Airways oxidative stress, lung function and cognitive impairment in aging

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Background. An altered balance of oxidants/anti-oxidants is one of the pathological mechanisms of many age-dependent disorders. We aimed to investigate the age-related airways oxidative stress, using non invasive, safe and repeatable techniques; to evaluate the correspondence between systemic and local oxidative stress in healthy subjects of different age ranges; to analyse the correlation between systemic and local oxidative stress with lung function and with cognitive impairment.

Methods. Thirty consecutive healthy high school graduated subjects (8 M, 22 F), divided in three ranges of age (<35; between 35 and 60; >60 years) were enrolled. All subjects underwent oxygen free radicals and exhaled nitric oxide measurement (by the diacron reactive oxygen metabolites test and by a rapid-response chemiluminescence nitric oxide analyzer), lung function tests, and cognitive impairment scales (Mini Mental State Examination and Geriatric Depression Scale).

Results. A significant increase of oxygen free radicals, exhaled nitric oxide, and Geriatric Depression Scale score and a significant decrease of forced expiratory volume in 1 second and forced expiratory vital capacity from younger to older subjects were identified. Moreover, the significant positive correlation between oxygen free radicals and exhaled nitric oxide, and between oxygen free radicals and exhaled nitric oxide with Geriatric Depression Scale score were found. The significant negative correlation between forced expiratory volume in 1 second and oxygen free radicals or exhaled nitric oxide was also demonstrated.

Conclusions. Our data supports the role of progressive local oxidative stress in damaging the lung function and in inducing depression symptoms.

Keywords: Elderly, Exhaled breath condensate, Oxidative stress, Cognitive impairment, Exhaled nitric oxide.

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Introduction

Oxidative stress has been a major topic for many years [1]. The role of free radicals in aging in fact, has been often studied. It is assumed that during aging, damaging effects of the oxygen free radicals may be accumulated in all components of the body. In fact, Reactive Oxygen Species (ROS) are normally generated by tightly regulated enzymes, such as NAD (P)H oxydase isoforms [2]. An overproduction of ROS is related to damage of cell structures such as lipids, proteins and DNA [2]. However, ROS are not only the cause of structural damage, but they are also physiologically important mediators in biological signalling processes, such as apoptosis [3].

During aging, the progressive deficit of antioxidant physiologic systems corresponds to this increase of free radicals, leading to an altered balance of oxidants/anti-oxidants that is one of the pathological mechanisms of many age-dependent disorders [4-6].

The role of ROS in lung diseases has been often studied. Lungs are continuously exposed to oxidants generated either endogenously or exogenously from air pollutants or cigarette smoke [7]. Cigarette smoke can damage lungs by various mechanisms such as depletion of glutathione and other anti-oxidants or initiation of redox cycling mechanisms [7]. The oxidation of proteins has an important role in the pathogenesis of chronic inflammatory lung diseases, such as Chronic Obstructive Pulmonary Disease (COPD), but high plasmatic levels of ROS have also been demonstrated in cystic fibrosis, asbestosis and idiopathic pulmonary fibrosis [8-10].

Lung inflammation has been found in healthy subjects during aging, as demonstrated by the increase of neutrophils in induced sputum of these subjects [11]. Oxidative stress is strictly related to lung inflammation because the recruited inflammatory cells are able to release free radicals, responsible for tissue damage [12, 13].

Usually, ROS are measured by assessing the plasmatic derivatives of reactive oxygen metabolites (D-ROMS). However, the possibility that the oxidative stress is also present in airways has been recently suggested [14, 15]. In the last decade, the interest
DESIGN OF THE STUDY

The systemic and local oxidative stress in different age ranges of healthy subjects and its correlation with lung function and cognitive impairment were investigated.

