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Review Article

Oxidative stress in cigarette smokers and patients with chronic obstructive pulmonary disease

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ABSTRACT

Cigarette smoking is the most important exogenous risk factor for chronic obstructive pulmonary disease (COPD). Oxidative stress induced by smoking is considered to play major roles in the pathogenesis of COPD. In this review, we describe the theory of oxidant/antioxidant imbalance and the cellular and molecular reactions induced by oxidative stress. In addition, we discuss the effects of cigarette smoking on oxidative stress in smokers and patients with COPD. Smoking leads to increased acute pulmonary and systemic oxidative stress in cigarette smokers. As chronic effects of smoking, both pulmonary and systemic oxidative stress, are increased in cigarette smokers and patients with COPD compared to non-smokers, the level of oxidative stress increases in association with duration of smoking history. Furthermore, pulmonary and systemic oxidative stress has been reported to be increased in response to exercise in cigarette smokers with a short smoking history, compared to non-smokers, although no difference was seen in baseline oxidative stress levels. Cigarette smoking affects the pulmonary and systemic oxidative stress response even in cigarette smokers with a short smoking history as well as smokers with a long smoking history and COPD patients.

KEY WORDS: Chronic obstructive pulmonary disease, oxidant/antioxidant imbalance, smokers, smoking cessation

INTRODUCTION

Patients with chronic obstructive pulmonary disease (COPD) have an obstructive ventilatory impairment. According to the World Health Organization, COPD was the sixth leading global cause of mortality in 1990 and is expected to surpass ischemic heart disease and cerebrovascular disease and be the third leading cause of mortality by 2020 [1]. The risk factors of COPD include endogenous genetic factors and exogenous factors such as cigarette smoking, air pollution, work-related inhalation of dust and chemicals, and passive smoking. Smoking is the most important exogenous risk factor of COPD [2], with the incidence of COPD in smokers increasing with age [3,4]. Regarding the pathology of the disease, chronic bronchitis develops due to lesions in the airway and alveolar destruction due to emphysematous lesions [5]. In smokers with cigarette sensitivity, the forced expiratory volume in one second (FEV1) rapidly decreases with age, but studies had found that when smoking was discontinued, the rate of decrease in FEV1 decreases, leading to slowing or stopping the progression of disease [6,7]. Smoking cessation has been considered essential for all patients with COPD, regardless of the degree of severity [8]. Several mechanisms, including oxidant/antioxidant imbalance, protease/antiprotease imbalance, and apoptosis, are thought be involved in the pathogenesis of COPD [9].

In this review article, we describe the theory of oxidant/antioxidant imbalance and the cellular and molecular reactions induced by oxidative stress, which is the predominant model for the pathogenesis of COPD. In addition, we discuss the effects of smoking on oxidative stress in young cigarette smokers as well as in older cigarette smokers and patients with COPD. In this review, we cite articles that define nonsmokers, smokers, and COPD patients as follows: (1) nonsmokers are individuals

who have never smoked, (2) smokers are individuals who smoke cigarettes regularly regardless of pack-year smoking history, but do not meet the diagnostic criteria of COPD, and (3) patients with the diagnosis of COPD are those individuals with the clinical signs of chronic bronchitis and irreversible airflow limitation (ratio of FEV1 to forced vital capacity of <70% after inhalation of a bronchodilator).

OXIDANT/ANTIOXIDANT IMBALANCE

Oxidant/antioxidant imbalance is a theoretical concept that best explains the pathogenesis of COPD. Oxidative stress is defined as the balance between reactive oxygen species (ROS) and antioxidants. Each inhalation of cigarette smoke contains 5×10^{14} ROS [10]. Cigarette smoke enters the lungs, and the ROS in the smoke directly causes oxidative stress in the lungs. In addition to this direct effect, smoking leads to accumulation of neutrophils and macrophages in the lungs [11], which release a larger amount of ROS in smokers than in nonsmokers [12]. Furthermore, cigarette smoke leads to a decrease not only in the level of antioxidants in alveolar epithelial cells but also in the level of antioxidants in plasma [13,14]. Thus, cigarette smoking directly and indirectly appears to cause both pulmonary and systemic oxidative stress, and the imbalance between ROS and antioxidants is involved in the pathogenesis of COPD [15].

