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The importance of vascular epithelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS) in rhinitis medicamentosa pathogenesis: An experimental rat model study

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Summary. Objective. Our aim in this study is to reveal the expression of Vascular Endothelial Growth Factor (VEGF) and inducible Nitric Oxide Synthase (iNOS) in the pathogenesis of rhinitis medicamentosa (RM), which occurs as a result of the overdose and long-term use of topical nasal decongestants.

Methods. In this study, 24 Wistar albino rats were divided into two groups as experimental and control groups. In the experimental group, 50 µl of 0.05% oxymetazoline (iliadin® merck) was applied intranasally to each nostril three times a day for 2 months with the help of a micropipette. 50 µl saline was applied to the control group. At the end of the second month, the rats were examined. RM was detected in the experimental group. Then the nasal tissues of the rats were removed and fixed with 10% phosphate buffered neutral formaldehyde (pH=7.4). Nasal tissues were decalcified in Morse's solution (10% sodium citrate and 22.5% formic acid). Histopathological evaluations of the preparations were stained using Masson Trichrome (TCM) and Hematoxylin Eosin (H&E) techniques and immunohistochemical examinations of the preparations were stained with VEGF and iNOS antibodies and photographed using the Leica DM6000B microscope and the Leica Application Suite Program.

Results. In the RM group, we found a significant increase in VEGF and iNOS expression in the nasal mucosa compared to the control group ($p<0.001$). We also observed the main histopathological changes in the nasal mucosa under a light microscope, including squamous metaplasia in the epithelium of the tunica mucosa, submucosal perivascular edema and degeneration of the submucosal glands.

Conclusions. According to these results, increased expression levels of VEGF and iNOS play an important role in rebound swelling in RM pathogenesis.

Key words: Rhinitis, Oxymetazoline, Nasal mucosa, Edema, Vascular Endothelial Growth Factor A

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Introduction

Rhinitis medicamentosa (RM), also known as rebound rhinitis is a type of non-allergic mucosal inflammation caused by overuse of topical nasal decongestants, especially oxymetazoline and xylometazoline. RM characteristically causes nasal congestion without rhinorrhea. This typically occurs after 5-7 days of use of topical decongestants. Patients often try to increase both the dose and the frequency of nasal sprays at the beginning of RM. This can lead to worsening of RM. The incidence of RM in otorhinolaryngology is known to be 1 to 9% (Graf, 2005).

RM can also occur with the use of antihypertensives, oral contraceptives and 5-phosphodiesterase inhibitors. Disusing nasal decongestant is the first-line treatment for RM. However, after discontinuation of nasal decongestants, withdrawal symptoms may occur including headache, restlessness and anxiety (Snow et al., 1980).

Nasal steroids and saline irrigation are generally used in the medical treatment of disease. RM causes hyperreactivity, swelling and congestion in the nasal mucosa. Edema is an important component of nasal obstruction in RM. Chronic vasoconstriction is seen in RM as a result of chronic inflammation in nasal soft tissue. Chronic vasoconstriction also causes hypoxemia then ischemia of the nasal mucosa which leads to dysfunction and obstruction. Nasal congestion resulting

from impaired nasal functions causes snoring and impaired sleep quality. RM can also predispose to chronic sinusitis and atrophic rhinitis.

RM is characterized by some histological changes, for instance, increased mucus production and an increase in the number of lymphocytes, plasma cells, fibroblasts, and presence of squamous cell metaplasia. However, the pathophysiology of rebound swelling is unclear, despite the high incidence of RM. VEGF is expressed by inflammatory cells as an adaptive response to inflammation. VEGF causes vascular permeability and angiogenesis (Melincovici et al., 2018).

Angiogenesis is an important physiological event for inflammation. VEGF is regulated by hypoxia both in vivo and in vitro settings (Goldberg and Schneider, 1994; Cohen and Gardner, 2016).

Nitric Oxide (NO) is a signaling molecule that plays an important role in chronic inflammation (Niu et al., 2018). The inducible Nitric Oxide Synthase (iNOS) protein is responsible for cellular NO production. Cellular NO production from L-arginine oxidation occurs via iNOS. Both iNOS and NO contribute to a large number of biological processes. The majority of cells do not express iNOS under normal conditions. Induction of iNOS usually occurs under oxidative stress. Increased NO levels lead to its reaction with superoxide leading to peroxynitrite formation which is known to be cytotoxic. These properties may define the roles of iNOS in host immunity, enabling macrophages to participate in anti-microbial and anti-tumor activities as an oxidative component (Mungrue et al., 2002). Unlike endothelial nitric oxide synthase (eNOS) or neuronal nitric oxide synthase (nNOS), iNOS is expressed only after cellular activation. In infectious diseases, human macrophages express the iNOS protein (Vouldoukis et al., 1995). There is increased iNOS expression in many chronic inflammatory and autoimmune diseases. iNOS increases in rheumatological diseases, especially systemic lupus erythematosus, rheumatoid arthritis and psoriasis and leads to NO production. Increased NO levels can also cause tissue damage (Simonetti et al., 2009). iNOS plays a role in the disease with chronic mucosal inflammation (Seimetz et al., 2011).