Subjects

The study population consisted of 30 healthy high school graduate adults (8 M, 22 F), divided in three groups on the basis of the age: 1) 10 subjects aged under 35 years (25.9±4.9 yr), (A), 2) 10 subjects aged between 35 and 60 years (52±5.9) (B), and 3) 10 subjects over 60 years (67.3±4.6) (C). All subjects were Caucasians, recruited from the Respiratory Disease Institute, University of Foggia. Informed written consent was obtained from all subjects and the study was approved by the Institutional Ethics Committee. All subjects were current non-smoker. Subjects with experienced respiratory symptoms or respiratory diseases and with clinical conditions associated with major elevation of inflammatory parameters (acute or chronic viral and bacterial infection, cancer, connective diseases, inflammatory bowel diseases, therapy with corticosteroids or immune-modulating agents, thromboembolic events or surgery in the preceding six months, endocrine diseases other that diabetes, liver or kidney diseases) were excluded.

On the first day, subjects underwent complete anamnesis, anthropometric and cognitive data and respiratory function tests collection. On the second day, subjects underwent exhaled NO measurement and blood tests. Subjects had not consumed anything and had not undergone any physical activity in the 60 minutes prior to the exhaled NO measurement.

Pulmonary function testing

Forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) and the FEV<sub>1</sub>/FVC ratio were measured using a spirometer (PK Morgan Ltd., Gillingham, UK). The best value of three manoeuvres was expressed as a percentage of the predicted normal value.

Measurement of exhaled NO

A rapid-response chemiluminescence NO analyser (NIOX) was used to quantify NO. Two-point calibrations were performed daily using 5.2-parts per million calibration gas. Exhaled NO (F<sub>e</sub>NO) was measured using a previously described restricted breath technique, which employed expiratory resistance and positive mouth pressure to close the velum and exclude nasal NO, and a constant expiratory flow of 45 mL/s. Subjects inhaled to total lung capacity, and exhaled while targeting a constant pressure of 20 mmHg. Exhalations proceeded until a clear NO plateau of at least 3s duration was achieved. Repeated exhalations were performed until three plateaus agreed within 5%.

Plasma measurements

Plasma reactive oxygen metabolites measurement

Systemic oxidative stress was measured by the diacron reactive oxygen metabolites (D-ROM) test (Diacron International, Italy). Values of D-ROM were expressed as Carratelli Units, where 1 U.CARR corresponds to 0.8mg/L H<sub>2</sub>O<sub>2</sub>.

Cognitive tests

Cognitive impairment was evaluated by the Mini Mental State Examination (MMSE) and...
Geriatric Depression Scale (GDS). The use of GDS also in younger groups of study population was justified by previous demonstration of validity of GDS performance compared with other well-validated and reliable measures of depression, such as Beck Depression Inventory, also in younger subjects [24].

MMSE is a 30 items test, evaluating orientation in the time and in the space, memory and calculation ability. A score between 24 and 30 was considered normal, while a score <24 was considered pathological.

GDS, a 30 items test, is a basic screening measure for depression. The score interpretation was: 0-9 normal, 10-19: mild depression, 20-30: severe depression.

The Italian authorised translation of these tests was self administered, according to the authors recommendations.

**Statistical analysis**

Data was expressed as means ± SD. A Mann-Whitney test was used to compare groups, and correlations between variables were performed using Pearson’s correlation test. The Significance was defined as a $p$ value of <0.05.

**Results**

**Subjects characteristics**

Anthropometric variables, functional, cognitive and oxidative stress data of studied subjects are shown in table 1.

**Pulmonary function testing**

FEV$_1$ showed significant decrease from group A to group C (3.65±0.16 l/m vs 2.67±0.18 l/m vs 1.89±0.18 l/m) (figure 1). The significant difference between group A and group B ($p<0.001$), between group B and C ($p<0.01$) and between group A and C ($p<0.0001$) was observed. FVC decreased significantly from group A to group C (4.4±1.0 vs 2.9±0.6 vs 2.3±0.6) (A vs B and A vs C respectively $p<0.05$ and $p<0.01$).

