Original Research Article

Antiangiogenic Effects Of Infliximab In In-Vivo Chick Chorioallantoic Membrane Model

ABSTRACT

Introduction: TNF-α plays a key role in initiating pathologic angiogenesis, however, literature on the effects of infliximab on angiogenesis is limited.

Objective: The study aimed to investigate the effects of infliximab on angiogenesis in an in vivo chick chorioallantoic membrane (CAM) model.

Methods: The study was approved by the local ethics committee on animal experimentation. Thirty fertilized specific pathogen-free eggs were incubated and kept under appropriate temperature and humidity control. On the third day of the incubation, infliximab (1 µmol) dissolved in phosphate-buffered saline in the first group, phosphate-buffered saline (negative control) (0.1 ml) in the second group, anti-VEGF (positive control) (1 µmol) in the third group were administered by injection. On the eighth day of incubation, the vascular structures of the CAMs were macroscopically examined, and standard digital photographs were taken. The digital images were analyzed, and data including mean vessel density, thickness, and number were compared between groups. $P < 0.05$ was considered statistically significant.

Results: Angiogenesis was significantly reduced in the anti-VEGF and infliximab groups compared to the saline-only group. Vessel thickness, vessel number, and vessel density were significantly less in the infliximab and anti-VEGF groups compared to the saline-only group (p=0.034, 0.029, 0.024, respectively).

Conclusions: Infliximab showed promising antiangiogenic effects in the chick CAM model. Thus, infliximab could be a treatment agent in pathological processes in which angiogenesis is responsible. The antiangiogenic effect of infliximab could be due to the inhibition of various angiogenesis-related cytokines and adhesion molecules. Further studies are needed to clarify the exact mechanisms.

Keywords: infliximab; angiogenesis; in-vivo model.

1. INTRODUCTION

Infliximab is a chimeric (mouse/human) immunoglobulin G1 monoclonal antibody that targets both soluble and transmembrane tumor necrosis factor-alpha (TNF-α). TNF-α is a potent proinflammatory cytokine that plays a role in immune dysregulation in various chronic inflammatory disorders including inflammatory bowel disease, rheumatoid arthritis,

ankylosing spondylitis, psoriatic arthritis, and chronic severe plaque psoriasis in adult patients [1].

Angiogenesis refers to the process of formation of new vessels from preexisting vascular structures. It is a multistep process that involves a series of events, and each step involves multiple growth factors, receptors, and molecules. Angiogenesis plays role in physiological conditions, such as embryonic development and wound healing, but is also a key player in a wide variety of pathological processes, including cancer, infection, arthritis, psoriasis, coronary artery disease, connective tissue diseases, and retinopathy [2-5].

In recent years, it has been shown that TNF-α plays a key role in initiating pathologic angiogenesis in a variety of clinical conditions [2, 6-8]. The high efficacy of TNF-α inhibitors in the treatment of chronic inflammatory diseases has been thought to be due primarily to the rapid reduction in the number of inflammatory cells, and little attention has been paid to their potential antiangiogenic effects.

Although some literature data are showing that infliximab decreases angiogenesis, experimental studies are scarce. Chick chorioallantoic membrane model is a low-cost and practical in-vivo model suitable for studying angiogenesis [9-11].

This study aimed to examine the effects of infliximab on angiogenesis in chick chorioallantoic membrane model.

2. MATERIAL AND METHODS

Thirty fertilized SPF eggs were included in the study. The study protocol was in compliance with current guidelines for the care of the laboratory animals and was approved by the Animal Experiments Local Ethics Committee. Eggs were randomly distributed and 3 groups of 10 eggs were formed. All fertilized eggs were incubated in the incubator under appropriate temperature and humidity control after their shells were sterilized. On the third day of incubation, infliximab (1 µmol) dissolved in phosphate-buffered saline in Group 1, phosphate-buffered saline (negative control) (0.1 ml) in Group 2, and bevacizumab (positive control) (1 µmol) in Group 3 was injected onto the chorioallantoic membrane. And all the eggs were placed back in the incubator by sticking a sterile film on the broken part of the shell. On the eighth day of incubation, the vascular structures in the chorioallantoic membranes were visualized ex-ovo, and all chick embryos were removed from the eggs and the process was terminated. Vascular structures in the digital photographs taken were evaluated with the Image J image analysis program and the angiogenesis differences between the groups were determined statistically. The number of vessels, vessel area and density (%), and vessel thickness (mm) were compared between 3 groups. SPSS v.18.0 program and Kruskal-Wallis test were used for statistical comparison. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