The aim of this study is to explain the role of VEGF and iNOS expression in the pathogenesis of rebound swelling in an experimental rat RM model created with oxymetazoline. Accurate diagnosis of RM can be done based on sufficient knowledge of the clinical and pathological features and critera. This is the first study to reveal VEGF and iNOS expression in the pathogenesis of RM within our literature knowledge.

Materials and methods

The study was carried out at Medipol University Faculty of Medicine, Experimental Animal Research and Application Center, with the approval of Medipol University Experimental Animals Ethics Committee dated 20/08/2020, numbered 38828770-772.02-E40132.

In the study, 24 6 month-old Wistar Albino rats weighing 250-300 g were used. The health conditions of the animals were checked before the study and no pathological conditions were found. The experimental animals were housed in metal cages in three or four at a temperature of 21°C, at humidity of 40 to 60%, with continuous warm and clean air, 12 hours light and 12 hours dark cycles. Rats were fed continuously with unlimited water and without any food restrictions. The rats were randomly selected and divided into two groups of 12 animals each. Control group (n=12) was treated with saline and experimental group (n=12) was treated with 0.05 % oxymetazoline (Iliadin® Merck). In the experimental group, 50 µl of 0.05% oxymetazoline (Iliadin® Merck) was applied intranasally to each nostril three times a day for 60 days with the help of a micropipette. 50 µl saline was applied to the control group three times a day for 60 days with the help of a micropipette.

Tissue collection and histological studies

Rats were dissected after 60 days with the decapitation technique. For histopathological and immunohistochemical assessments, tissue samples were fixated in 10% neutral formaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. After the fixation, the nasal mucosa tissues were decalcified in Morse's solution (22.5% formic acid and 10% sodium citrate). Decalcified tissues were processed through alcohol series and they were embedded in par-affin. The 5 µm thick paraffin-embedded tissues were sectioned by using a rotary microtome (Leica RM 2245 model; Leica Instruments, Germany). For histopathological examinations, the tissue sections were mounted on poly-L-lysine coated slides and they were stained with TCM and H&E.

Histopathological analysis and scoring

Slides were evaluated and photographed under a Leica DM6000B microscope by the help of Leica Application Suite image analysis program. For histopathological evaluation, the nasal mucosa sections were stained with TCM Staining and H&E Techniques.

Two histologists, who were blind to the groups, graded the sections semi-quantitatively. Histo-pathological scoring was done with the scoring criteria in Table 1.

As shown in the table, epithelial degeneration, basal membrane thickening, submucosal edema, basal membrane detachment, infiltration, capillary congestion and submucosal gland degeneration were used as histopathological change parameters in nasal mucosa sections. These parameters are scored as 0 (none); 1 (mild); 2 (moderate) and 3 (severe). The scores of the parameters were collected for each subject in the control and RM groups. Averages were taken within the group (Dokuyucu et al., 2014) .

VEGF and iNOS immunohistochemistry processing and scoring

For immunohistochemical examinations, 5 µm thick sections of nasal mucosa tissue samples were transferred to Poly-L-Lysine coated slides, which stayed in 0.1% sodium citrate and 0.1% Triton X-100 for 4 minutes at 4°C. After that, citrate buffer (0.01 M; pH=6.0) was applied for 45 seconds in a microwave oven. Slides stayed in 0.3 % H_2O_2 solution for 30 minutes at 21°C to stop endogenous peroxidase activity. Vectastain Universal Quick kit (RTU Vectastain; Vector Laboratories, Burlingame, CA, USA) was used to block nonspecific binding. iNOS antibody (code n. ab 15323, Abcam, Cambridge, UK.) at 1:200 dilution and VEGF antibody R2 (code n. 55B11 cell signaling, Danvers, MA.) at the 1:500 dilution was used for probing. Lastly, chromogen diaminobenzidine tetrahydrochloride (TA 125 TD; Thermo Fisher Scientific, Leicestershire, UK) was used as an indicator. Counter-staining was performed for 10 minutes with Mayer's hematoxylin (Kohen et al., 2018).

