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RESEARCH

Scavenger receptor B1 involvement in chronic obstructive pulmonary disease pathogenesis

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Abstract

Objective: Chronic obstructive pulmonary disease (COPD) is one of the main causes of morbidity and mortality in the United States. Oxidative stress due to cigarette smoking seems to be one of the major driving mechanisms in COPD pathogenesis. Since the scavenger receptor B1 (SR-B1) appears to play a key role in mediating the uptake for α-tocopherol and other antioxidants in lung tissue, we aimed to investigate its role in COPD pathogenesis.
Methods: Lung tissue biopsies were obtained from 12 subjects; 6 of these had a diagnosis of COPD in a stable clinical state, the others 6 were current ($n = 1$) or ex-smokers ($n = 5$) with normal lung function (controls). 4-Hydroxynonenal (4-HNE)–SR-B1 adducts were detected by immunoprecipitation. α-tocopherol concentration was determined by HPLC.
Results: SR-B1 levels were lower in COPD patients and these results parallel with lower levels of vitamin E in lung tissue found in COPD patients. This effect can be the consequence of oxidative posttranslational modifications, confirmed by the binding of the peroxidation product 4-HNE to SR-B1 possibly leading to its degradation.
Conclusions: The loss of SR-B1 may be involved in lung α-tocopherol content decrease with the consequence of making lung tissue more susceptible to oxidative damage as suggested by the SR-B1–4-HNE adduct formation, and more prone to COPD development. Thus, our findings suggest a novel role of SR-B1 in pathomechanisms underlying COPD.

Significance statement

Chronic obstructive pulmonary disease (COPD) is one of the main causes of morbidity and mortality in the United States. Oxidative stress has been suggested to be the major driving mechanism in COPD pathogenesis. Loss of scavenger receptor BI (SR-B1) significantly decreases tocopherol lung content making lung tissue more susceptible to oxidative damage. The results of our study show that SR-B1 levels were lower in COPD patients and these results parallel with lower levels of vitamin E in lung tissue. Our findings suggest a novel role of SR- B1 in pathomechanisms underlying COPD.

Keywords

- oxidative stress
- cigarette smoking
- 4-hydroxy-2-nonenal

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Introduction

It is well established that cigarette smoking (CS) is a risk factor for many chronic diseases mainly affecting cardiovascular and respiratory systems (PDQ Screening and Prevention Editorial Board 2002). Today, it has been well demonstrated that CS is the main cause for developing COPD, although not all the smokers are affected by this pathology, evidencing a possible genetic predisposition (Cho *et al.* 2022).

COPD is a major health issue and one of the three most common causes of death worldwide (Adeloye *et al.* 2022). The abnormal inflammatory response to chronic exposure to CS components is believed to be the key component of COPD pathogenesis (Caramori *et al.* 2016). However, inflammation is not the only pathogenic mechanism underlying COPD. Oxidative stress has been also shown to play a primary role in COPD onset and clinical progression (Nucera *et al.* 2022). This chronic inflammation of the lower airways is characterized by a progressive loss of lung parenchyma and an accelerated decline of organ function.

Cigarettes smoke contains over 4700 compounds in gaseous and particulate states that are able to induce oxidative stress to cells, and its toxic effect is mainly due to the presence of oxidants, including volatile electrophilic compounds such as α,β-unsaturated aldehydes (Szparaga *et al.* 2021). Among these, 4-hydroxy-2-nonenal (HNE), a lipid peroxidation product, is highly reactive and potentially toxic. These aldehydes form covalent adducts with various proteins, thus affecting a variety of biochemical processes, including transcription factor activation, gene and protein expression, production of inflammatory cytokines, and cell death (Sharma *et al.* 2022).

Clinical trials with antioxidants have not been able to definitely prove the ability of micronutrients to prevent tobacco-related diseases such as COPD (Barnes 2020). The reason behind these controversial results could depend on both genetic background and the personal ability to uptake micronutrients in lung cells.