It was observed that angiogenesis was normal in the saline-only group, and angiogenesis was significantly reduced in the anti-VEGF and infliximab groups. Compared to the negative control, vessel thickness, vessel number, and vessel density were significantly less in the infliximab and anti-VEGF groups (p=0.034, 0.029, 0.024, respectively).

The effects of anti-TNF-alpha drugs on angiogenesis is a current issue. In some studies, it has been mentioned that these drugs may have antiangiogenic effects besides anti-

inflammatory effects [6-8]. However, there is no previous study examining the antiangiogenic potentials of these agents in the chick embryo angiogenesis model. This study is a first in this regard and provides crucial data regarding the potential benefit of infliximab in the treatment of pathological processes in which angiogenesis plays a role.

Anti-TNF-α drugs offer high efficacy in the treatment of chronic inflammatory disorders by promoting a rapid reduction in cell number at the site of inflammation through apoptosis, cytotoxicity, decreased cell flow, or reduction in chemotactic molecules [6].

TNF-α is one of the several pro-inflammatory cytokines that have been reported to promote angiogenesis [6-8, 12-23]. TNF-α stimulated-immune cells can release some angiogenesis-related cytokines and adhesion molecules such as vascular endothelial growth factor (VEGF), Tie-2, basic fibroblast growth factor (bFGF), Interleukin-8 (IL-8), intercellular adhesion molecule (ICAM-1), Leucine-rich-alpha-2-glycoprotein1 (LRG1), Angiopoietin-2 (Ang-2) and vascular cell adhesion molecule (VCAM-1) [6, 15-20].

In mouse corneas, TNF-alfa shows a synergistic effect in soluble VCAM-1-induced angiogenesis [15]. In psoriatic skin, TNF-α increases VEGF expression, promoting angiogenesis, which performs an important role in altering the morphology of blood vessels observed [24-26]. Furthermore, TNF-α increases angiogenic potential in a co-culture system of dental pulp cells and human umbilical vein endothelial cells (HUVECs) [19]. In the peritoneal angiogenesis model, TNF-α up-regulates the gene expression of VEGF, Angiopoietin-2, and Tie2 and significantly promotes angiogenesis [20]. TNF-α is upregulated in İnterferon-induced protein with tetratricopeptide repeats 2 (IFIT2)-depleted metastatic oral squamous cell carcinoma (OSCC) cells, which exhibit enhanced metastatic activity, and chemoresistance. And, blocking TNF-α inhibits angiogenesis, growth and metastases of OSCC cells [23].

It has been suggested that the antipsoriatic mechanism of TNF-α inhibitors may be due to the dual inhibition of inflammation and angiogenesis [6]. Administration of anti-TNF-α antibody to K14-VEGF transgenic mice had both significant treatment efficiency in psoriasis and inhibited blood vessels formation directly and indirectly [6]. Anti-TNF-α antibodies could suppress the TNF-α induced inflammatory cascade, which activated immune cells and promoted the release of some angiogenesis-related cytokines and adhesion molecules, thereby inflammation-induced angiogenesis was inhibited. Moreover, anti-TNF-α antibodies may also have a direct role in suppressing vascular formation, which has been proved by the endothelial cells tube formation in vitro [6].

There are some literature data showing the antiangiogenic effects of infliximab in various pathological conditions. Administration of infliximab downregulates mucosal angiogenesis in patients with Chron's disease and restrains the production of VEGF-A by mucosal fibroblasts. It is proposed that this ameliorates inflammation-driven angiogenesis in the gut mucosa and contributes to the therapeutic efficacy of blockade of TNF-α [27]. Furthermore, in the synovial membrane of patients with psoriatic arthritis, infliximab induced consistent changes in several factors involved in angiogenesis regulation, in parallel with the clinical response. The pattern of reduced VEGF with increased Ang-2 as well as Tie2 reduction suggested vascular regression as a potential mechanism underlying the antiangiogenic effect of infliximab [28].

