# Is VEGF a potential therapeutic target in asthma?

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**Abstract**

*Asthma is currently affecting approximately 300 million people worldwide and is expected to rise to 400 million over the next couple decades. Airway remodelling in asthma consists of mucus hypersecretion, epithelium erosion, extracellular matrix protein deposition under the basement membrane, smooth muscle proliferation and angiogenesis. The vascular endothelial growth factor (VEGF) plays a part in remodelling through its effect upon angiogenesis, but the extent of its contribution to asthma pathology is not yet completely understood. Irreversible airway remodelling seems to be an important contributor to asthma progressive worsening, so further developments in preventing remodelling are warranted. A better understanding of VEGF mechanisms in asthma could aid such therapeutic developments.*
***Keywords:*** *asthma, VEGF, airway remodelling, therapy*

**Rezumat**

***Este VEGF o potențială țintă terapeutică în astm?***
*Astmul afectează, în prezent, aproximativ 300 de milioane de oameni din întreaga lume și este de așteptat ca acest număr să crească la 400 de milioane în următoarele decenii. Remodelarea căilor respiratorii în astm este caracterizată de hipersecreție de mucus, eroziuni epiteliale, depuneri de proteine ale matricei extracelulare în membrana bazală, proliferarea musculaturii netede și angiogeneză. Factorul de creștere a endoteliului vascular (VEGF) joacă un rol în remodelare, prin efectul său asupra angiogenezei, dar gradul său de contribuție la fiziopatologia astmului nu este încă pe deplin înțeles. Remodelarea ireversibilă a căilor respiratorii pare să contribuie semnificativ la agravarea progresivă a astmului, astfel încât sunt justificate studii ulterioare în prevenirea remodelării. O mai bună înțelegere a mecanismelor VEGF în astm ar putea ajuta astfel de evoluții terapeutice.*
***Cuvinte-cheie:*** *astm, VEGF, remodelarea căilor aeriene, terapie*

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## Introduction

Asthma entails worldwide thousands of hospital admissions, work and school days missed and patients experience a significantly decreased quality of life, therefore this disease has profound direct and indirect costs. Susceptibility to the development asthma is dependent upon a combination of environmental and genetic factors which also determine asthmatic phenotype, severity, and age of onset. The literature suggests there may be well over 100 contributing genes which interact to produce the onset of asthma[1], and although asthmatic phenotype is shaped by differences in adaptive and innate immunity within each individual patient, observations in families suggest genetics have a large influence.

Asthma airway remodelling comprises multiple structural alterations such as mucus hypersecretion, epithelial basement membrane thickening, smooth muscle hypertrophy, and most important in relation to vascular endothelial growth factor (VEGF), angiogenesis. Remodelling is thought to be caused by the abnormal chronic allergic inflammation which entails repeated injury and repair of the airway walls. Several recent developments suggest remodelling and inflammation occur simultaneously[2], indicating that structural remodelling can be detected even in the early stages of asthma. Furthermore, several papers provide data implicating its importance to disease severity[3, 4].

### *Bronchial angiogenesis*

Changes in airway vasculature could contribute to bronchial hyperresponsiveness[5]. In normal tissues, vascularisation is maintained by the dynamic interaction of pro and anti-angiogenic factors. However, in chronic inflammation conditions, this balance is altered and causes abnormal neoangiogenesis. This increased airway vascularisation is well documented in asthma and is correlated with disease progression from mild and moderate asthma to severe forms[4,6-7]. The clinical significance of vascular alterations lies in the fact that the associated cell infiltration and oedema lead to airway wall thickening and narrowing of the lumen as well as further remodelling[8]. Among the number of inflammatory modulators involved in angiogenesis and the most potent in regulating asthmatic airway remodelling is VEGF, whose excessive expression is detected in the asthmatic airways.

Considering the consequent clinical effects of airway remodelling, VEGF inhibition could represent a promising potential target for asthma treatment.

## Overview of VEGF involvement in asthma

VEGF is an abundant polypeptide within the lung where it has a key role in mediating the angiogenesis. VEGF is expressed by eosinophils and other cell types in the lung and acts as an inducer of endothelial cell activation, broadly impacting their functions and causing oedema and increased vascular permeability[9]. Contrary to reports claiming it lacks angiogenic effects in the respiratory tract[10] there is considerable evidence to suggest that angiogenesis and chronic inflammation are co-dependent. Thus, VEGF is a potent remodelling mediator and its inhibition has potential therapeutic effects.

### *Structure and receptors*

The VEGFR-2 receptor has the primary role in transmitting the VEGF signal. An alternatively spliced soluble form of VEGFR-1 is seemingly inert and has been shown to be a natural inhibitor of VEGF activity in humans and mice[11]. This soluble form is assumed to be upregulated during overactive signalling in order to act as a decoy for VEGF, consequently decreasing VEGF/VEGFR-2 signalling. The specificity of

VEGF binding further implies that the soluble decoy receptor may be a good biomarker for asthma progression and treatment response.