Two decades ago, Acton *et al.* (Acton *et al.* 1996) identified the scavenger receptor class B1 (SR-B1) as a HDL receptor. This transmembrane protein mediates the selective uptake of HDL cholesteryl esters and facilitates the trafficking of these lipids in the tissues (Gillard *et al.* 2018). Additional functions of this receptor have been shown, such as its ability to uptake the lipophilic antioxidant α-tocopherol (AT), which suggests an indirect role of the receptor in cell defensive mechanisms against oxidative stress challenges. More specifically, it has been observed that SRB1 KO mice had 64% less vitamin E levels in the lung (Mardones *et al.* 2002), therefore being more susceptible to oxidative damage. In addition, SR-B1 is very susceptible to oxidative damage, which induces its degradation via proteasome (Sticozzi *et al.* 2013, Crivellari *et al.* 2017).

Therefore, we have hypothesized that upon lung exposure to SHS and its attendant OS, a positive feedback loop is induced where the lipid soluble antioxidants consumption increases (to fight against oxidative stress) and SR-B1 expression diminish as a consequence of oxidative damage. This combination results in reduced lung vitamin E contents, making the target tissue even more vulnerable to further insults.

These premises provide the rationale of the current study, which addresses the hypothesis that the expression of SR-B1 is downregulated in COPD lung as an effect of oxidative burden potentially caused by CS. We found that the level of SR-B1 is significantly lower in COPD patients compared to control smokers with normal lung function, and this alteration may occur via oxidative posttranslational modification.

Materials and methods

Study approval

All patients were recruited from the Respiratory Diseases Clinic of the University Hospital of Ferrara (www.ospfe.it). The diagnosis of COPD was based on the GOLD (www.goldcopd.org) criteria (a compatible history and spirometry, a post-bronchodilation forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio < 70%)).

Patients (i) who had pulmonary function test report within 3 days, (ii) with diagnosis stable COPD (for more than 2 weeks), and (iii) who did not report any treatment with oxygen, antibiotics, glucocorticoids, and theophylline within the last 1 month were included in the study. The exclusion criteria were as follows: (i) patients having been treated with immunosuppressive drugs in the past month; (ii) patients having other airflow-limited diseases, (iii) patients having severe diseases, and (iv) patients having infectious diseases other than that of the respiratory system.

We obtained and studied peripheral lung tissue from 12 subjects: six of these had a diagnosis of COPD in a stable clinical state, the others six were current ($n = 1$) or ex-smokers ($n = 5$) with normal lung function (controls).

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The study conformed to the Declaration of Helsinki and was approved by the ethics committees of the University Hospital of Ferrara, Italy; written informed consent was obtained from each participant, and nonneoplastic peripheral lung tissue sampling was performed during lung resection surgery for a suspected malignancy according to the guidelines of the local ethics committee.

Harvesting and preparation of lung samples

Lung tissue preparation was performed as previously detailed (Valacchi *et al.* 2007). Briefly, lung tissue biopsies were homogenized at 4 °C in radioimmunoprecipitation assay (RIPA) buffer (150 mM $NaCl_2$, 50 mM Tris–HCl, pH 7.4, 1% NP-40, 1 mM EDTA, 1 mM EGTA, 0.1% sodium dodecyl sulfate, 5 mM dithiothreitol, 5 mM NaF, 1 mM phenylmethyl sulfonyl fluoride, 10 mg/mL leupeptin, 10 µg/mL aprotinin, 10 mg/mL iodoacetamide) (Merck KGaA), incubated on ice for 1 h, and separated by centrifugation at 21,000 ***g*** for 10 min. Supernatants were stored at −80°C until further processing.

Western blot analysis

Total cell lysates were extracted in RIPA buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Nonidet P-40, 1 mM EGTA, 0.1% SDS, 5 mM N-ethylmaleimide (Merck KGaA), protease and phosphatase inhibitor cocktails (Merck KGaA) as described before (Valacchi *et al.* 2007), Briefly, 60 µg boiled proteins were loaded onto 10% sodium dodecyl sulfate–polyacrylamide electrophoresis gels. The gels were electroblotted onto nitrocellulose membranes and then blocked for 1 h in 3% milk. Membranes were incubated overnight at 4 °C with the primary antibody SR-B1 (Novus Biologicals, Inc., Littleton, CO, USA) and with horseradish peroxidase-conjugated secondary antibody (Bio-Rad). The blots were stripped and reprobed with β-actin (Cell Signaling; Celbio, Milan, Italy) as the loading control. Images of the bands were digitized and the densitometry of the bands were performed using Image J software.

