Chronic Obstructive Pulmonary Disease and Pulmonary Vascular Disease

A Comorbidity?

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of death worldwide, and in addition to its impact on the patient's health, it poses a major socioeconomic burden. Tobacco smoke, indoor cooking, and air pollution are major triggers of the disease. This article summarizes evidence for the concept that lung microvascular molecular alterations

can be a driver of lung emphysema. If findings from preclinical models allow a transfer to the human situation, this concept can offer new approaches for curative treatment of lung emphysema.

Keywords: chronic obstructive pulmonary disease; pulmonary hypertension; lung regeneration; inducible nitric oxide synthase; soluble guanylate cyclase

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Chronic obstructive pulmonary disease (COPD) is a leading health problem worldwide. The World Health Organization predicts that COPD will be ranked the third most important cause of death by 2030. The term “chronic obstructive pulmonary disease” or “COPD” summarizes chronic bronchitis with airway obstruction (that is not fully reversible upon application of bronchodilators or inhaled steroids) and lung emphysema. The latter is defined as a destruction of the alveoli distal of the terminal bronchioles. Lung emphysema and chronic bronchitis can occur simultaneously but also independently. Major risk factors for development of COPD are genetic determination (e.g., alpha-1 antitrypsin deficiency), exposure to airborne dusts and chemicals, and infections. However, the major cause of COPD is tobacco smoking. In this article, the possible contributions of pulmonary vascular remodeling and lung molecular vascular alterations in the

development and preclinical treatment of lung emphysema are discussed.

Classically, COPD is seen first as an airway disease that secondarily has consequences on the entire body, causing systemic effects such as cardiopulmonary disease and muscle wasting. However, more recent literature suggests that such systemic effects can also be caused directly by, for example, tobacco smoking (1). Tobacco smoke chemicals can pass from the lung's alveoli into the systemic circulation and exert systemic effects. Moreover, activation/release of mediators or cells from the pulmonary circulation may contribute to such effects. Accordingly, vascular dysfunction can be a feature of COPD (2). Against this background, research also focused on the pulmonary circulation. Inhaled noxious agents can directly affect the lung vasculature because there is an intimate structural linkage between the lung

capillaries and the precapillaries at the alveolar level (3).

Pulmonary Vascular Alterations and Remodeling in COPD

Early studies already found structural alterations of the pulmonary vasculature in rodent models of tobacco smoke exposure, and studies in guinea pigs finally showed that a vascular phenotype can precede emphysema-like airspace enlargement in this species (4, 5). Research in this field gained momentum when Barberà's group investigated human lungs from smokers who had not developed COPD and found severe pulmonary vascular remodeling with prominent narrowing of the pulmonary vascular lumen, resembling the characteristics of pulmonary vascular remodeling in pulmonary hypertension

(PH) (6). This group later showed that early vascular alterations such as smooth muscle cell proliferation also precede the development of airspace enlargement in smoke-exposed guinea pigs (7). Because pulmonary vascular remodeling can precede emphysema development, my group and others hypothesized that lung vascular alterations may even trigger lung emphysema development. In addition to pulmonary vascular remodeling, pulmonary endothelial dysfunction has been shown to occur upon smoke exposure (7, 8).

The occurrence of PH in patients with COPD is still under discussion, however. Although severe PH is rather rare in COPD and numbers range between 1% and 4% of patients with COPD, mild to moderate PH occurs much more often, and it was calculated that if the definition of PH were to be expanded to a mean pulmonary artery pressure greater than 20 mm Hg, the prevalence would be approximately 91% (9). Numerous studies showed that the presence of mild PH is of prognostic relevance in patients with COPD (10). For an overview, the reader is referred to a thorough review by Kovacs and colleagues (11).