***Biological function***

Because of its wide distribution, VEGF affects the properties of endothelial cells in different organs, but most relevantly in the asthmatic lungs. In endothelial cells, VEGF promotes the secretion of chemokines, the expression of adhesion molecules such as E-selectin and the production of anti- apoptotic factors, such as survivin. VEGF also activates endothelial nitric oxide synthase (NOS) which causes vasodilation through the synthesis of nitric oxide (NO). VEGF gene expression and its secretion can be induced by many growth factors and cytokines. TGF–β and IL–13 were shown to regulate VEGF secretion in fibroblasts, airway smooth muscle cells, and epithelial cells whereas GM-CSF has a similar action in eosinophils. In addition, VEGF secretion can be regulated by environmental factors. For example hypoxia is a potent stimulus for VEGF production in endothelial cells with the aim of promoting vessel formation to increase gas exchange. As mentioned previously, the balance in VEGF regulation is disrupted in the asthmatic airway remodelling phenotype. Inhibitors of the VEGF pathway include endostatin, angiostatin, and TIMPs. These molecules which would usually inhibit the VEGF effects are less potent in asthma, either because of their downregulation in the chronically inflamed airway or from an imbalance in the production in relation to pro-angiogenic factors.

High VEGF levels have been found in asthmatic BAL and sputum samples[6]. Although its main action is in angiogenesis, VEGF also impacts other stages of remodelling increasing its profile potential therapeutic target. Thus therapeutic agents that could antagonize the effects of VEGF may be clinically useful in the treatment of asthmatic symptoms through prevention of irreversible airway remodelling.

## Remodelling phenotype of asthma

There are a large percentage of patients who do not respond completely to the classical treatment of asthma. Some of these patients display significant airway remodelling characterised by progressively thickened basement membranes with little to no improvement despite adherence to treatment. Better knowledge of VEGF mechanisms within the airways is useful in perhaps recognizing a VEGF-specific phenotype in which a distinct form of inflammation entailing remodelling exists. In light of the expected increase in asthma incidence, potentially as a result of alterations in air pollution, distinct phenotypes may emerge. Given the correlation with increased disease severity, therapies which target airway remodelling could represent a significant development.

## New proposed treatments

Recognition of the VEGF pathway as a key regulator of angiogenesis has led to the development of therapeutic strategies designed to inhibit VEGF production. Given the involvement of this messenger in cancer angiogenesis, VEGF inhibitors have been developed for cancer in order to prevent tumour metastasis. Thus treating tumours by inhibiting their ability to develop new blood vessels is based on the premise that cancer growth is dependent on angiogenesis. A similar concept could be applied to the angiogenesis that characterises airway remodelling in asthma inflammation.

***Anti-VEFG antibodies***

Some VEGF–neutralizing monoclonal antibodies have been developed to block VEGF-A from binding to VEGFR-2 but not to VEGFR-1. They have shown impressive antitumor activity in preclinical and early stage clinical studies. These antibodies induce the apoptosis of the endothelial cells of newly formed vessels which are dependent on VEGF-A to maintain cell adhesion and survival, while maintaining the integrity of fully developed vessels.

Of the several antibodies developed to target VEGF most clinical experience was obtained with bevacizumab[8], a humanized antibody which acts by binding and neutralizing the biologically active VEGF-A to prevent it from binding VEGFRs. Generally, it is well tolerated in cancer patients; a double-blinded phase two trial of 116 patients with metastatic kidney cancer showed bevacizumab was able to majorly slow down progression[12]. Another bifunctional antibody has been designed using domains from both an anti-VEGFR-2 antibody and an anti-VEGFR-1 antibody[13]. This new antibody is able to bind both VEGFR-1 and VEGFR-2 and is capable of blocking the interaction between the receptors and their respective ligands, resulting in a more potent inhibition of VEGF-stimulated endothelium. Although these findings suggest that monoclonal antibodies such as bevacizumab could be used therapeutically in preventing asthmatic angiogenesis, their use has not yet been attempted clinically. Using novel technical advances in developing monoclonal antibodies, researchers might be able to further develop these drugs to allow them to bind specifically to vessels within the asthmatic airway.