Immunoprecipitation of SR-B1 and detection of 4-HNE adducts

The antibody for SR-B1 (5 µg) (Thermo Fisher Scientific Inc.) was precoupled to 50 µL of magnetic Dynabeads Protein G (Novex, Life Technologies). Excess antibody was washed by placing the tube on a DynaMag™ magnet and removing the supernatant. Then, cell protein extracts (500 µg) were incubated with the antibody-coated beads for 10 min at room temperature (RT). After washing, the immunocomplexes were mixed with reducing sample buffer, boiled, and analyzed by SDS-PAGE and immunoblotting with 4-HNE antibody (Millipore).

Quantification of α-tocopherol

AT concentrations were determined by HPLC using a Waters Spherisorb ODS2 C-18 (4.6 × 100 mm, 3 µm particle size) column with electrochemical detection, as described by Valacchi *et al.* (Valacchi *et al.* 2000). Tissue AT was extracted following saponification with alcoholic potassium hydroxide in the presence of 1% ascorbic acid. AT was detected electrochemically using an oxidizing potential of 500 mV and quantitated by calculation from a standard curve of authentic AT standards.

Results

The main characteristics of the study subjects are shown in Table 1.

SR-B1 has been shown to be involved in many regulatory functions, including the ability to indirectly protect from the oxidative stress-related damage cause by CS. Owing to this, we assessed the SR-B1 protein levels in the peripheral lung tissue of both COPD patients and control smokers with normal lung function, using a specific SR-B1 antibody able to recognize both, the mature (82 KDa) and the immature form (not glycosylated form, 66 KDa) of SR-B1. As shown in Fig. 1, COPD patients have high levels of SR-B1 immature form and very low level of the functional form (82 KDa).

Increased levels of peroxidation (Sticozzi *et al.* 2014) and oxidative stress (OS) (Solak *et al.* 2005, Prieux *et al.* 2020) have been widely related to the high levels of pro-oxidants contained in CS. In particular, we have previously shown that posttranslational modification via formation to 4-HNE protein adducts deeply affect SR-B1, by increasing the rate subsequent ubiquitination and proteasome degradation, as we showed in previous studies (Sticozzi *et al.* 2013, Ferrara *et al.* 2022). For this reason, we evaluated the presence of this highly reactive aldehyde and the eventual formation of adducts with SR-B1 in lung tissue from COPD patients and control subjects. As shown in Fig. 2A, in COPD peripheral lung tissues there was a significant increase in 4-HNE protein adducts (top panel) resulting in an increment of almost 50% (bottom panel).

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Table 1 Characteristics of subjects for the study on peripheral lung parenchyma. Data are expressed as mean ± S.E.M.

Subjects	*n*	Age	Sex		Smoking history		Pack-years	Chronic bronchitis	FEV_1 % pred	FEV_1/ FVC %
			Male	Female	Ex-smokers	Current smokers				
Control smokers	6	71.2 ± 3.9	4	2	5	1	35.8 ± 5.4	0	90.2 ± 8.2	83.5 ± 4.3
COPD	6	71.3 ± 1.1	5	1	3	3	45.5 ± 3.2	3	75.3 ± 5.5	65.2 ± 1.5

Immunoblotting assay (Fig. 2B upper panel) evidenced that the interaction between SR-B1 and 4-HNE was clearly stronger in COPD patients compared to the control smokers with normal lung function resulting in almost twofold increase (Fig. 2B bottom panel).

Loss of SR-B1 due to oxidative modification could affect vitamin E uptake in lung cells, as this receptor is a key player in this process (Valacchi *et al.* 2011). To address this hypothesis, we measured the levels of AT in peripheral lung tissue of COPD and control smokers with normal lung function. As shown in Table 2, the levels of this lipophilic vitamin were significantly lower in COPD peripheral lung tissue compared to control smokers with normal lung function.

Discussion

In the present study, we found for the first time that COPD patients have lower levels of mature (and biologically functional) SR-B1 in the peripheral lung parenchyma compared to control smokers with normal lung function. The collected data suggest that this alteration may be the result of oxidative stress-mediated posttranslational modification which, as previously shown in other experimental settings, may increase the rate of proteasomal degradation of this receptor.