Immunohistochemical expressions of VEGF and iNOS were evaluated for both distributions of staining (H-score) and intensity. Immunohistochemical labeling was evaluated with these two parameters. H-score was calculated with a modified method (Kohen et al., 2018). The intensity was explained based on H-score, by using 0 for negative staining, 1+ for weak staining, 2+ for moderate staining, and 3+ for strong staining. The following formula produces an H-score in the range of 0-300, where 300 equals 100 % VEGF and iNOS positive cells had intense staining (i.e., 3+): H-score=(% of cells stained at intensity category 1×1) + (% of cells stained at intensity).

Table 1. Histopathological Scoring Criteria.

Score	Finding
0 none; 1 mild; 2 moderate; 3 severe	epithelial degeneration
0 none; 1 mild; 2 moderate; 3 severe	basement membrane thickening
0 none; 1 mild; 2 moderate; 3 severe	submucosal edema
0 none; 1 mild; 2 moderate; 3 severe	basement membrane detachment
0 none; 1 mild; 2 moderate; 3 severe	infiltration
0 none; 1 mild; 2 moderate; 3 severe	capillary congestion
0 none; 1 mild; 2 moderate; 3 severe	submucosal gland degeneration
0 none; 1 mild; 2 moderate; 3 severe	cartilage surface disconutinity

Statistical analysis

Statistical analyses were administered by using SPSS 22 software. Results were assessed with a confidence interval of 95% and a p value less than 0.05 was accepted as statistically significant. Descriptive statistical methods (mean ± standard error of the mean.)

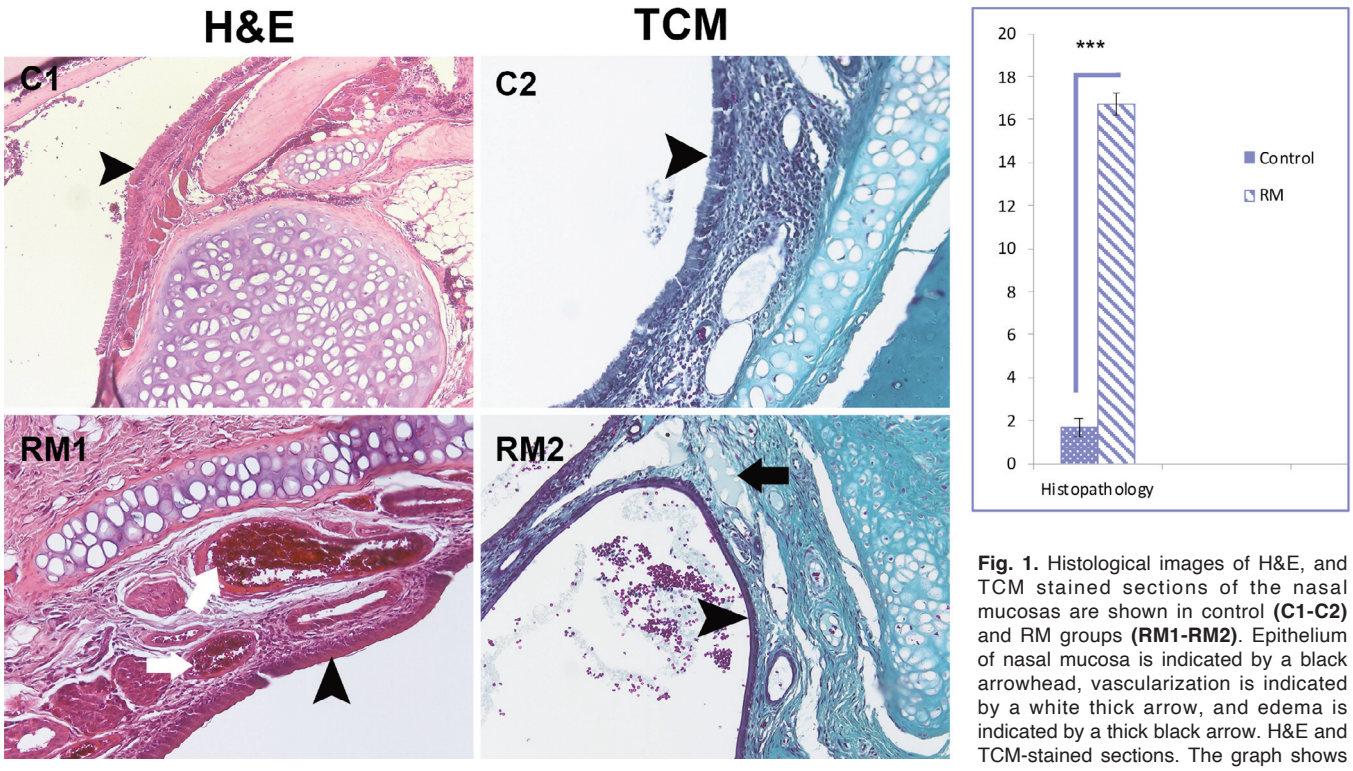

Fig. 1. Histological images of H&E, and TCM stained sections of the nasal mucosas are shown in control **(C1-C2)** and RM groups **(RM1-RM2)**. Epithelium of nasal mucosa is indicated by a black arrowhead, vascularization is indicated by a white thick arrow, and edema is indicated by a thick black arrow. H&E and TCM-stained sections. The graph shows the histopathological score of the nasal mucosa in control and RM groups. *** p<0.001 vs. control. x 20.

were used as independent t test for the control and experimental group. Post hoc power is the retrospective power of an observed effect based on the sample size and parameter that estimates from a given data set. In our study, we performed post hoc power analysis with software G*Power, (Version 3.0.10).