In a rabbit model of corneal neovascularization induced by alkali burn, the topical administration of infliximab inhibited corneal neovascularization and decreased inflammation and fibroblast activity. And infliximab has been suggested to be a promising alternative for the ocular topical antiangiogenic therapy [29]. Furthermore, in experimental choroidal

neovascularization and age-related macular degeneration rat models,intravitreal infliximab reduced angiogenesis and inhibited choroidal neovascularization growth [30, 31].

4. CONCLUSION

[In conclusion, TNF-a promotes angiogenesis through various angiogenesis-related cytokines and adhesion molecules. And anti-TNF-α agents offer promising antiangiogenic effects. Our study findings showing decreased angiogenesis with infliximab in an in vivo chick chorioallantoic membrane model support the previous literature data on the antiangiogenic effects of infliximab. Therefore, the study findings suggest that infliximab can be studied as a potential treatment agent in other pathologies with increased angiogenesis.

CONSENT AND ETHICAL APPROVAL

The study protocol was approved by the Local Ethics Committee (AKÜHADYEK-267-17).

REFERENCES

1. Melsheimer R, Geldhof A, Apaolaza I, Schaible T. Remicade® (infliximab): 20 years of contributions to science and medicine. Biologics. 2019;13:139-178.

2. Shin MR, Kang SK, Kim YS, Lee SY, Hong SC, Kim EC. TNF-α and LPS activate angiogenesis via VEGF and SIRT1 signalling in human dental pulp cells. Int Endod J. 2015;48(7):705-16.

3.Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003;9(6):653-660.

4. Folkman J, Klagsburn M. Angiogenetic factors. Science. 1987;235(4787):442-7.

5.Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017 Jun 28. doi: 10.1007/s10456-017-9562-9.

6. Liu Y, Yang G, Zhang J, Xing K, Dai L, Cheng L, et al. Anti-TNF-α monoclonal antibody reverses psoriasis through dual inhibition of inflammation and angiogenesis. Int Immunopharmacol. 2015;28 (1):731-43.

7. Whitmore HAB, Amarnani D, O'Hare M, Delgado-Tirado S, Gonzalez-Buendia L, An M, et al. TNF-α signaling regulates RUNX1 function in endothelial cells. FASEB J. 2021;35(2):e21155.

8 Fajardo LF, Kwan HH, Kowalski J, Prionas SD, Allison AC. Dual role of tumor necrosis factor-alpha in angiogenesis. Am J Pathol. 1992;140(3):539-544.

9.Ribatti D. The chick embryo chorioallantoic membrane (CAM). A multifaceted experimental model. Mech Dev. 2016;141:70-7.

10.Deryugina EI, Quigley JP. Chapter 2. Chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. Methods Enzymol. 2008;444:21-41.

11.Ribatti D. Chicken chorioallantoic membrane angiogenesis model. Methods Mol Biol. 2012;843:47-57.

12. F. Balkwill, Tumour necrosis factor and cancer. Nat Rev Cancer. 2009;9(5): 361–371.

13. Pan, P. An, R. Zhang, X. He, G. Yin, W. Min, Etk/Bmx as a tumor necrosis factor receptor type 2-specific kinase: role in endothelial cell migration and angiogenesis, Mol Cell Biol. 2002;22(21):7512-7523.

14.R. Zhang R, Xu Y, Ekman N, Wu Z, Wu J, Alitalo K, Min W. Etk/Bmx transactivates vascular endothelial growth factor 2 and recruits phosphatidylinositol 3-kinase to mediate the tumor necrosis factor-induced angiogenic pathway. J Biol Chem. 2003;278(51):51267-76.

15. Nakao S, Kuwano T, Ishibashi T, Kuwano M, Ono M. Synergistic effect of TNF-alpha in soluble VCAM-1-induced angiogenesis through alpha 4 integrins. J Immunol. 2003;170(11):5704-11.

