can also stimulate the repatriation of inflammatory cells, enhance sensitization of the antigen and play a decisive role for adaptive inflammation [18]. VEGF may play a role in the pathogenesis of atopy and could be involved in the regulation of the development of lesions in the presence of an atopic process, possibly with erythema and edema, due to prolonged capillary dilatation and increased permeability [19]. Several studies have been conducted indicating a significant increase in plasma VEGF levels in patients with atopic conditions [32, 33]. At the same time, these authors did not find any correlation between levels of VEGF in plasma and the number of cells that promote secretion of this growth factor (mast cells, platelets). In our study, the expression of VEGF was increased in cells of lamina propria; in addition, partial expression was observed in cells, which were located in epithelial layer.

It should be noted that in the named work, the level of VEGF in the blood is not considered to be a suitable prognostic marker for the development of the atopic state [32], but we have found literary sources that assess the predictive significance of blood changes in another [17, 29, 34], and indicate that the level of VEGF correlates with the degree of severity of the atopic state. At the same time (quite unexpectedly) in the last paper, there is a lack of correlation between the severity of atopy and blood serum concentrations such factors as IL-6, IL-8, IL-15, TNFα, or CCL5 specifies the risk of developing cardiovascular disease in the presence of such changes [34].

In any case, tissue changes in VEGF in the oral cavity in the presence of an atopic process are almost not described. There are separate data on changes in the skin that indicate the ratio of the level of VEGF intensivity to the disease activity [35, 36]. The authors suggest that vascular changes in the skin of patients with atopy may be associated with the inflammatory process [35], and the main sources of an abundance of angiogenic and lymphangiogenic factors are effector cells of inflammation (mast cells, basophils, eosinophils, macrophages, lymphocytes etc.,); the role of lymphagenogenesis in the onset of atopy remains largely unknown [35].

In this regard, our data with the immunohistochemical determination of VEGF in the epithelium are detectable in accord with the above works [36] should be considered as a marker for assessing the severity of lesions by the atopic process, given that there is a connection between the VEGF and the estimated symptoms of localized injury [36]. A special feature of our study is identification of VEGF not only in lamina propria but epithelium also. Last, as given the distribution of the CD20 and CD23 proteins, also is combined with our results. Some of those cells (mast cells for example) appear to be not only a source of VEGF, but also a target for that factor [35, 37]. VEGF level with proven or potential roles in vascular development and remodeling have been identified including some that are associated with inflammatory condition with most important role of VEGF in inflammation as mediating of the angiogenic response [38].

Thus, we have described the morphological changes in the tissues of the oral mucosa which are usually regarded as a manifestation of atopic process with the development of

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inflammatory, degenerative, dyscirculatory processes, metabolic disorders, development of which has been involved active vascular endothelial growth factor with it influence on angiogenesis and which can serve as a basis for the development of preventive measures in patients with atopic diseases.

CONCLUSIONS

An atopic process in the oral cavity is characterized by morphological picture with inflammatory, degenerative, dyscirculatory changes which are accompanied by activation of vascular endothelial growth factor in oral mucosa. Twice increased expression activity of VEGF has been observed in the affected areas of the lamina propria and it is associated with perivascular inflammatory microinfiltration. Pronounced correlations have been detected between VEGF and CD23 (r = 0.91), VEGF and CD20 (r = 0.87), CD23 and CD20 (r = 0.89). So, activation of vascular endothelial growth factor is connected with accumulation of inflammatory infiltrate represented by B-lymphocytes, activated macrophages, eosinophils which are typical in atopic process.

Authors contribution: NR–manage of research, literature search, data analysis; KL–plan and performing experiment, data analysis, wrote the paper; GV–morphological investigation, selected and provided histological samples, wrote the paper. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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