Results

Histopathological results

The total score of epithelial degeneration, basement membrane thickening, submucosal edema, basement membrane detachment, infiltration, capillary congestion and submucosal gland degeneration parameters were statistically significantly higher in the RM group compared to the control group in the nasal mucosa sections stained with H&E (16, 72±0.506 and 1.66±0.432; p <0.001; Figs. 1, 3). In the RM group, it was observed that the pseudo-stratified ciliated columnar epithelium transformed into a stratified squamous epithelium in the lamina epithelialis of the tunica mucosa and disappeared goblet cells and cilia. In the RM group, the degeneration of the submucosal glands and the proliferation of the vessels in this area were followed by edema around the vessels.

In the RM group, similar findings were found in TCM-stained nasal mucosa sections as in H&E-stained samples, additionally, irregularity of collagen fibers in network tissues were observed.

VEGF expression

In the RM group, the immunohistochemical staining of nasal mucosa tissue with VEGF was evaluated with the H-Score. In the RM group, VEGF expression in the nasal mucosa was detected in the tunica mucosa and vascular structures in submucosa regions. VEGF showed a positive reaction in the edematous areas. VEGF expression in RM group was significantly higher than the control group (37.90±1.365 and 15.83±0.521; p<0.001; Fig. 2).

iNOS expression

The immunohistochemical analyzes of the tissue samples of the RM group stained with iNOS antibody were evaluated with the H-Score technique. The iNOS activation in the nasal mucosa of the RM group showed a positive reaction in the tunica mucosa and connective

