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Integrative characterization of chronic cigarette smoke-induced cardiopulmonary comorbidities in a mouse model☆

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ABSTRACT

Cigarette smoke-triggered inflammatory cascades and consequent tissue damage are the main causes of chronic obstructive pulmonary disease (COPD). There is no effective therapy and the key mediators of COPD are not identified due to the lack of translational animal models with complex characterization. This integrative chronic study investigated cardiopulmonary pathophysiological alterations and mechanisms with functional, morphological and biochemical techniques in a 6-month-long cigarette smoke exposure mouse model. Some respiratory alterations characteristic of emphysema (decreased airway resistance: Rl; end-expiratory work and pause: EEW, EEP; expiration time: Te; increased tidal mid-expiratory flow: EF50) were detected in anaesthetized C57BL/6 mice, unrestrained plethysmography did not show changes. Typical histopathological signs were peribronchial/perivascular (PB/PV) edema at month 1, neutrophil/macrophage infiltration at month 2, interstitial leukocyte accumulation at months 3–4, and emphysema/atelectasis at months 5–6 quantified by mean linear intercept measurement. Emphysema was proven by micro-CT quantification. Leukocyte number in the bronchoalveolar lavage at month 2 and lung matrix metalloproteinases-2 and 9 (MMP-2/MMP-9) activities in months 5–6 significantly increased. Smoking triggered complex cytokine profile change in the lung with one characteristic inflammatory peak of C5a, interleukin-1α and its receptor antagonist (IL-1α, IL-1ra), monokine induced by gamma interferon (MIG), macrophage colony-stimulating factor (M-CSF), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) at months 2–3, and another peak of interferon-γ (IFN-γ), IL-4, 7, 13, 17, 27 related to tissue destruction. Transient systolic and diastolic ventricular dysfunction developed after 1–2 months shown by significantly decreased ejection fraction (EF%) and deceleration time, respectively. These parameters together with the tricuspid annular plane systolic excursion (TAPSE) decreased again after 5–6 months. Soluble intercellular adhesion molecule-1 (sICAM-1) significantly increased in the heart homogenates at month 6, while other inflammatory cytokines were undetectable.

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This is the first study demonstrating smoking duration-dependent, complex cardiopulmonary alterations characteristic to COPD, in which inflammatory cytokine cascades and MMP-2/9 might be responsible for pulmonary destruction and sICAM-1 for heart dysfunction.

List of abbreviations

BALF	bronchoalveolar lavage fluid
BLC	B-lymphocyte chemoattractant
COPD	chronic obstructive pulmonary disease
EEP	end-expiratory pause
EEW	end-expiratory work
EF%	ejection fraction
EF50	tidal mid-expiratory flow
f	frequency
IL-1α	interleukin-1 alpha
IL-1ra	interleukin-1 receptor antagonist
IL-16	interleukin-16
I-TAC	interferon-inducible T-cell chemoattractant
KC	keratinocyte chemoattractant
LAA/TLV	low attenuation area/total lung volume ratio
L_m	mean linear intercept (chord) length
LV	left ventricular
MCP-1	monocyte chemoattractant protein-1 (JE)
M-CSF	macrophage colony-stimulating factor
MIG	monokine induced by gamma interferon
MMP	matrix metalloproteinase
MV	minute ventilation
PB/PV	peribronchial/perivascular
PEF	peak expiratory flow
PIF	peak inspiratory flow
RANTES	regulated on activation normal T cell expressed and secreted
Rl	airway resistance
RT	relaxation time
SDF-1	stromal cell-derived factor 1
sICAM-1	soluble intercellular adhesion molecule-1
TAPSE	tricuspid annular plane systolic excursion
Te	expiratory time
Ti	inspiratory time
TIMP-1	tissue inhibitor of metalloproteinase-1
TNF-α	tumor necrosis factor-alpha
TREM-1	triggering receptor expressed on myeloid cells-1
TV	tidal volume
WBP	whole-body plethysmography

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major global health problem that in 2020 is projected to rank fifth worldwide in terms of economic and social burden of disease and third in terms of mortality. According to the most recent definition and description of the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2017) from the Global Strategy for the Diagnosis, Management and Prevention of COPD, it is characterized by persistent respiratory functions and airflow limitation. It is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung due to airway and/or alveolar abnormalities usually caused by noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity (Vestbo et al., 2013). Functional respiratory disorders result from chronic obstructive bronchiolitis narrowing the small airways and emphysema due to lung parenchymal destruction. COPD adversely affects both the structure and function of the right ventricle due to pulmonary arterial hypertension, the phenomena known as *cor pulmonale*. It is known that chronic hypoxia leads to pulmonary arteriolar constriction that represents an increased afterload for the right ventricle. In addition chronic hypoxia may induce functional contractile impairment of the left ventricle as well. Therefore, the potential effect of carbon-monoxide, an important toxic compound of cigarette smoke should also be emphasized, which may greatly contribute to the development of hypoxic conditions and related diseases. Cigarette smoking is the most common cause of COPD accounting for approximately 95% of cases in developed countries besides other predisposing factors, such as air pollutants and occupational exposure (Salvi and Barnes, 2009).

There is no curative treatment, the available therapy is restricted to corticosteroids, adrenergic β_2 receptor agonists and acetylcholine muscarinic receptor antagonists that can only slow down the progression and alleviate the symptoms (Vestbo et al., 2013). However, these have limited effect in a relatively small patient population (Restrepo, 2015). Therefore, there is an urgent need to find novel therapeutic targets in COPD. Due to the extensive interest in this area of research, our knowledge of the underlying mechanisms has remarkably expanded. Cigarette smoke and other airway irritants induce an abnormal inflammatory response involving $CD8^+$ lymphocytes, neutrophils and macrophages. These immune cells release chemotactic factors, colony stimulating factors and proinflammatory cytokines, thus sustain and enhance inflammation and immune cell recruitment. Furthermore, proteases like neutrophil elastase, cathepsins and matrix metalloproteinases (MMPs) are responsible for elastin destruction resulting in emphysema formation (Barnes et al., 2003; Yao et al., 2013). However, the complex pathophysiological mechanism, the inflammatory cascades and the role of the immune cells, sensory nerves and neuro-immune interactions, as well as the key mediators need to be determined to identify potential novel therapeutic targets (Canning and Spina, 2009).

Besides human studies to analyse tissue samples, translational animal models are particularly important to define the pathophysiological processes underlying the molecular pathways. Many species like rodents, sheep, dogs, guinea pigs, and monkeys have been investigated for modeling COPD (Helyes and Hajna, 2012; Leberl et al., 2013; Wright and Churg, 2008), but considering the possibilities of genetic engineering, easier handling and less compound requirement, mouse models seem to be most suitable and promising to elucidate the pathophysiological pathways and the complexity of the mechanisms (Martorana et al., 2006; Mercer