Morphological changes positive correlates with oxidative stress in COPD. Preliminary data of an experimental rat model – study and literature review

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Abstract
It is well known that nicotine that is a major toxic component of cigarette smoke induces oxidative stress which is responsible for the lung damages in COPD and cancer. There have been reported some cases of COPD in never smoking patients exposed to air pollutants. The aim of our study is to evaluate the morphological pulmonary changes in rats exposed to cigarette smoke respectively to solid combustible smoke and to establish the relationship between the exposure and the level of oxidative stress measured through serum (s) and pulmonary tissue (l) MDA in rats (TBARS method). Thirty Wistar rats were divided into three groups (n = 10): (1) the control group (C), (2) the cigarette smoke group (CS), and (3) the solid combustible smoke group (SC). Apart from the control group, these were treated with solid combustibles smoke or cigarette smoke for six months. We collected blood for serum determination of MDA and the lungs were removed for histopathological analysis and to determine the levels of malondialdehyde (MDA). The levels of serum and lung MDA were significantly higher in CS and SC groups compared with C group, but not significantly differences between CS and SC group were detected. These findings are positively correlated with histopathological changes (squamous metaplasia and clear cell hyperplasia in the bronchium epithelium, emphysema) found in pulmonary tissue. Preliminary data of our study confirms that not only the cigarette smoke but also the environmental pollutants are involved in the major pathways of COPD.

Keywords: COPD, oxidative stress, cigarette smoke, solid combustible smoke, rat experimental model.

Introduction
COPD is an extremely complex disease. Lots of studies were made during the last 40 years concerning COPD pathogenesis, physiopathology and morphological changes but they did not obtain clear and unitary conclusions. The main risk factor incriminated in COPD pathogenesis is cigarette smoking but only 15% of active smokers develop airway flow obstruction and COPD. On the other hand, epidemiological studies showed that 5–12% of COPD patients never smoked. Only a little is known about the pathophysiology of the constant airway flow obstruction in these patients. These data suggest the implication of some factors, other than cigarette smoking in pathogenesis of COPD [1].

A major impediment in the study of COPD is that the fundamental morphological changes that produce the major pulmonary dysfunction are placed in the peripheral airways, at bronchiole-alveolar junction, that are difficult to be explored by optic fiber endoscopies. That is why the animal models had an important role in the modern era of research in COPD, after Gross P et al. [2] discovered that the intratracheal administration of papain determine emphysema. On the other hand, Laurell CB and Erikkson SE [3] determined that the deficit of alpha1-antitrypsin determine a high risk for emphysema. These two findings are the scientific fundament for the hypothesis elastase–antielastase in the pathogenesis of emphysema and even after 40 years animal models are important tools for the studies of the pulmonary morphological changes in COPD [4].

It has been lately reported COPD cases in patients who never smoked. A coherent morphological study on human tissue is practically impossible because of the major pulmonary dysfunction of the COPD patients that do not permit optic fiber endoscopies or surgical procedures. That is why we have proposed to make a comparative study on experimental rat model consisting in rats exposed to cigarette smoke, respectively to solid combustible burning smoke. The aim of our study is to analyze the morphological changes induced by smoke in the central and peripheral airway walls, respectively the pulmonary parenchyma and to evaluate the oxidative stress.

Material and methods
We used 30 Wistar adult male rats, divided in three groups of 10 rats each. The CS (cigarette smoke) lot was daily exposed to the smoke resulted from burning of 10 cigarettes.
The SC (solid combustible smoke) was exposed to the smoke resulted from the burning of 300 g of solid combustible (wood, coal). The third lot, C (control) was unexposed. The exposure was made in one 50/50/50 cm precinct, 60 minutes daily.

The slaughter of the animals was performed two days after the last exposure, confirm the EC rules. The rats were anesthetized with Thiopental and then sacrificed. Blood and tissues (lungs, heard, kidneys) were removed. Lung tissue was processed by usual histopathological technique: 10% formalin fixed, paraffin embedded, Hematoxylin–Eosin and Van Gieson stained.

We have considerate malondialdehyde (MDA) as a marker of oxidative stress induced by the cigarette smoke respectively the solid combustible smoke exposure. MDA levels were determined using TBARS method. Unfortunately we did not have the technical possibility to perform spirometry tests.