Findings from Preclinical Models

Against this background, my group recently investigated a possible causative role of pulmonary vascular structural and molecular alterations in a C57BL/6 mouse model of tobacco smoke-induced emphysema (12). Intriguingly, pulmonary vascular remodeling and PH clearly preceded the development of airspace enlargement. Upon long-term tobacco smoke exposure, airspace enlargement was detectable at the earliest time point after 6 months of smoke exposure, as determined by alveolar morphometry as well as by lung functional parameters. PH was present already after 3 months of smoke exposure, however, concomitant with pulmonary vascular remodeling (12). Pulmonary vascular remodeling was characterized by an increased smooth muscle cell layer of the vessel media, and the phenotype was very similar to vascular remodeling seen in mouse models of hypoxia-induced PH. However, the smoke-exposed mice were not exposed to hypoxia; the smoke exposure chamber was not hypoxic, and arterial oxygenation was not reduced (12). Along these lines, gene

expression patterns of small vessels differed greatly when hypoxia-exposed and smoke-exposed mice were compared (12).

The Inducible Nitric Oxide Synthase–Nitric Oxide–Peroxynitrite Axis as a Driver of PH and Emphysema upon Smoke Exposure

Because oxidative and nitrosative stress have been implicated in the development of lung emphysema, my group focused on the regulation of endothelial nitric oxide synthase (eNOS; NOS3) and inducible nitric oxide synthase (iNOS; NOS2) as possible underlying mechanisms. My group identified an upregulation of iNOS that was restricted to the pulmonary vasculature, as shown by immunostaining and quantitative polymerase chain reaction of laser capture-microdissected vessels. The functional relevance of the iNOS upregulation was shown by the fact that iNOS-but not eNOS-knockout mice were completely protected from PH and the development of airspace enlargement upon smoke exposure (12). However, bone marrow transplant with reconstitution of irradiated wild-type mice of bone marrow from iNOS-knockout mice and vice versa revealed that iNOS in bone marrow-derived cells triggered PH, whereas iNOS in non-bone marrow-derived cells was responsible for the development of airspace enlargement (12) (Figure 1). My group concluded that iNOS, even in the absence of vascular remodeling, can trigger emphysema and thus suggested that lung vascular molecular alterations are a driver of lung emphysema development. My group proposed that iNOS and subsequent nitric oxide (NO) upregulation exert their effect via peroxynitrite formation that occurs in the presence of superoxide (13) (Figure 1). Accordingly, peroxynitrite increased apoptosis of alveolar epithelial type II cells and endothelial cells and decreased proliferation of type II cells. This is in line with the loss of alveoli and small vessels characteristic of emphysema. My group further linked peroxynitrite signaling to previously described key pathways of lung emphysema development: 1) upregulation of Rtp801, an inhibitor protein of mammalian target of rapamycin, shown to be essential for lung emphysema development (14); and 2) downregulation of vascular endothelial growth factor, which has been suggested to negatively impact the lung maintenance program (15).

Preclinical Treatment of Lung Emphysema with an iNOS Inhibitor

Using an iNOS inhibitor available for oral application, *N*⁶-(1-*iminoethyl*)-*L*-lysine dihydrochloride (*L*-NIL), my group found in tobacco smoke-exposed mice that this treatment not only prevented the development of lung airspace enlargement and PH in smoke-exposed mice but also, astonishingly, even reversed existing lung emphysema and PH. For the latter (“curative”) approach, treatment with *L*-NIL was started only after full establishment of the disease for 3 months without any further smoke exposure. Full restoration of lung structure and function was determined by functional measurements, design-based stereology, and electron microscopy, showing complete alveolar restoration (12).

Do Established PH Therapies Affect the Development of Airspace Enlargement in Preclinical Models?

Following the concept of pulmonary vascular alterations as a possible driver of lung emphysema, we asked whether other therapies established for the treatment of PH also positively affect lung airspace enlargement in smoke-exposed mice. In a preventive approach, smoke-exposed mice and guinea pigs were treated with soluble guanylate cyclase inhibitors riociguat (approved for treatment of idiopathic as well as chronic thromboembolic PH) and BAY 41-2272, respectively. At least in mice, this treatment fully prevented the development of PH as well as lung airspace enlargement. In guinea pigs, vascular remodeling as well as pulmonary vascular resistance was improved, and lung airspace enlargement was prevented (16) (Figure 1).