**Exhaled NO**

Concentrations of exhaled NO were progressively higher from group A to group C (5.3±1.0 vs 8.8±1.0 vs 11.0±1.2 ppb) (figure 2). The significant difference between groups (A vs B and A vs C respectively $p<0.05$; $p<0.005$) was observed.

**ROMs**

Concentrations of oxygen free radicals (ROMS) were progressively increasing from group A to group C (186.7±7.6 U.CARR vs 273.6±8.4 U.CARR vs 391.7±16.5 U.CARR) (figure 3). The significant difference between group A and group B ($p<0.0001$), between group B and C ($p<0.0001$) and between group A and C ($p<0.0001$) was identified.

**Cognitive tests**

No significant difference was observed between the three groups analysed regarding MMSE scores (30±0 vs 30±0 vs 30±0). The significant

<table>
<thead>
<tr>
<th>Table 1. - Anthropometric, functional and systemic inflammation data in the population studied</th>
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<tbody>
<tr>
<td>&lt;35 (n=10)</td>
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<td>Age (yr)</td>
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<td>MMSE</td>
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<td>GDS</td>
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Group of 35-60 and over 60 vs <35 = * $p<0.05$; ** $p<0.01$; *** $p<0.0001$. FEV$_1$: forced expiratory volume in 1 second; FVC: forced expiratory vital capacity; GDS: Geriatric Depression Scale; MMSE: Mini Mental State Examination; NO: exhaled nitric oxide; ROMS: reactive oxygen metabolites.
difference was observed regarding GDS scores (5.8±0.6 vs 8.4±0.6 vs 11.5±0.8) (A vs B: \( p < 0.01 \); B vs C: \( p < 0.05 \); A vs C: \( p < 0.001 \)) (figure 4).

**Correlations**

The relationships between systemic oxidative stress markers (plasmatic ROMS), airways oxidative stress markers (exhaled NO), lung function indices (FEV\(_1\)) and cognitive impairment (MMSE and GDS) were analyzed. The results are shown in table (table 2).

The significant positive correlation was observed between plasmatic ROMS and exhaled NO \((r=0.5; p<0.01)\); furthermore, the significant positive correlation was observed between plasmatic ROMS and GDS scores \((r=0.7, p<0.0001)\) and between exhaled NO and GDS score \((r=0.5, p<0.01)\) (figure 5).

The significant negative correlation was found between ROMS and FEV\(_1\) \((r=-0.77, p<0.0001)\) (figure 6) and between exhaled NO and FEV\(_1\) \((r=-0.52, p<0.01)\) (figure 7).

**Discussion**

Little available data explain our interest in studying oxidative stress in elderly.

The strong correlation between lung inflammation and oxidative stress in lung diseases is well known. Inflammation is a protective response to tissue injury, but if this protection occurs in an uncontrolled manner, it results in a chronic inflammation [25]. During chronic inflammation, the recruitment of inflammatory cells, such as neutrophils and phagocytes, leads to ROS release and tissue damage [12, 26]. In fact, activated neutrophils, macrophages and eosinophils generate O\(_2^-\), which is converted into H\(_2\)O\(_2\) by superoxide dismutase, and hydroxyl radicals, as secondary reaction. Lung cells in response to ROS release cytokines such as IL-8 and TNF-\(\alpha\) that induce neutrophil recruitment and activation of transcription factors, increasing the inflammatory process [27, 28]. ROS are highly reactive and they are able to deplete intracellular GSH (a physiological antioxidant) and oxidise membrane phospholipids, causing disruption of its function and cell death. ROS are also able to damage nuclear DNA and proteins in many inflammatory lung diseases [29].

Inhaled environmental oxidants may exacerbate the underlying inflammation in inflammatory lung diseases [25]. Ozone, the major component of air pollution particulates, is able to cause cellular damage by lipid peroxidation, neutrophil infiltration, increased airway responsiveness and reduced pulmonary function also in normal subjects [30]. Moreover, cigarette smoking contains many oxidants and free radicals and it causes airways neutrophils and macrophages infiltration, leading to oxidants release and inflammation [31].