**Results**

The rats' medium weight was at the beginning of the study 250 g. All the animals were standard feed during the whole period of experiment. In the end, medium weight of the C lot was 350g, 289g for CS lot, respectively 297g for the SC lot. We can see a loss of weight in smoke exposed rats (Figure 1).

Both the two smoke exposed lots presented lesions at the large and small, peripheral, airways and also at the pulmonary parenchyma comparative with those that are characteristic for COPD. We have found clear cell hyperplasia (Figures 2 and 3) and squamous metaplasia (Figures 4 and 5) of the small airway mucosal epithelium, mononuclear inflammatory infiltrate in the small airway walls (Figures 6 and 7) and alveolar walls destructions corresponding for emphysema (Figures 8 and 9).

The serum level of MDA was 6.29+ 2.76 nmole/ml in the C lot; in the pulmonary homogenate the MDA level was 0.42+ 0.23 nmole/mg protein.

The serum level of MDA was 8.06+ 1.32 nmole/ml in the CS lot; in the pulmonary homogenate the MDA level was 0.84+ 0.82 nmole/mg protein.

The serum level of MDA was 7.3+ 1.34 nmole/ml in the SC lot; in the pulmonary homogenate the MDA level was 0.79+ 0.56 nmole/mg protein.

**Discussions**

There is no longitudinally study on COPD experimental model until 1997, when Wright JL et al. [5] showed that the pulmonary function of the rat normally decrease during 12 months, because of rat aging and the cigarette smoke accelerates this effect similarly in human, as also demonstrated Gold DR et al. in 1996 [6].

Wright JL et al. [5] demonstrated that the chronic cigarette smoke exposure determine airflow obstruction. The vital capacity (VC) and the forced vital capacity (FVC) are increased in the smoke exposed animals comparative with the control, according to the increased statically pulmonary compliance.

Usually, the vital capacity is decreased in human COPD patients. This paradox in animals is explained by transpulmonary positive pressure of the air income rather than active inspire [7].

The forced expiratory volume in one second (FEV₁) physiologically decrease during the 4th and the 12th month, but the decrease is more significant in the exposed group than in the unexposed one. FEV₁/FVC decreased significantly in the smoke exposed group in the 4th–8th month period meanwhile the aging decreasing of the airflow was produced in the 8th–12th month period.

It is evident that is a net discrepancy between the smoking effects on the pulmonary volumes and compliance and, respectively on the airflow, pulmonary volumes and compliance being precarious influenced comparative with airflow possible because of the damaged pulmonary matrix.

Some authors suggested in their studies that the chronic cigarette smoke exposure determine a dynamic alteration of the lung collagen with secondary pulmonary dysfunction [8, 9]. Snider GL et al. have demonstrated using biochemical methods, increased levels of the pulmonary collagen that positive correlated with the decrease of the FEV₁ in experimental model emphysema [10].

Wright JL et al. (1997) sustain that the alteration of the pulmonary volumes followed by decreasing of the airflow indicates a remodeling process of the matrix in rats [5]. It is well known that the matrix is damaged during lifetime [11]; changes observed in cigarette smoke exposed rats are analogue to the rapidly aging process like in smoking humans [6].

In their study, Wright and Churg showed that rats were used in lot of COPD studies [6]. Huber GL et al. [12] and Heckman CA and Dalbey WE [13] have exposed rats for 6 to 30 months. Both of them determine a small but significant raise of the aerial space volume at a 10 cigarettes per day exposure level and demonstrated the decrease of the elastic recoil.

Heckman CA and Dalbey WE have noticed that the volumetric proportion of the aerial space was not significantly increased when the exposure level decreased at seven cigarettes per day, even at prolonged period [13].

Ofulue AF et al. found a relatively stabile increase of the aerial space (130%) after six months of 10 per day cigarette smoke exposure [14].

Rubio ML et al. exposed rats to the two cigarettes smoke per day for 10 weeks and morphometrically demonstrated the thickening of the bronchiolar walls in bronchioles less than 100 μm in diameter that was associated with an abnormal N₂ elimination curve [15].

