Vascular Pharmacology 57 (2012) 113–118

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

NO and EDHF pathways in pulmonary arteries and veins are impaired in COPD patients

Qin Yang [a,*,1], Norihisa Shigemura [b,1], Malcolm John Underwood [b], Michael Hsin [b], Hong-Mei Xue [b], Yu Huang [c], Guo-Wei He [d], Cheuk-Man Yu [e,**]

[a] Division of Cardiology, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong & TEDA International Cardiovascular Hospital, Medical College, Nankai University, Tianjin, China
[b] Division of Cardiothoracic Surgery, Department of Surgery, Hongkong
[c] School of Biomedical Sciences; The Chinese University of Hong Kong, Hong Kong
[d] TEDA International Cardiovascular Hospital, Medical College, Nankai University, Tianjin, China & Department of Surgery, Oregon Health and Science University, Portland, OR, USA
[e] Division of Cardiology, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 17 January 2012
Received in revised form 29 March 2012
Accepted 9 May 2012

Keywords:
Chronic obstructive pulmonary disease
Endothelium
Nitric oxide
Pulmonary vasculature

ABSTRACT

We investigated endothelial function of both pulmonary arteries and veins in patients with chronic obstructive pulmonary disease (COPD) of varying severity in regard to the role of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Lung tissues were obtained from patients undergoing lobectomy or pneumonectomy. Patients were grouped to control, moderate COPD, and severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. Pulmonary arteries and veins were studied for endothelium-dependent relaxations. NO concentration was measured by electrochemical method. Protein expressions of eNOS and phosphorylated eNOS were determined by Western-blot. Endothelium-dependent relaxation was more significant in pulmonary arteries than in veins. The vasorelaxation was decreased in patients of moderate COPD and further decreased in severe COPD. The severity of endothelial dysfunction in both pulmonary arteries and veins correlated with the degree of airflow obstruction. COPD patients exhibited reduced endothelial NO production, decreased eNOS protein expression and decreased eNOS phosphorylation. The EDHF component was abolished in the pulmonary vasculature of patients with severe COPD. NO and EDHF pathways are both involved in the regulation of vascular tone in human pulmonary arteries and veins. Both pathways are impaired in COPD patients and the severity of the impairment increases with the progress of the disease. Downregulation of eNOS expression and inhibition of eNOS activation underlie the reduction of NO in COPD patients.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading health problems in the world and continues to be a major cause of morbidity and mortality in developed countries. COPD is now considered as an inflammatory disease mainly caused by cigarette smoking with pulmonary vasculature affected during the course of the disease (Barberà et al., 1994; Pauwels et al., 2001; Voelkel and Cool, 2003; Wright et al., 1992). Studies of pulmonary arteries showed the impairment of endothelium-dependent vasorelaxation in patients with COPD (Dinh-Xuan et al., 1991, 1993; Peinado et al., 1998). However, to date, despite the knowledge of the essentiality of venous system in pulmonary circulation, endothelial function of pulmonary veins has been barely studied in COPD patients. In addition, little work has been done regarding pulmonary endothelial function in patients with COPD of varying severity.

As a potent relaxing factor released from endothelium, nitric oxide (NO) plays an important role in modulating pulmonary vascular tone. With the findings of the reduction in pharmacologically stimulated NO-mediated vasodilatation, previous studies have suggested the compromised NO function in pulmonary arteries in patients with COPD (Cremona et al., 1999; Dinh-Xuan et al., 1991; Peinado et al., 1998). The decreased plasma concentration of NO supported the concept of some form of endothelial cell dysfunction in COPD patients (Cella et al., 2001). Nevertheless, there is lack of direct measurement of NO release from pulmonary endothelium in

Abbreviations: COPD, chronic obstructive pulmonary disease; EDHF, endothelium-derived hyperpolarizing factor; Indo, indomethacin; L-NNA, N^G-nitro-L-arginine; NO, nitric oxide; eNOS, nitric oxide synthase; HbO, oxyhemoglobin; PMSF, phenylmethylsulfonyl fluoride; p-eNOS, phosphorylated eNOS; SNAP, S-nitroso-N-acetyl-D,L-penicillamine.