***RTK blockers***

VEGF function can also be inhibited by small molecules that block activation of the VEGFR tyrosine kinase activity and subsequently interfere with the VEGF signal transduction pathway. Several drugs have been developed for this purpose and SU5416 was one of the first of this kind to be explored clinically in cancer. Adverse effects in phase one trials of SU5416, however, have dampened initial enthusiasm in addition to its intravenous administration[14]. Several new smaller and orally available tyrosine kinase inhibitors are currently in clinical development, but their recorded antitumor activity is limited. Tumours may not be inhibited by these drugs as expected due to their ability to maintain the angiogenic process through interaction with an array of growth factors[15]. Despite this, VEGF-blocking therapy may be promising in the treatment of asthma, since it is directed solely against proliferating capillary endothelial cells at the site of angiogenesis and because in asthma VEGF seems to be the primary factor involved. Furthermore, it is debatable whether current studies in cancer are utilizing the VEGF inhibitors for a sufficient period of time. It appears that angiogenic inhibitors must be administered continuously and for longer than is usual with conventional chemotherapy, as the regression of a quickly growing capillary bed is slower than the destruction of tumour cells. Further research could potentially allow these drugs to express high specificity for lung endothelial cell surface proteins and VEGFRs.

***Soluble FLT-1 receptor***

Another potential approach for inhibiting VEGF is the use of the soluble form of VEGFR-1, or FLT-1, administered with an adenovirus. As acknowledged earlier, VEGFR-1 functions as an endogenous inhibitor of VEGF, by binding to it with the same affinity as VEGFR-2 but without activating the signalling pathway. This tactic was experimented for attacking tumour neoangiogenesis in ovarian cancer[16]. By constructing a recombinant adenovirus designed to express soluble FLT-1, researchers were able to monitor tumour growth within two different mouse models. What the authors appropriately found was that the treatment was able to successfully inhibit ovarian tumour growth and increase survival rate by blocking the VEGF pathway. Results in both models showed significant disruption of local vasculature at doses which did not lead to any signs of toxicity. These results may look promising for the prevention of asthmatic angiogenesis, but using adenoviruses as carriers is problematic. There are several difficulties posed by adenoviral therapy; for example, the expression of the transgene is relatively short, lasting only 5 – 10 days. The vectors are known to have an inability to differentiate between dividing and non-dividing cells, suggesting their use might not be successful for specifically preventing new vessel growth. The success of the treatment in syngenic animal models may not be easy to reproduce in humans, due to the vast variations in humans. Furthermore, the human immune system can easily fight off the viral vector, seeing it as a pathogenic threat[17].

***VEGF and endostatin***

Overactivity of VEGF can result from an imbalance between its expression and that of its opposing factors. One of these factors, endostatin, is a collagen-like globular protein produced endogenously by varied cell types which is a strong inhibitor of angiogenesis[18]. Authors simultaneously measured levels of endostatin and VEGF in the induced sputum of asthmatic patients and evaluated their correlation and balance compared to healthy control subjects. They concluded that the ratio of VEGF to endostatin was significantly higher in asthmatics, but could be reversed back to control levels by treatment with beclomethasone dipropionate (BDP). These results support the hypothesis that inhaled corticosteroids (ICS) may suppress asthmatic airway remodelling by inhibiting angiogenesis.

***Corticosteroids as VEGF inhibitors***

With VEGF antagonism emerging as a prospective treatment in asthma, several researchers decided to take the previous steroid findings one step further by analysing them in well characterised experimental asthma models.

One team investigated the ability of budesonide to inhibit airway remodelling[9]. Inhaled budesonide is known to alter protein synthesis in asthma and its effects on an airway (Calu-1) and an alveolar (A549) epithelial cell line were observed. The group established that the drug was able to inhibit VEGF secretion and mRNA expression in both cell types in a dose dependent manner. Budesonide action on the glucocorticosteroid receptor was confirmed by using the receptor antagonist mifepristone. Addition of this blocked the budesonide-dependent inhibition of VEGF production in these cell lines. Another group also investigated the use of corticosteroids to inhibit VEGF action. ICS treatment using dexamethasone led to decreased remodelling, fewer VEGF-expressing cells and lower basement membrane thickness[8]. However, determining the effects of steroid use on patients who perhaps have not yet developed noticeably symptomatic asthma is essential; though the experiments use a control asthmatic mouse, any possible adverse side effects of these drugs on a wild-type mouse or relatively healthy patient will have to be considered. Likewise, further support for dexamethasone use could be gained from dose comparison trials in order to determine the minimum effective dose needed. As previously mentioned, glucocorticosteroids are highly effective as anti-asthmatic drugs, but can be ineffective in patients with steroid resistance; this must also be taken into account.