Soluble guanylate cyclase stimulation is suggested to exert its effect via increased cyclic guanosine monophosphate (cGMP) production, and the soluble guanylate cyclase–cGMP system has been shown to be essential for vascular homeostasis and the regulation of vascular tone. In the emphysema mouse model, it was shown that the functionally essential β 1-subunit of the soluble guanylate cyclase was downregulated (16). A possible link to the above-described iNOS–NO–peroxynitrite signaling pathway was detected as a variety of peroxynitrite

Figure 1. In tobacco smoke-exposed mice, pulmonary hypertension and pulmonary vascular remodeling precede the development of lung airspace enlargement. iNOS deletion and oral treatment with the iNOS inhibitor L-NIL prevented smoke-induced pulmonary hypertension and lung airspace enlargement. iNOS from bone marrow-derived cells triggered pulmonary hypertension, whereas iNOS from non-bone marrow-derived cells was responsible for development of lung airspace enlargement. L-NIL treatment fully reversed both pulmonary hypertension and airspace enlargement. The respective effects are suggested to be mediated via ONOO^- formation derived from the reaction of NO with the hitherto not identified O_2^- source. Similarly, sGC stimulation prevented pulmonary hypertension and development of airspace enlargement in rodents exposed to tobacco smoke. This may have been due to downregulation of sGC in the tobacco smoke-exposed mice. If these results are transferable to the human situation, treatment of lung vascular molecular alterations may allow development of new treatment concepts for lung emphysema. BM = bone marrow; iNOS = inducible nitric oxide synthase; L-NIL = N^6 -(1-iminoethyl)-L-lysine dihydrochloride; NO = nitric oxide; ONOO^- = peroxynitrite; O_2^- = superoxide; PH = pulmonary hypertension; sGC = soluble guanylate cyclase.

effects; for example, the increased apoptosis of endothelial cells and type II alveolar epithelial cells from mice was reduced by riociguat (16). In addition, riociguat prevented the increase of 3-nitrotyrosine, suggesting an inhibitory effect on peroxynitrite generation (16). The positive effects of the cGMP-increasing drug riociguat and its related compound are supported by the fact that phosphodiesterase 5 inhibitors also prevented PH and prevented or attenuated airspace enlargement in smoke-exposed mice and guinea pigs, respectively (17, 18). In addition, phosphodiesterase 4 inhibitor treatment also had positive effects on PH and emphysema development in mice and improved mitochondrial dysfunction in smoke extract-exposed Beas-2B cells (18, 19). However, currently, no direct link of phosphodiesterase 4 inhibition and iNOS-derived signaling is established.

Parallels of the Mouse Model to the Human Disease

Of course, one has to be very cautious when transferring findings from mouse models to

humans. The mouse lung shows a much higher regenerative potential than the human lung. Spontaneous lung regeneration has been found in adult, non-smoke-exposed, pneumotomized mice, demonstrating a high regenerative potential in this species (20, 21). However, smoke-exposed mice do not spontaneously regenerate from long-term smoke exposure-induced airspace enlargement. Such differences in the regenerative potential between smoke- and non-smoke-exposed mice could be key to detecting mechanisms of lung regeneration (12). Importantly, lung regeneration seems to be possible in adult humans, as shown in a recent case report (22).

With regard to iNOS-NO-peroxynitrite, studies by my group showed that there are large similarities in terms of structural and molecular alterations. Even smokers without COPD showed pulmonary vascular remodeling, upregulation of iNOS, and increased levels of an end product of peroxynitrite formation, nitrotyrosine (12). Similarly to mice, the soluble guanylate cyclase subunit- $\beta 1$ was downregulated in human smokers with and without PH (18).

Although not a direct proof of the transferability of rodent model findings into

human lung disease, the concept of pulmonary vascular molecular alterations as a possible driver of lung emphysema may offer new concepts for the pathobiology and hopefully the development of new treatment of a major lung disease, hitherto treated only with regard to symptoms. Speculatively, iNOS inhibition could be a better strategy to treat COPD than treatment approaches with standard PH therapies. A major difference from these therapies, which mainly target pulmonary vascular remodeling, would be the concept of peroxynitrite signaling discussed herein that is derived from vascular iNOS but targeting the alveolar integrity and maintenance. In addition, vasodilator therapy can worsen gas exchange owing to ventilation/perfusion mismatch (11). At the current stage, however, this is difficult to judge because only few randomized controlled trials investigated PH treatment in this regard, and real long-term studies that allow evaluation of improvement of gas exchange surface are missing in patients with COPD with PH as well as in patients with COPD without PH (11, 23–26). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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