16. Shi JX, Su X, Xu J, Zhang WY, Shi Y. MK2 posttranscriptionally regulates TNF-α-induced expression of ICAM-1 and IL-8 via tristetraprolin in human pulmonary microvascular endothelial cells. Am J Physiol Lung Cell Mol Physiol. 2012;302(8):L793-9.

17. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. Nature. 1987;329(6140):630-2.

18. Wang Y, Xu J, Zhang X, Wang C, Huang Y, Dai K, Zhang X. TNF-α-induced LRG1 promotes angiogenesis and mesenchymal stem cell migration in the subchondral bone during osteoarthritis. Cell Death Dis. 2017;8(3):e2715.

19. An L, Shen S, Wang L, Li Y, Fahim S, Niu Y, et al. TNF-alpha increases angiogenic potential in a co-culture system of dental pulp cells and endothelial cells. Braz Oral Res. 2019;33:e059.

20. Yuan J, Fang W, Lin A, Ni Z, Qian J. Angiopoietin-2/Tie2 signaling involved in TNF-α induced peritoneal angiogenesis. Int J Artif Organs. 2012;35(9):655-62.

21. DeBusk LM, Chen Y, Nishishita T, Chen J, Thomas JW, Lin PC. Tie2 receptor tyrosine kinase, a major mediator of tumor necrosis factor alpha-induced angiogenesis in rheumatoid arthritis. Arthritis Rheum. 2003;48(9):2461-71.

22. Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cellsurvival in vivo. Proc Natl AcadSciUSA. 2002;99:11205-10.

23. Lai KC, Liu CJ, Lin TJ, Mar AC, Wang HH, Chen CW, et al. Blocking TNF-α inhibits angiogenesis and growth of IFIT2-depleted metastatic oral squamous cell carcinoma cells. Cancer Lett. 2016;370(2):207-15.

24. Yang HL, Chang HC, Lin SW, Senthil Kumar KJ, Liao CH, Wang HM, et al. Antrodiasalmonea inhibits TNF-α-induced angiogenesis and atherogenesis in human endothelial cells through the down-regulation of NF-κB and up-regulation of Nrf2 signaling pathways, J Ethnopharmacol. 2014;151(1):394-406.

25. Noort AR, van Zoest KP, Weijers EM, Koolwijk P, Maracle CX, Novack DV, et al. NF-κB inducing kinase is a key regulator of inflammation-induced and tumor-associated angiogenesis. J Pathol. 2014;234(3):375-385.

26. Jung K, Lee D, Lim HS, Lee SI, Kim YJ, Lee GM, Kim SC, et al. Double anti-angiogenic andanti-inflammatory protein valpha targeting VEGF-A and TNF-α in retinopathy and psoriasis, J Biol Chem. 2011;286(16):14410-14418.

27. Rutella S, Fiorino G, Vetrano S, Correale C, Spinelli A, Pagano N, et al. Infliximab therapy inhibits inflammation-induced angiogenesis in the mucosa of patients with Crohn's disease. Am J Gastroenterol. 2011;106(4):762-70.

28. Cañete JD, Pablos JL, Sanmartí R, Mallofré C, Marsal S, Maymó J, et al. Antiangiogenic effects of anti-tumor necrosis factor alpha therapy with infliximab in psoriatic arthritis. Arthritis Rheum. 2004;50(5):1636-41.

29. Voiculescu OB, Voinea LM. Infliximab eye drops treatment in corneal neovascularization. J Med Life. 2015;8(4):566-7.

30. Regatieri CV, Dreyfuss JL, Melo GB, Lavinsky D, Farah ME, Nader HB. Dual role of intravitreous infliximab in experimental choroidal neovascularization: effect on the expression of sulfated glycosaminoglycans. Invest Ophthalmol Vis Sci. 2009;50(11):5487-94.

31. Olson JL, Courtney RJ, Mandava N. Intravitreal infliximab and choroidal neovascularization in an animal model. Arch Ophthalmol. 2007;125(9):1221-4.