***VEGF signalling through FABP4***

One prospective treatment involving the VEGF induced angiogenesis pathway would be to target components downstream, such as fatty acid binding protein 4 (FABP4). FABP4 is known as an intracellular chaperone molecule involved in angiogenesis, highly specific to the lung, and induced by VEGF *in vitro*. It is suggested that FABP4 also plays a role in the regulation of asthmatic airway angiogenesis *in vivo*[19]. In mouse models VEGF overexpression (VEGF–TG) correlated positively with elevated levels of FABP4 proving their connection; in addition, bronchial biopsies from asthmatic patients show higher levels of the protein in blood vessels compared to control subjects. Knockout (KO) of FABP4 expression in mice resulted in significantly diminished VEGF-induced inflammation, neovascularisation, and endothelial cell proliferation. Furthermore, VEGF overexpression in these KO mice produced lower levels of NOS, a mediator of VEGF inflammation, and consequently exhaled NO (eNO) levels. These results show that FABP4 is responsible for modulating VEGF responses within mouse airways, and uncover a possible target for inhibition in humans. In the asthmatic subjects, researchers found that vessels expressing FABP4 were in close proximity with the bronchial endothelium, and that this indicates vessels which are undergoing angiogenic remodelling; the protein serves as a highly valid biomarker for neovascularisation. The authors also suggest that lower airway inflammation could be attributed to a decreased inflammatory cell influx due to a decreased amount of blood vessels.

***VEGF signalling through NO***

Some studies accredit the asthmatic abilities of VEGF to its connection with NO[20]. NO is a free radical gaseous regulator produced by a wide variety of cells and essential to maintenance of several biological processes. In asthma, elevated levels of eNO are indicative of asthma and VEGF has been found to be a potent stimulator of enzyme NOS and thus NO production. Although the role of NO in the inflammatory response has been well documented, its role in modulating the effects of VEGF had not adequately been investigated and so is questioned in this study. The authors hypothesized that VEGF induces its extravascular responses in asthma specifically through NO-dependent mechanisms.

Using NOS inhibitor L-arginine methyl ester (L-NAME), the authors blocked VEGF-induced oedema, neovascularisation, and cell infiltration, proving that these mechanisms are at least in part NO-dependent. Through these results, authors demonstrate that VEGF induces angiogenesis, but also has effects such as mucus metaplasia, oedema, and AHR

on nonvascular tissue through NO-dependent mechanisms, whilst having no effect on induction of dendritic cells; dendritic cells can be omitted from consideration of potential targets. Therefore the VEGF-NO pathway could serve as a useful pharmaceutical target for VEGF-induced asthma and remodelling prevention.

***VEGF and baicalin***

Though many believe herbal remedies have less medical importance, there is increasing evidence for their usefulness in the treatment of asthma and the prevention of remodelling[21]. Baicalin can be extracted from the root of *Scutellaria baicalensis*, a plant used for years in Chinese medicine to combat conditions such as cancer and liver disease. Researchers tested increasing doses of the compound on asthmatic mouse models and found improved lung function in a dose dependent manner. The results suggest that baicalin has significant anti-remodelling effects *in vivo* by decreasing VEGF protein expression, and may very well do so in clinical trials as well. Being a direct plant derivative, it will be more readily metabolized by the body and patients avoid the risk of the major side effects they may come across in more common chemical pharmaceuticals. Moreover, baicalin is orally distributed and considerably lower in cost, making its incorporation in asthma treatment plans advantageous.

## Conclusion

Although we have some knowledge about the triggers of airway remodelling in asthma, including VEGF, there is still a lack of drugs that could be effective for remodelling prevention or treatment. Despite the improvements they provide, current therapies do not significantly influence airway remodelling. This poses an issue for asthmatics whose symptoms result from factors other than the standard asthmatic inflammation. Though a cure for asthma will not likely be found in the very immediate future, further research in addition to that which has been discussed can make incremental advances towards one. Trials using treatments targeting VEGF could help to classify patients by phenotype and create more specific and effective treatment plans. Expansion of this type of treatment could prevent patients from developing irreversible airflow obstruction.

The discovery of a VEGF specific phenotype will allow for patient–specific treatment. The cause of airway remodelling is multifactorial, which indicates that use of drugs optimized towards any one pathway may not be effective on their own. Nevertheless, antiangiogenic therapy is a promising approach and its use in conjunction with others may reduce the adverse effects of high dose steroids in use currently. Further clinical and preclinical trials are required to test various treatment strategies and to determine their toxicity profiles. In order to be thorough, researchers can create trials comparing the treatments discussed earlier in order to discover which of the compounds is most beneficial to the inhibition of VEGF-mediated remodelling. Along with the new technologies which provide highly specific genetic modification and targeting it should be possible to construct a drug with an effective active moiety. On the basis of this review, it is suggested that VEGF signalling is a suitable target for blockade with expectations of a lessened asthmatic remodelling response. Specialized treatment plans which focus on the prevention of airway remodelling, in particular through interference of the VEGF pathway, could have major benefits for this specific phenotype by altering the course of disease, and could point towards a cure for symptomatic asthma. ■

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