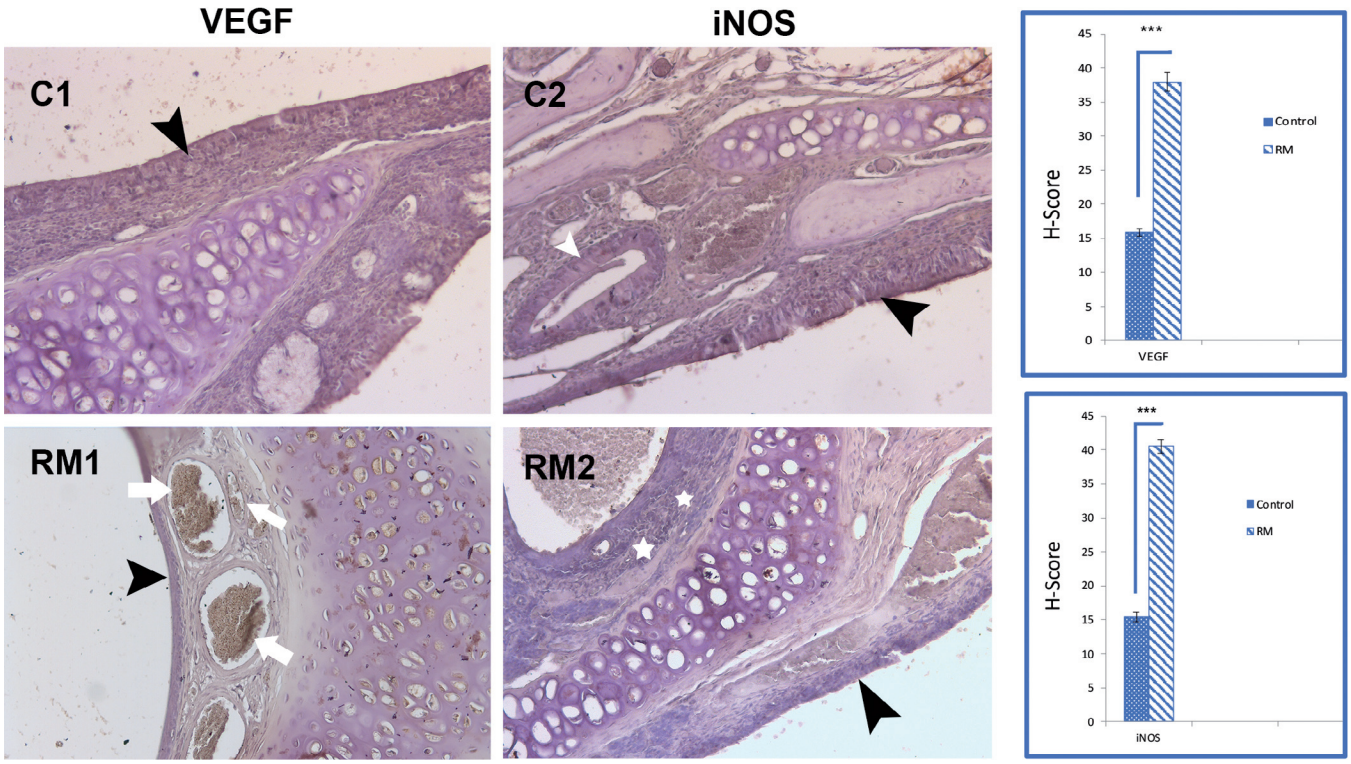

Fig. 2. Immunohistochemical analysis images of iNOS and VEGF stained sections of the nasal mucosas are shown in control **(C1-C2)** and RM groups **(RM1-RM2)**. Epithelium of nasal mucosa is indicated by a black arrowhead, vascularization and VEGF activity are indicated by white thick arrow, infiltration and iNOS expression are indicated by a white asterisk. Serousmucous gland is indicated by a white arrowhead in C2. These graphs show H-score values of iNOS and VEGF expressions in control and RM groups. iNOS and VEGF immunohistochemical-stained sections. Graph shows the histopathological score of the nasal mucosa in control and RM groups. *** p<0.001 vs. control. x 20.

tissue in the submucosa. Similar to VEGF expression, iNOS activity was found to be statistically higher in the RM group compared to the control group (40.50±0.992 and 15.50±0.745; p <0.001; Table 2, Fig. 2).

Discussion

In this study, we demonstrate the central role of VEGF and iNOS in the RM in nasal mucosa cells. We found a significant increase in VEGF and iNOS expression in the nasal mucosa in RM compared to the control group. In consequence of our literature search, our study is the first study to reveal VEGF and iNOS expression in RM.

Under light microscopy, we observed a loss of cilia in the epithelium of the nasal mucosa, edema in the epithelial cell layer, thickening of the epithelial basement

Table 2. Comparison of histopathology, VEGF and iNOS expressions in nasal septum tissue between control and experimental groups.

Group Name	Histopathology	H-Score of VEGF expression	H-Score of iNOS expression
Control	1,66±0,432	15,83±0,521	15,50±0,745
RM	16,72±0,483***	37,96±1,248***	39,86±1,105***

Values are expressed as mean±standard error of the mean (n=12).