Huber GL et al. [12] and Heckman CA and Dalbey WE [13] also demonstrated the existence of an inflammatory mononuclear infiltrate with peribronchiolar distribution. Huber GL et al. [12] found an increased metaplastic secretor cells in the tracheal epithelium. Also, Hayashi M et al. showed increased mucous glands volume after 30 consecutive days of smoke exposure, data that sustain the results of the Jones and all study from 1973 [16, 17].
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Figure 1 – Comparative evolution of the weight of the three groups

Figure 2 – Pulmonary morphological changes in CS group. Clear cell hyperplasia

Figure 3 – Pulmonary morphological changes in SC group. Clear cell hyperplasia

Figure 4 – Pulmonary morphological changes in CS group. Squamous metaplasia of the small airway epithelium

Figure 5 – Pulmonary morphological changes in SC group. Squamous metaplasia of the small airway epithelium

Figure 6 – Pulmonary morphological changes in CS group. Mononucleate inflammatory infiltrate in the small airway wall
Figure 7 – Pulmonary morphological changes in SC group. Mononucleate inflammatory infiltrate in the small airway wall

Figure 8 – Pulmonary morphological changes in CS group. Emphysema

Figure 9 – Pulmonary morphological changes in SC group. Emphysema

Figure 10 – Serum values of MDA are significantly lower in control group comparing the CS and SC groups. There are no significant differences for serum MDA between CS and SC group

Figure 11 – MDA levels in pulmonary tissue are significantly higher in both SC and CS groups comparing the control group. The insignificantly difference between SC and CS group appears also in pulmonary tissue as well as in serum
Shore S et al. [18] and Finlay GA et al. [19] reported COPD attributes emphysema or bronchitis, on rat experimental models. The emphysema or chronic bronchitis in rats was generally obtained using chemical substances and they do not reproduce exactly the biological and pathological aspects of the human COPD as chronic inflammation. Kodavanti UP et al. [20] obtained emphysema using elastase instillation and the persistent chronic inflammation of bronchi with sulfur dioxide. Finally, the lesions and clinical manifestations were similar with those of human COPD.

Lots of irritant substances were used to induce COPD in experimental models: lipopolysaccharides, cadmium chloride, O₃, silica dust. Of course, the cigarette smoke exposure is the one who determine the complex pulmonary changes in COPD. Lots of species were exposed to cigarette smoke during these years: dog, rabbit, Guinea pig and rodents [21].

Like in human patients with COPD, the weight of the animals with chronic exposure to smoke is lower than of the unexposed during the whole period of experiment [7, 22, 23].

In our study, rats that were exposed in the same conditions to the cigarette smoke respectively to solid combustible smoke had a similar body weight evolution. It is unanimous accepted the loss of the muscular mass in COPD, probably through apopotic mechanisms.

Recently, the developing of COPD is associated with intense oxidative stress, respectively with reduced antioxidant resources [24, 25]. Important from this point of view are the oxidant-antioxidant balance disequilibrium and the role of the oxidative metabolism in the systemic effects of COPD. Systemic and pulmonary oxidative metabolism disturbances that determine loss of muscular mass and carcinogenesis are obviously in COPD patients [26].

The oxidative stress determines increasing in proteolysis susceptibility through amino acid chains changes resulting in protein aggregates and peptide connections cleavage. Human plasmatic proteins contain carbonil groups and loose sulfhidril groups as result of the action of the saturated and unsaturated aldehydes contained in the cigarette smoke after cigarette smoke exposure [27, 28–30].

The plasmatic proteins can be degraded by the reactive oxygen species (RSA) through nitration and oxidation. The smokers have higher serum levels of nitric proteins as fibrinogen, transferyn, ceruloplasmin and plasminogen respectively oxidated proteins comparing with non-smokers [31].

Cigarette smoke is a rich source of oxidant agents. Cigarette smoke tar (particles that lay down of the filter) contains 10¹⁵ spin/gram tar. Generates radicals are sufficiently stable to permit their detection through ESR (electron spin resonance). Oxidant agents from the cigarette smoke are semiquinone, carbon and nitric reactive species, reactive olefins and dienes, NO. Oxidative stress may be quantified through measurement of some biomarkers.