We decided to focus our attention on SR-B1 in COPD for two main reasons: (i) evidence suggests that SR-B1 may be involved in many physiological processes in lung, including some that are altered in COPD and (ii) SR-B1 is highly vulnerable to oxidative challenges caused by CS, the main risk factor and pathogenic player of COPD (Valacchi *et al.* 2015).

SR-B1 is mostly known and studied for its critical role in reverse cholesterol transport (RCT) and HDL homeostasis (Shen *et al.* 2018). The possible role of HDL in the development of COPD has been proposed, on the basis on the observed epidemiological association between the clinical severity of the disease and low levels of these lipoproteins (Valacchi *et al.* 2015, Zafirova-Ivanovska *et al.* 2016, Vicol *et al.* 2022). However, these data are mixed and, in any case, did not support a role of SR-B1 in the etiology and pathogenesis of COPD but rather in the frequent cardiovascular complications of the lung disease.

There are several mechanisms, besides the well-characterized chronic inflammation of the airways, that are involved in COPD onset and progression, and SR-B1 may play a role in some of them. This multifunctional receptor is able to recognize a vast variety of ligands, including apoptotic cells, seemingly facilitating their disposal, and pathogens (Gillard *et al.* 2018) (10.1016/j.jacl.2018.04.001). Notably, the increase in apoptotic alveolar epithelial and endothelial cells in the lung tissue of COPD patients has been referred to as a potential upstream event in COPD pathogenesis (Demedts *et al.* 2006). Moreover, lungs of patients affected by this disease are abnormally sensitive to respiratory viral and bacterial

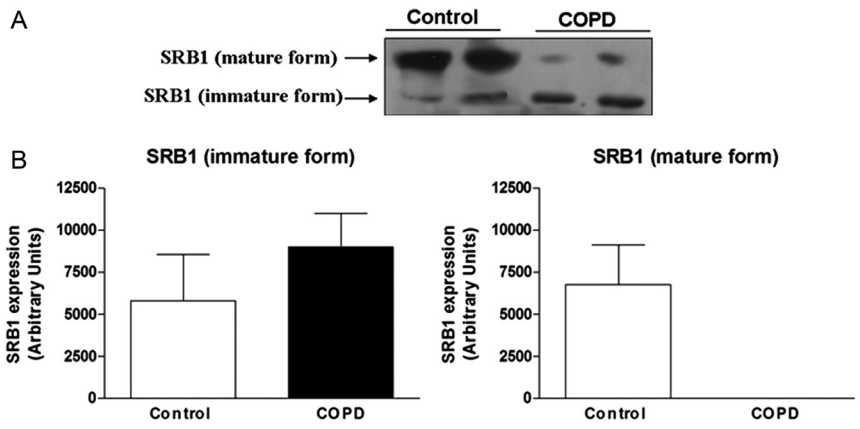

Figure 1
Peripheral lung parenchyma tissue samples of COPD patients showed low levels of mature SR-B1 compared to those of control smokers with normal lung function. Data are expressed as mean ± S.E.M. (*$P < 0.05$). (A) Western blot (top panel) is a representative of five different patients. (B) SR-B1 band quantification is shown in the right panel. Data are expressed in arbitrary units (averages of five different experiments, *$P < 0.05$). β-Actin was used as loading control.