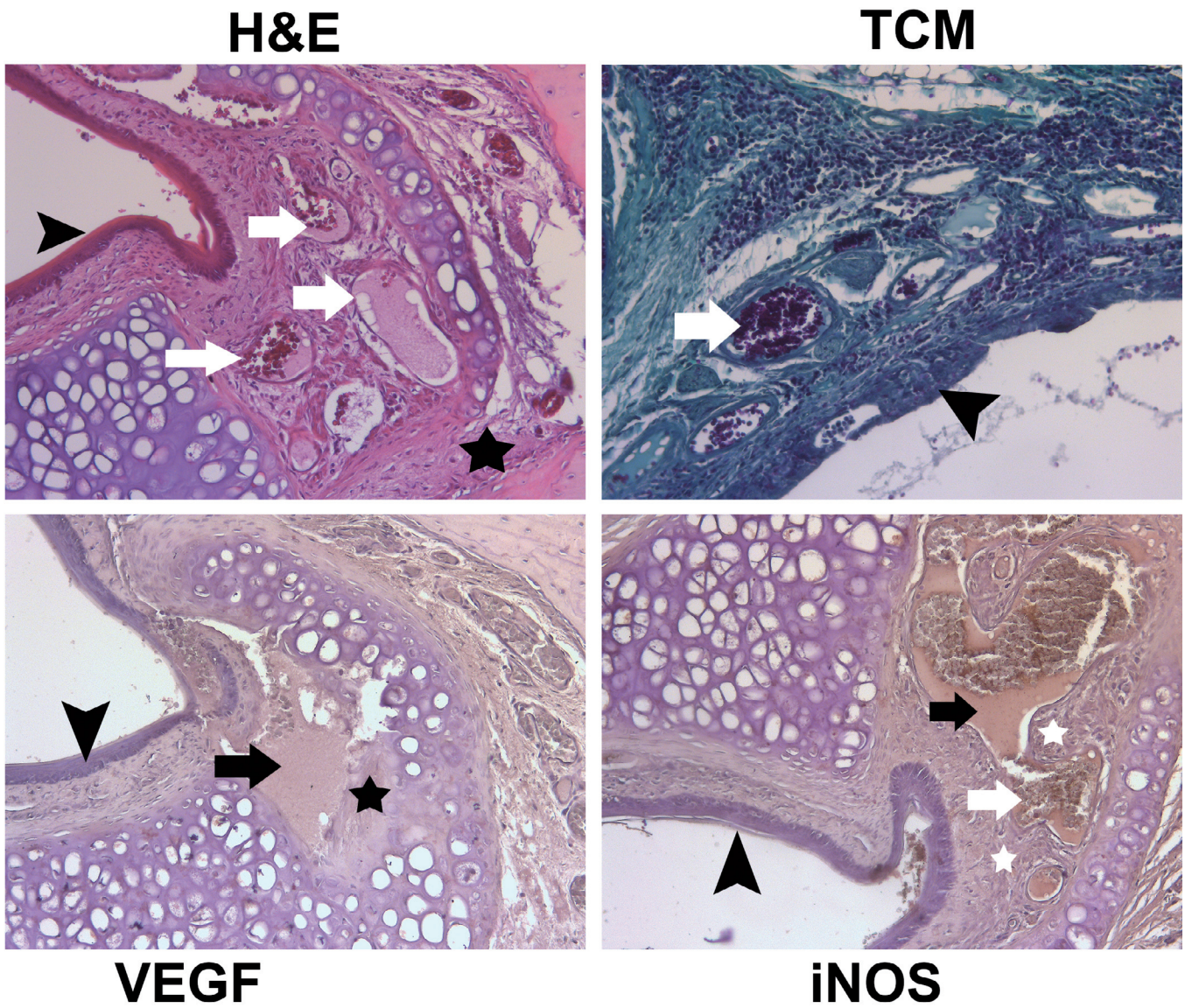

Fig. 3. Both Histological images of H&E, and TCM stained sections and Immunohistochemical analysis images of iNOS and VEGF stained sections of the septal cartilages are shown in the RM group. Epithelium of nasal mucosa is indicated by a black arrowhead, vascularization is indicated by white thick arrow, infiltration is indicated by a white asterisk and cartilage degeneration is indicated by a black asterisk. x 20.

membrane, separation in the basal layer, submucosal perivascular edema, mononuclear cell infiltration, interstitial edema, epithelial hyperplasia, squamous metaplasia, submucosal gland degeneration, and also an increase in fibroblasts, lymphocytes, macrophages and widening of the intercellular space. Furthermore, we detected increased vascularity in the perichondrium in the cartilage, degeneration in the cartilage and hypertrophy in the lacunae of chondrocytes in RM. In the damage of the cartilage, an increase in the vessels in the submucosa feeding the cartilage, fibrous connective tissue changes, edema and mononuclear cell increase may occur.

In a previous study of interstitial edema changes in cilia cell counts and cilia ultrastructure and separation of the basal layer in the nasal mucosa of RM patients were reported (Zucker et al., 2019). In another study, epithelial hyperplasia and degeneration of mucus secreting cells and submucosal glands in the nasal decongestant group were demonstrated by microscopic examination. Moreover, mucosal changes, ulceration and goblet cell hyperplasia have been demonstrated (Lin et al., 2004). Interstitial edema in the RM has been reported as the main pathological change in a previous experimental study (Elwany and Abdel-Salaam, 2001).

We also found congestion and edema as the main pathological changes in the nasal mucosa in the RM group. In another study, rebound nasal congestion in RM was demonstrated by an increase in vascular permeability and mononuclear cells (Scadding, 1995). However, the cause of rebound swelling has not been explained so far.

Some hypotheses regarding the pathogenesis of RM have been suggested in previous studies. The first hypothesis is that chronic vasoconstriction causes ischemia of the nasal mucosa leading to interstitial edema. A second hypothesis is that with the emergence of these constructive mechanisms, there is reactive hyperemia and congestion, the sensitivity of endogenous catecholamines decreases and adrenoreceptors start to show resistance to nasal decongestants. A third hypothesis is that there is a change in vasomotor tone with an increase in vascular permeability and edema. A fourth hypothesis is that beta-adrenoreceptor activity can overcome alpha activity leading to rebound vasodilation (Ramey et al., 2006).

In our study, we measured increased expression levels of both VEGF and iNOS in the nasal mucosa in the RM group. Similarly, co-expression of VEGF and iNOS in skin tissue has been demonstrated in psoriasis. (Simonetti et al., 2009).

We consider that increased VEGF and iNOS may be the cause of rebound swelling in RM pathogenesis. It has been reported that rebound swelling in the RM causes vascular permeability and edema formation (Elwany and Stephanos, 1983). Additionally, overproduction of angiogenic factors such as VEGF due to hypoxia has been reported (Rini and Small, 2005). We think that the high VEGF and iNOS expression is related to tissue hypoxia in RM. It has been reported that hypoxia induces VEGF expression via hypoxia-inducible factor (HIF) production (Semenza, 1998).