METABOLIC PATHWAYS OF ROS AND CELLULAR AND MOLECULAR REACTIONS INDUCED BY ROS

ROS are chemically active oxygen-bearing molecules that have unpaired electrons and are stabilized by receiving an electron from antioxidants. ROS such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\bullet -}$), and hydroxyl radicals ($^{\bullet}OH$) are produced during normal cellular activity. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and GSH peroxidase (GSH-Px) are present in cells and blood. $O_2^{\bullet -}$ are produced from oxygen by xanthine oxidase and are rapidly converted into H_2O_2 by SOD. H_2O_2 subsequently becomes $^{\bullet}OH$ after receiving an electron and is finally converted into water (H_2O) [Figure 1]. Moreover, reduced GSH and GSH-Px reduce H_2O_2 and hydroperoxide into H_2O and alcohol, respectively [16]. Even under normal conditions, ROS are produced during cellular metabolism, and the organism is always exposed to oxidative stress. If there is an imbalance between ROS and antioxidants that favor ROS, they become toxic, which may lead to many types of disease, including COPD [17-19].

Oxidative stress induced by smoking has been thought to initiate a series of cellular and molecular reactions. Oxidative stress activates kinase cascades and transcription factors such as activator protein 1 and nuclear factor Kappa B, resulting in the release of inflammatory mediators such as cytokines, leukotrienes, and prostaglandins. Consequently, ROS promotes inflammation, cell injury, and apoptosis [20,21].

Systemic oxidative stress has been assessed in samples of blood and urine. Moreover, pulmonary oxidative stress has been assessed on samples of bronchoalveolar lavage fluid (BALF)

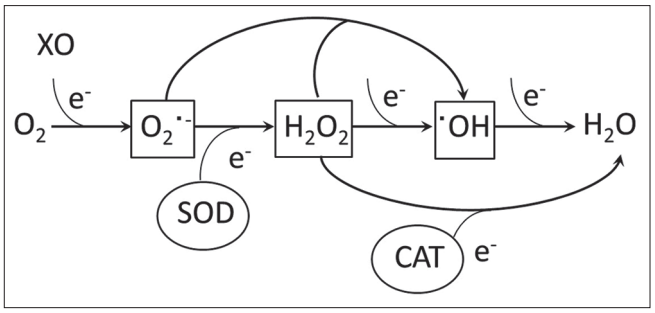

Figure 1: Metabolic pathway of oxidative stress. Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\bullet -}$), and hydroxyl radicals ($^{\bullet}OH$) are continuously produced during normal cellular metabolism in living cells. Antioxidants such as superoxide dismutase (SOD) and catalase (CAT) reduce these ROS to ultimately form water (H_2O)

and less invasively, on samples of exhaled breath condensate (EBC) [22]. EBC is a liquid sample obtained by cooling exhaled breath, which contains vapors and aerosols from the respiratory tract [23-40]. Table 1 lists the indicators of oxidative stress that have been found in BALF, EBC, and blood and urine samples from cigarette smokers and patients with COPD [25-69].

ACUTE EFFECTS OF CIGARETTE SMOKING ON PULMONARY AND SYSTEMIC OXIDATIVE STRESS IN CIGARETTE SMOKERS

The acute effects of smoking on pulmonary oxidative stress have been reported. The consumption of a single cigarette leads to significant increases in the H_2O_2 concentration in EBC from cigarette smokers [31,32]. We conducted a pilot study to elucidate the acute effects of smoking on antioxidant markers as well as oxidant markers in the lung [29,30]. The concentration of H_2O_2 in EBC samples from 5 smokers was significantly increased after smoking, whereas the levels of 8-isoprostane and thiobarbituric acid reactive substances (TBARS) were undetectable. The biological antioxidant potential (BAP), an indicator of overall antioxidant activity in EBC, was significantly decreased after smoking, whereas SOD levels were undetectable [70,71]. We also measured H_2O_2 and BAP in EBC samples from 5 smokers before, immediately after and 1, 2, 4, 8, and 12 h after smoking to examine serial changes in oxidative stress [30]. The H_2O_2 concentration in EBC was significantly increased immediately and 1 h after smoking compared to baseline, and then gradually decreased and returned to baseline levels within 12 h. By contrast, BAP in EBC was significantly decreased immediately after smoking compared to baseline, then gradually increased and returned to baseline levels within 8 hours. These results indicate that smoking led to acute increases in oxidative stress in the lungs.