By contrast, poor data is available regarding airways inflammation and oxidative stress in healthy subjects during aging, although several papers investigated systemic inflammation in elderly.

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![Fig. 1. FEV\(_1\) values in three analysed groups. (Group A: <35 years; B: >35 and <60 years; C: >60 years).](image1)

![Fig. 2. Exhaled NO levels in three analysed groups (Group A: <35 years; B: >35 and <60 years; C: >60 years).](image2)

![Fig. 3. ROMS plasmatic levels in three analysed groups (Group A: <35 years; B: >35 and <60 years; C: >60 years).](image3)
in 1 second (FEV1) during aging. The negative vital capacity (FVC) and forced expiratory volume demonstrated the progressive decrease of forced ment in airways studies.

underlying the systemic and local oxidative stress (ROMS) suggests a common mechanism un-

ing airways inflammation observed in elderly hypothesis that exhaled NO probably reflects under-

stress markers (exhaled NO), lung function indices between the progressive increase of systemic and airways oxidative markers (plasmatic ROMS), airways oxidative metabolites supports the role of oxidative stress, due to pollutants and cigarette smoke exposure during life, in damaging the lung function.

Several studies demonstrated progressive cognitive impairment during aging and they have re-

Table 2. - Correlations between systemic oxidative stress markers (plasmatic ROMS), airways oxidative stress markers (exhaled NO), lung function indices (FEV1) and cognitive impairment (MMSE and GDS)

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<th>MMSE</th>
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<tr>
<td>Exhaled NO</td>
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<td>R=0.5***</td>
<td>R=-0.52**</td>
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<tr>
<td>ROMS</td>
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<td>R=0.7****</td>
<td>R=0.77***</td>
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* p<0.05; ** p<0.01; ****p<0.0001. FEV1; forced expiratory volume in 1 second; GDS: Geriatric Depression Scale; MMSE: Mini Mental Status Examination; NO: exhaled nitric oxide; ROMS: reactive oxygen metabolites.

In agreement with previous publications, we demonstrated the progressive increase of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) during aging. The negative correlation with systemic and airways oxidative metabolites supports the role of oxidative stress, due to pollutants and cigarette smoke exposure during life, in damaging the lung function.

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Little damage to a progressive increase of the systemic oxidative stress [34, 35]. The EVA study showed that subjects with the highest levels of TBARS (thiobarbituric acid reactant substances, an indicator of liperoxidation) presented the increased risk of cognitive decline, measured using the Mini Mental Status Examination (MMSE) [36]. Negative correlation between cognitive degree and anti-oxidant status was identified in this study [36]. It is probable that the increased levels of oxidative stress and/or antioxidant deficiencies may represent risk factors for cognitive decline, that is a major component of age-related dement-

These studies can be limited by the population sample: in fact the enrolled patients were often in the age-range between 60 and 80 years and the follow up of three or four years was done. To our knowledge, no sufficient data is available on the progressive cognitive impairment during aging from younger to older subjects. In contrast with previous studies, analysing the cognitive impairment degree with MMSE, we found no differences in MMSE score between three groups, although the progressive increase of systemic and airways oxidative stress was observed. We have tested cogni-

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tering neurotransmission, ultimately contributing to depression [38, 39]. On this basis, it is possible to suggest that oxidants level and oxidants-anti-oxidants balance alterations are, at least in part, responsible also for the depression increase observed in healthy subjects during aging [40]. In accordance with this data, our results not only show a correlation between oxidative stress and airway inflammation in healthy subjects during aging, but they also could suggest a role of oxidative stress in progressive cognitive impairment. Our data, which is not conclusive due to limitations of the study, such as the small number of enrolled patients, is nevertheless suggestive of the importance of oxidative stress in cognitive functions and new studies are necessary in order to clarify the mechanisms underlying these correlations in healthy aging individuals.

References