VEGF-A has been shown to be a stimulus for the progression of other angiogenesis-related diseases such as coronary artery disease, diabetic retinopathy and arthritis (Carmeliet and Jain, 2000). The effect of anti-VEGF-A therapy on macular degeneration has been shown (Rosenfeld et al., 2006). A neutralizing antibody against VEGF-A called bevacizumab has been reported to be the most useful drug for countering the progression of various human tumors (Ferrara and Kerbel, 2005).

It has been reported that increased iNOS expression causes tissue damage and dysfunction in inflamed lung epithelial cells and macrophages. The role of peroxynitrite formation by iNOS has been demonstrated in the pathogenesis of asthma (Meurs et al., 2003).

We also consider that the increase in iNOS expression leads to damage to the nasal mucosa cells. In addition, high iNOS expression has been reported in pulmonary fibrosis, emphysema, and other airway diseases (Leberl et al., 2013). Two pathways have been shown as the main mechanism in hypoxic iNOS stimulation. In the first pathway, HIF, which is an important factor in the cellular response to hypoxic conditions, binds directly to the hypoxia sensitive element (HRE) located in the promoter region of iNOS. In the second pathway, hypoxia activates NF-κB through activation of the inhibitory κB kinase, leading to classical NF-aktivB signal transduction and iNOS induction. NFkB is the major regulator of cytokine-induced iNOS expression (Robinson et al., 2011).

A study reported that iNOS inhibition prevented the development of chronic obstructive pulmonary disease (Seimetz et al., 2011). However, it has been revealed that iNOS induction contributes to inflammatory bowel diseases by increasing pro-inflammatory cytokines in the intestinal mucosa (Kolios et al., 2004). In another study, it was shown that iNOS expression had increased in human colitis tissue. This demonstrated that it could be the target of a possible therapeutic inhibition (Gochman et al., 2012).

We consider that rebound nasal obstruction is associated with increased VEGF and iNOS expression in the nasal mucosa. We can explain the increase in VEGF and iNOS expression in our study with the increase of tissue hypoxia. Cell damage may occur as a result of increased tissue hypoxia in the nasal mucosa which has the potential to create important problems in terms of nasal health. However, hypoxia alone or in combination with pro-inflammatory cytokines have been reported to increase iNOS expression (Macciò and Madeddu, 2012).

There is a relationship between VEGF and iNOS expression (Simonetti et al., 2009). In our study, we measured that the expression of VEGF and iNOS increased together in the nasal mucosa in RM, which play a key and essential role in rebound swelling in RM. VEGF and iNOS expression increases play an important role in RM pathogenesis. Although the physiopathology has not been elucidated, the increase in VEGF and iNOS

expression we observed in this study supports the relationship between hypoxia and RM. Vasoconstrictor drops should not be prescribed indiscriminately by physicians and should not be misused by patients.

Conclusion

According to these results, increased expression levels of VEGF and iNOS play an important role in rebound swelling in RM pathogenesis. VEGF and iNOS expression in nasal mucosa tissue as a response to hypoxia play a role in the pathogenesis of RM. Determining the correct diagnostic criteria in RM is also important for treatment. Understanding the VEGF and iNOS regulatory mechanisms in the nasal mucosa may provide new treatment modalities for RM.

References

Carmeliet P.J. and Jain R.K. (2000). Angiogenesis in cancer and other diseases. Nature 407, 249-257.

Cohen S.R. and Gardner T.W. (2016). Diabetic retinopathy and diabetic macular edema. Dev. Ophthalmol. 55, 137-146.

Dokuyucu R., Cevik C., Ozler G.S., Ozgur T., Arli C., Sefil F.Y. and Yonden Z. (2014). Determination of oxidative stress and effect of erdosteine on rhinitis medicamentosa in a rat model. Eur. J. Pharmacol. 742, 153-157.

Elwany S. and Abdel-Salaam S. (2001). Treatment of rhinitis medicamentosa with fluticasone propionate-an experimental study. Eur. Arch. Otorhinolaryngol. 258, 116-119.

Elwany S.S. and Stephanos W.M. (1983). Rhinitis medicamentosa. An experimental histopathological and histochemical study. ORL J. Otorhinolaryngol. Relat. Spec. 45, 187-194.

Ferrara N. and Kerbel R.S. (2005). Angiogenesis as a therapeutic target. Nature 438, 967-974.

Gochman E., Mahajna J., Shenzer P., Dahan A., Blatt A., Elyakim R. and Reznick A.Z. (2012). The expression of iNOS and nitrotyrosine in colitis and colon cancer in humans. Acta histochem. 114, 827-835.