The acute effects of smoking on systemic oxidative stress have also been reported. Cigarette smoke contains large amounts of free radicals such as $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$ [72], and 8-hydroxydeoxyguanosine (8-OHdG) has been found to increase in plasma 30 min after smoking [53]. TBARS also was found to increase in plasma at 1 hour after smoking [60]. The

Table 1: Indicators of oxidative stress in specimens of BALF, EBC, plasma, and urine from patients with COPD and from cigarette smokers

Source	Oxidant markers	Antioxidant markers
EBC	Hydrogen peroxide [25-32], products of lipid peroxidation[a] [26,33,34], leukotriene B4 [35-38], 8-isoprostane [27,36,38-40]	BAP [29,30]
BALF	Xanthine oxidase activity [41], superoxide [42], products of lipid peroxidation [42], oxidized glutathione [43]	Total antioxidant capacity [42], reduced glutathione [42,44,45]
Plasma	Products of lipid peroxidation [28,46-52], 8-OHdG [53], carbonyls [54-56], leukotriene B4 [38], F2-isoprostane [57], 8-isoprostane [38], myeloperoxidase [58], superoxide anion [59], ROS-induced DNA damage [28], xanthine [47,54], hypoxanthine [47,54], xanthine oxidase activity [62], oxidized GSH [47,55]	Total antioxidant capacity [46,54-56,60-62], reduced glutathione [47,48,55,63], GSH-Px [64-66], CAT [64-66], SOD [61,64-66], uric acid [28,54, 61], vitamin C [67], vitamin E [48,59,61,67]
Urine	Products of lipid peroxidation [28], 8-OHdG [66], F_2-isoprostane [68], 8-isoprostane [69]	

[a]Products of lipid peroxidation includes MDA, TBARS, and conjugated dienes. 8-OHdG: 8-hydroxydeoxyguanosine. BALF: Bronchoalveolar lavage fluid, EBC: Exhaled breath condensate, TBARS: Thiobarbituric acid reactive substances, BAP: Biological antioxidant potential, MDA: Malondialdehyde, COPD: Chronic obstructive pulmonary disease, ROS: Reactive oxygen species, GSH-Px: Glutathione peroxidase, CAT: Catalase, SOD: Superoxide dismutase

plasma levels of antioxidants such as ascorbic acid, cysteine, methionine, and urate have been found to decrease 5 min after smoking a single cigarette [14]. These reports suggest that smoking leads to rapid increases in pulmonary and systemic oxidative stress.

CHRONIC EFFECTS OF CIGARETTE SMOKING ON PULMONARY OXIDATIVE STRESS IN SMOKERS AND PATIENTS WITH COPD

Pulmonary oxidative stress in smokers with a long smoking history has been shown to be higher than in nonsmokers. H_2O_2 and 8-isoprostane levels in EBC from smokers were higher than the levels in nonsmokers [73]. The levels of oxidized GSH in BALF samples from older smokers (mean age 57 years, mean smoking history 55 pack/years) were also higher than the levels in young smokers (mean age 23 years, mean smoking history 5 pack/years) and nonsmokers (mean age 23 years) [43]. Moreover, the H_2O_2 concentration in EBC was positively correlated with pack-year smoking history [32]. Nitrosothiol, which is produced when nitric oxide reacts with reduced GSH, was reported to be higher in EBC samples from smokers than in samples from nonsmokers; the nitrosothiol level in EBC was positively correlated with pack-year smoking history [74].

Pulmonary oxidative stress in patients with COPD is higher than in nonsmokers. Xanthine oxidase activity was higher in BALF samples from patients with COPD than in samples from nonsmokers [41]. EBC levels of H_2O_2, isoprostanes, and leukotrienes, which are indicators of oxidative stress, were reported to be higher in patients with COPD than in nonsmokers [36,39,73]. Moreover, compared with healthy smokers, levels of isoprostanes were increased in EBC samples [39] and levels of reduced GSH were decreased in BALF samples from patients with COPD [44]. These results suggest that pulmonary oxidative stress is increased in cigarette smokers and patients with COPD compared to nonsmokers. In addition, patients with COPD may have higher levels of pulmonary oxidative stress than healthy smokers.