* Correspondence to: Q. Yang, Division of Cardiology, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Block B, 5A, Prince of Wales Hospital, Shatin, N.T., Hong Kong. Tel.: +852 26450519; fax: +852 26451762.
** Correspondence to: C.-M. Yu, Division of Cardiology, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.
E-mail addresses: yangqs@cuhk.edu.hk (Q. Yang), cmyu@cuhk.edu.hk (C-M. Yu).
[1] Contributed equally.

1537-1891/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2012.05.004

patients with COPD. Furthermore, although in smokers, the protein level of endothelial nitric oxide synthase (eNOS) was found decreased in pulmonary arteries (Barberà et al., 2001), it remains poorly studied whether endothelial dysfunction in COPD patients is associated with alterations in eNOS expression and/or eNOS activation in pulmonary vasculature.

In contrast to NO, the existence of another endothelium-derived vasodilator, endothelium-derived hyperpolarizing factor (EDHF), remains a matter of controversy in human pulmonary arteries (Kozłowska et al., 2007; Lawrence et al., 1998; Norel et al., 1996). Furthermore, to date, no data has been reported concerning the role of EDHF in pulmonary arteries and veins in patients with COPD.

Therefore, in the present study, we investigated endothelial function in both pulmonary arteries and veins in COPD patients of varying severity. With the presence of inhibitors of NO synthase and cycloxygenases, as well as NO scavenger, oxyhemoglobin, we were able to study the authentic role of EDHF (Ge et al., 2000; Yang et al., 2003) in human pulmonary circulation with its role further explored in COPD patients. Furthermore, both eNOS expression and eNOS phosphorylation, as well as NO release were determined in pulmonary arteries of patients with COPD.

2. Materials and methods

2.1. Selection of patients

Twenty-eight patients (24 males and 4 females) who underwent lobectomy or pneumonectomy for localized non-obstructive peripheral lung carcinomas were studied. Pulmonary function tests were performed during the days prior to surgery. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, patients were classified into three groups. 1) Control (non-COPD). Patients with normal lung function: forced expiratory volume in one second/forced vital capacity (FEV1/FVC) > 70% and FEV1 ≥ 80% predicted. 2) Moderate COPD. Patients with air flow obstruction: FEV1/FVC < 70% and 50% ≤ FEV1 < 80% predicted. 3) Severe COPD. Patients with deteriorated airflow obstruction: FEV1/FVC < 70% and FEV1 < 50% predicted. COPD patients and the control subjects were well matched for age, sex and smoking habit (Table 1). No patient had clinical evidence of pulmonary hypertension and none received statin or oral corticosteroids within at least 4 months before the study entry.

2.2. Tissue collection and vessel preparation

Human lung tissues were obtained from patients during surgery. The study was approved by Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee (Hong Kong). Pulmonary arteries and veins with diameter of 1–2 mm were carefully dissected under a microscope, with care taken to protect the endothelium. The vessels were cleaned of fat and connective tissue and cut into cylindrical rings of 2-mm length for vasorelaxation study and electrochemical measurement of NO concentration, or snap frozen for Western analysis of eNOS expression.

Table 1
Characteristics of the patients.

	Control	Moderate COPD	Severe COPD
Patient number (male/female)	11 (9/2)	9 (8/1)	8 (7/1)
Age, year	64 ± 8	61 ± 9	67 ± 6
Tobacco consumption, pack-years	46 ± 18	51 ± 20	50 ± 14
Ex-smokers	6	6	5
Current smokers	5	3	3
FEV_1, % predicted	91 ± 6	68 ± 10*	38 ± 6†#
FEV_1/FVC %	80 ± 9	64 ± 7*	46 ± 9†#
Pa_{O2} (mm Hg)	83 ± 6	76 ± 8	69 ± 7*
Pa_{CO2} (mm Hg)	36 ± 3	38 ± 4	41 ± 5

Data are presented as mean ± SD. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; Pa_{O2}: partial pressure of arterial oxygen; Pa_{CO2}: partial pressure of arterial carbon dioxide. *$p<0.05$, †$p<0.01$ compared with control; #$p<0.05$ compared with moderate COPD.