The most convincing method in the direct measurement of the oxygen radicals in the pulmonary tissue and in the exhaled air but this method is also the most difficult because of the high reactivity of the short life species free oxygen radicals.

On the other hand, it is difficult to use direct techniques and ESR on the lung tissue. The alternative for these direct methods is the measurement of the oxygen radical effects on the variety of pulmonary bio molecules, lipids, proteins or DNA [32].

Free radicals generate lipid peroxidation resulting in lipidic radicals who react with oxygen in aerobic cells resulting peroxyl radical. The peroxyl radical starts up a reaction chain through which the polyunsaturated fat acids (free or belonging to lipids) are transformed in lipidic hydroperoxids. Lipid peroxidation determine membrane dysfunctions, inactivates membrane sites of the receptors and enzymes, increase the membrane permeability [33].

The reaction between the lipid hydroperoxids with the antioxidants (α-tocopherol), iron or copper ions, or haemoglobin, results in gaseous hydrocarbonated and unsaturated aldehydes (malondialdehyde) [34].

Lipid peroxidation products have increased plasmatic and pulmonary lavage liquid levels in healthy smokers and in emphysema, chronic bronchitis and asthma patients. The high level of the lipid peroxidation products positive correlates with the small airway obstruction degree in COPD patients [35].

COPD is considerate lately a systemic disease. One of the systemic manifestations of COPD is the presence in COPD patients’ blood of oxidative stress markers. This is morphological reflected in high sequestration of neutrophils in pulmonary microcirculation during smoking and COPD exacerbation periods, oxidant mediated phenomenon.

Cell membrane polyunsaturated lipids and the fatty acids are major targets for the free radicals attack resulting in lipid peroxidation, a process that continues as a chain reaction and generates peroxides and aldehydes. Lipid peroxidation reaction products may be measured in the body fluids as substances that react with the thioarbituric acid (TBARS – thiobarbituric acid reactive substances).

Plasmatic level of TBARS is significantly increased in healthy smokers and in COPD patients comparing with healthy nonsmokers. Oxidative stress measured as TBARS plasmatic level inversely correlated with the percentage of the predicted VEMS indicating the fact that the lipid peroxidation is associated with airflow limitation [36].

The data analyze showed that the serum MDA levels are significantly lower in the C lot than in the CS and SC lots. There are no significant differences between the two smoke exposed lots (Figure 10). Concerning the pulmonary tissue MDA level, the value in the C lot is again significantly lower than in the two smoke exposed lots. The differences between the two smoke exposed lots are insignificant (Figure 11). The comparative increased of serum and lung tissue MDA levels in the two chronic smoke exposed lots, significantly higher than those stand out in the M lot, indicates a high level of the oxidative stress induced by the environmental pollutants. The increased serum MDA suggests the whole body implication in COPD.
Actually, COPD appears to be a general disease not an only one organ one.

Although the majority of information about the pathogenesis of COPD is focused on the emphysema development, is well-known that COPD includes also chronic bronchitis and small airway lesions. There are not so many informations concerning these aspects but, presumably, factors that initiate the inflammation and the effects of the proteolitic and oxidant aggression are also implicated. Emphysema animal model elastase-induced is associated with airway epithelium clear cell hyperplasia and mucous hyper secretion that is characteristic for chronic bronchitis [36]. The consistent alveolar wall destructions in rats were a surprise for us considering that the literature data suggests the rat seems to be the most resistant to emphysema [21].

Histopathology aspects positive correlate with the serum and tissue levels of MDA.

Conclusions

These preliminary data of our study strongly suggest the fact that the environmental pollutants could also be implicated in pathogenesis of COPD. This kind of aggression could be an explication for the cases of COPD in patients that never smoked.

It becomes more obviously that COPD is a complex disease that implicates the whole body. The very numerous studies made during the last 50 years in their disease that implicates the whole body. The very aggression could be an explication for the cases of COPD includes also chronic bronchitis and small airway lesions. There are not so many informations concerning these aspects but, presumably, factors that initiate the inflammation and the lack of a unitary vision determined the existence still of a lot of obscure aspects concerning COPD pathogenesis and, secondarily, the inefficiency of the treatment.

References


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