CHRONIC EFFECTS OF CIGARETTE SMOKING ON SYSTEMIC OXIDATIVE STRESS IN SMOKERS AND PATIENTS WITH COPD

Systemic oxidative stress has been reported to be higher in smokers with a long smoking history and in patients with COPD than in nonsmokers. Morrow *et al.* [57] reported that levels of F2-isoprostanes, which are indicators of oxidative stress, are higher in the blood and urine of smokers than in nonsmokers. By contrast, levels of SOD and CAT were decreased in smokers compared to nonsmokers [75]. With longer duration of smoking and higher number of daily cigarettes, the levels of F2-isoprostanes were found increased and plasma levels of SOD, CAT, vitamin C, and vitamin E were found decreased in blood and urine samples from smokers [67,68]. Patients with COPD were found to have higher levels of plasma malondialdehyde (MDA) [51,76,77] and urine 8-isoprostane [69], lower SOD and CAT activity and lower levels of ascorbic acid and reduced GSH than currently healthy smokers, former smokers, and nonsmokers [76-78]. These results suggest that smokers have higher systemic oxidative stress levels than nonsmokers and that patients with COPD have especially higher levels of systemic oxidative stress than healthy smokers, former smokers, and nonsmokers.

EFFECTS OF SMOKING CESSATION ON OXIDATIVE STRESS IN PATIENTS WITH COPD

The effects of smoking cessation on pulmonary and systemic oxidative stress in patients with COPD have been reported. The difference between 8-isoprostane EBC concentrations in smoking COPD patients versus those in COPD patients who had stopped smoking for 6 months or longer was not significant [39]. There were also no significant differences between the levels of plasma GSH, vitamin C, and MDA levels in smoking COPD patients versus those in COPD patients who had not smoked for 1 year or longer [51]. However, the plasma concentrations of conjugated dienes and TBARS were reported to be lower in COPD patients who had stopped smoking for 3 months than the levels in smoking COPD patients [52]. The

limited number of studies that have examined the effects of smoking cessation on pulmonary and systemic oxidative stress in patients with COPD did not reach a consensus on the effects of smoking cessation on pulmonary and plasma oxidative stress.

PULMONARY AND SYSTEMIC EFFECTS OF SMOKING IN YOUNG CIGARETTE SMOKERS

Young smokers with a short smoking history have been found to have smoking-induced pulmonary and systemic oxidative stress. A significant difference between the oxidative stress in young smokers versus oxidative stress in non-smokers was observed after exercise stress testing, although the difference in baseline oxidative stress between the 2 groups of participants was not significant.

Bloomer *et al.* [79] measured plasma MDA, protein carbonyls, and 8-OHdG levels in young smokers (mean age 24 years, mean smoking history 6 pack-years) before and after a graded exercise test. The levels of MDA and protein carbonyls were higher after the exercise test in smokers than in non-smokers. Moreover, Gochman *et al.* [80] reported that the plasma concentrations of conjugated dienes, which are an indicator of ROS, after a treadmill exercise test were higher in young smokers (mean age 23.7 years) than in nonsmokers. We measured plasma hydroperoxide concentrations in young smokers (mean age 25.9 years, mean smoking history 3.2 pack/years) before and after 30 s of maximal exercise and found that the plasma hydroperoxide levels after exercise were higher in smokers than in nonsmokers, although the differences in baseline plasma hydroperoxide levels were not significant [49].

There have been few reports on the pulmonary oxidative stress response in young cigarette smokers. We examined H_2O_2 concentrations in the lungs of young smokers and nonsmokers after 30 s of maximal exercise and found that the EBC H_2O_2 concentrations were significantly higher after exercise in the smokers than in the nonsmokers [29]. These findings suggest that the pulmonary and systemic oxidative stress response can be observed even in smokers with a short smoking history.

CONCLUSION

In this review, we reported the effects of smoking-induced oxidative stress, which is thought to be a cause of COPD, in smokers and patients with COPD. Smoking increases acute pulmonary and systemic oxidative stress in smokers. Moreover, the chronic effects of smoking include increased pulmonary and systemic oxidative stress in smokers and patients with COPD compared with nonsmokers, and oxidative stress increases with longer duration of smoking. Pulmonary and systemic oxidative stress were also found increased in young cigarette smokers with a short smoking history.

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