Goldberg M.A. and Schneider T.J. (1994). Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. J. Biol. Chem. 269, 4355-4359.

Graf P. (2005). Rhinitis medicamentosa: a review of causes and treatment. Treat Respir. Med. 4, 21-29.

Kohen M.C., Tatlipinar S., Cumbul A. and Uslu Ü. (2018). The effects of bevacizumab treatment in a rat model of retinal ischemia and perfusion injury. Mol. Vis. 24, 239-250.

Kolios G., Valatas V. and Ward S.G. (2004). Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology 113, 427-437.

Leberl M., Kratzer A. and Taraseviciene-Stewart L. (2013). Tobacco smoke induced COPD/emphysema in the animal model-are we all on the same page? Front. Physiol. 4, 91.

Lin C.-Y., Cheng P.-H. and Fang S.-Y. (2004). Mucosal changes in rhinitis medicamentosa. Ann. Otol. Rhinol. Laryngol. 113, 147-151.

Macciò A. and Madeddu C. (2012). Inflammation and ovarian cancer. Cytokine 58, 133-147.

Melincovici C.S., Boşca A.B., Şuşman S., Mărginean M., Mihu C., Istrate M., Moldovan I.M., Roman A.L. and Mihu C.M. (2018). Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. Rom. J. Morphol. Embryol. 59, 455-467.

Meurs H., Maarsingh H. and Zaagsma J. (2003). Arginase and asthma: novel insights into nitric oxide homeostasis and airway hyperresponsiveness. Trends Pharmacol. Sci. 24, 450-455.

Mungrue I.N., Husain M. and Stewart D.J. (2002). The role of NOS in heart failure: lessons from murine genetic models. Heart Fail Rev. 7, 407-422.

Niu Y., Chen R., Xia Y., Cai J., Lin Z., Liu C., Chen, C. Peng L., Zhao Z., Zhou W., Chen J. and Kan H., (2018). Personal ozone exposure and respiratory inflammatory response: The role of DNA methylation in the arginase-nitric oxide synthase pathway. Environ. Sci. Technol. 52, 8785-8791.

Ramey J.T., Bailen E. and Lockey R.F. (2006). Rhinitis medicamentosa. J. Investig. Allergol. Clin. Immunol. 16, 148-155.

Rini B.I. and Small E.J. (2005). Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. J. Clin. Oncol. 23, 1028-1043.

Robinson M.A., Baumgardner J.E. and Otto C.M. (2011). Oxygen-dependent regulation of nitric oxide production by inducible nitric oxide synthase. Free Radic. Biol. Med. 51, 1952-1965.

Rosenfeld P.J., Brown D.M., Heier J.S., Boyer D.S., Kaiser P.K., Chung C.Y. and Kim R.Y. (2006). Ranibizumab for neovascular age-related macular degeneration. N. Engl. J. Med. 355, 1419-1431.

Scadding G.K. (1995). Rhinitis medicamentosa. Clin. Exp. Allergy 25 5, 391-394.

Seimetz M., Parajuli N., Pichl A., Veit F., Kwapiszewska G., Weisel F.C., Milger K., Egemnazarov B., Turowska A., Fuchs B., Nikam S., Roth M., Sydykov A., Medebach T., Klepetko W., Jaksch P., Dumitrascu R., Garn H., Voswinckel R., Kostin S., Seeger W., Schermuly R.T., Grimminger F., Ghofrani H.A. and Weissmann N. (2011). Inducible NOS inhibition reverses tobacco-smoke-induced emphysema and pulmonary hypertension in mice. Cell 147, 293-305.

Semenza G.L. (1998). Hypoxia-inducible factor 1: master regulator of O2 homeostasis. Curr. Opin. Genet. Dev. 8, 588-594.

Simonetti O., Lucarini G., Campanati A., Goteri G., Zizzi A., Marconi B., Ganzetti G., Minardi D., Di Primio R. and Offidani A. (2009). VEGF, survivin and NOS overexpression in psoriatic skin: critical role of nitric oxide synthases. J. Dermatol. Sci. 54, 205-208.

Snow S.S., Logan T.P. and Hollender M.H. (1980). Nasal spray “addiction” and psychosis: a case report. Br. J. Psychiatry 136, 297-299.

Vouldoukis I., Riveros-Moreno V., Dugas B., Ouaaz F., Bécherel P., Debré P., Moncada S. and Mossalayi M.D. (1995). The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the Fc epsilon RII/CD23 surface antigen. Proc. Natl. Acad. Sci. USA 92, 7804-7808.

Zucker S.M., Barton B.M. and McCoul E.D. (2019). Management of rhinitis medicamentosa: A systematic review. Otolaryngol. Head Neck Surg. 160, 429-438.

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