2.3. Vasorelaxation study

The rings were mounted in a four-channel Mulvany myograph (Model 610M; J.P. Trading, Aarhus, Denmark) as described previously (Yang et al., 2002, 2005, 2008, 2011; Zhang et al., 2004, 2006). The Krebs solution was aerated with a gas mixture of 95%O_2–5%CO_2 at 37 °C and had the following composition (in mM) : Na^+, 144; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 128.7; HCO_3^-, 25; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.2; and glucose, 11. The rings were equilibrated for 45 min before and after normalization in the myograph and were subsequently set at the internal circumference that was equivalent to 90% of the circumference at a passive transmural pressure of 40 mm Hg (for pulmonary arteries) and 30 mm Hg (for pulmonary veins) (Zhang et al., 2004, 2006). This pressure was maintained throughout the experiments.

2.3.1. Endothelium-dependent relaxation

Endothelium-dependent relaxations were evaluated by bradykinin (−10 to −6.5 LogM) in U_{46619}-precontracted pulmonary arterial and venous rings in control subjects and patients with moderate or severe COPD.

2.3.2. Endothelium-dependent relaxation mediated by EDHF

To determine the EDHF-mediated relaxation, production of NO and PGI_2 were eliminated by inhibitors of cycloxygenase and NO synthase as well as scavenger of the residual NO (Ge et al., 2000; Yang et al., 2003). Indomethacin (Indo, 7 μM), N^G-nitro-L-arginine (L-NNA, 300 μM), and oxyhemoglobin (HbO, 20 μM) were added into the myograph chamber 30 min before the U_{46619}-precontraction (Yang et al., 2002, 2003, 2005; Zhang et al., 2004, 2006). Cumulative concentration–relaxation curves to bradykinin (−10 to −6.5 LogM) were then established in both pulmonary arterial and venous rings in patients with/without COPD.

2.4. Electrochemical measurement of NO — direct measurement with NO sensor

Pulmonary arterial rings from control subjects and patients with COPD were mounted in an organ chamber and equilibrated for 1 h before the measurement of NO release from vascular endothelium. NO concentrations upon the stimulation of bradykinin (−6.5 logM) were measured by using a NO-specific sensor that is connected to a NO meter (ISO-NO, World Precision Instruments, Sarasota, FL, USA), as previously published (Ge et al., 2000; He and Liu, 2001; Huang et al., 2011; Liu et al., 2000; Yang et al., 2003, 2005). In brief, the NO microsensor (ISO-NOP30L, WPI) was calibrated prior to experiment by using S-Nitroso-N-acetyl-D,L-penicillamine (SNAP) in combination with a catalyst, copper sulfate, to generate known amounts of NO in solution. A calibration curve was constructed by plotting the signal output (pA) vs. concentration (nM) of SNAP. After calibration, the NO microsensor was slowly introduced into the vessel lumen by means of a micromanipulator (WR-6, Narishige International). NO release stimulated by bradykinin was then calculated from the amperage generated by comparison with the calibration curve.

2.5. Western-blot analysis of eNOS and phosphorylated eNOS ($p\text{-}eNOS^{Ser1177}$)

Pulmonary arterial rings were snap frozen in liquid nitrogen and kept in −80 °C freezer and subsequently homogenized with ice-cold RIPA lysis buffer, containing 1 μg/ml leupetin, 5 μg/ml aprotonin, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, 1 mM EGTA, 1 mM EDTA, 1 mM sodium fluoride, and