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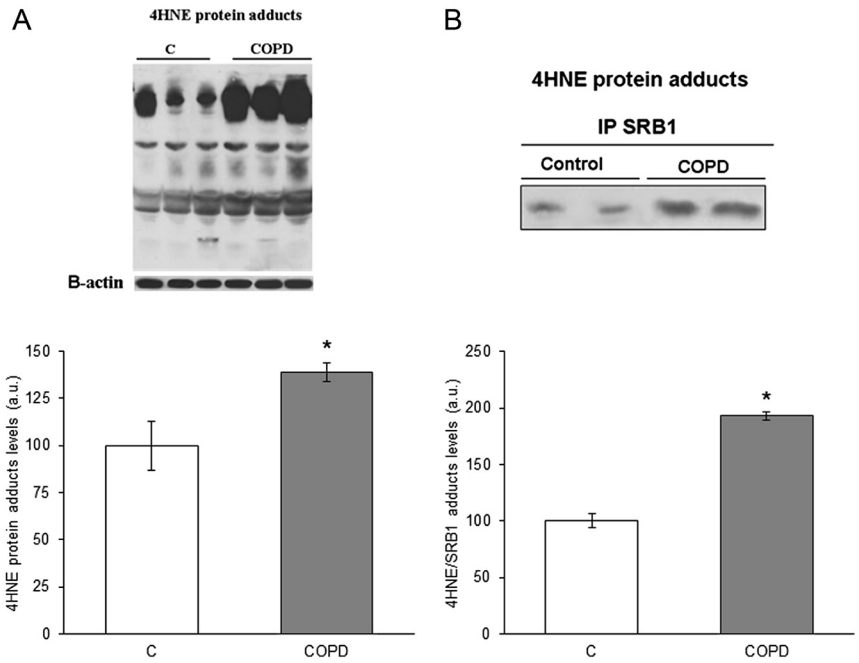

Figure 2
COPD peripheral lung tissue showed high levels of HNE protein adducts (A) and the presence of HNE adducts on SR-B1 (B) compared to controls. (A) Lung lysates were immunoblotted for 4-HNE adducts. Showed is a representative Western blot of five experiments from five different patients. (B) Samples were immunoprecipitated with SR-B1 Ab and immunoblotted with anti-4-HNE. Western blot is representative of five independent experiments.

infections, which can greatly worsen the prognosis (D'Anna *et al.* 2021). In particular, preclinical evidence suggests that downregulation of SR-B1 may facilitate the entry and the replication of virus. SR-B1 may also be able to recognize bacteria and also to orchestrate neutrophilic host defense response to inhaled noxious compounds, included those present in COPD patients (Gowdy *et al.* 2015).

This receptor may also be indirectly involved in the regulation of redox homeostasis in lung cells. Abnormal elevation in reactive oxygen species (ROS) has been well documented in COPD and may occur as direct consequence of inhaled toxicants and/or as result of activation of leukocytes and epithelial cells (Barnes 2022). A functional SR-B1 seems to contribute to preserve the oxidative balance by, among others, mediating the uptake AT and carotenoids, from HDL and other lipoproteins. This vitamin seems to play an important protective role in human lung. Accordingly, large trials have shown that AT supplements significantly decrease the risk of developing COPD and other chronic pulmonary diseases (Agler *et al.* 2011) and higher vitamin E intake prevents COPD development (Liu *et al.* 2023). Preclinical evidence pints to direct link between SRB1 and intracellular levels of the potent lipophilic antioxidant. Indeed, it has been shown that in SR-B1-null mice a significant increase in levels of circulating ATol is accompanied by a concomitant reduction in several organs, including lung (Mardones *et al.* 2002). Owing the important contribution in the antioxidant defensive mechanism, the reduction in the levels of AT observed in stable COPD lungs could result in their major vulnerability to oxidative challenge.

Decline in AT could be a cause of the detected decrease of functional SR-B1 in diseased lungs. Our hypothesis is that the increase in oxidative stress (witnessed by the observed increase in 4-HNE protein adduct levels) may lead to the observed posttranslational change related to the covalent binding with 4-HNE. This highly reactive aldehyde tends to form covalent bonds with amino acid residues such as lysine, histidine, and cysteine present in the proteins (Pecorelli *et al.* 2016). The loss of SR-B1 may be ascribed to this modification. Indeed, we have previously shown that this significantly accelerates proteasome-mediated degradation of the receptor in cultured cells following CS or ozone exposure (Sticozzi *et al.* 2012, 2018, 2020).

Table 2 COPD lungs showed lower levels of α-tocopherol compared to control smokers with normal lung function. Data expressed as mean ± S.E.M.

	Control smokers with normal lung function	COPD
α-tocopherol (nmol/mg tissue)	70 ± 2.34	46 ± 4.56 ($P < 0.01$)

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

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Author contribution statement
CC conceived the study and wrote the paper; GV conceived the study and reviewed the manuscript; AP and CS performed experiments and analyzed data; GC conceived the original idea and reviewed the final draft; AP supervised the project and reviewed the final draft; PC and FN supervised the project, analyzed the data, and reviewed the final draft.

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