Experimental COPD induced by solid combustible burn smoke in rats: a study of the emphysematous changes of the pulmonary parenchyma

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Abstract
According to the GOLD 2006 definition, COPD is a preventable and treatable pathological situation characterized by the partially reversible airflow limitation determined by a variable proportion mixture of small airways disease (obliterative bronchiolitis) and parenchyma destruction (emphysema). A major impediment in the study of the COPD is represented by the fact the fundamental morphological changes that determine the major pulmonary dysfunction take place in the small, peripheral, airways, at the bronchiolo-alveolar attachments. That is why the experimental model of COPD developed progressively to the transgenic mouse. There are many experimental studies on the animal models that have obtained emphysema rapidly through intratraheal instillation of elastasis or bronchitis/bronchiolitis through intratraheal instillation of particles. It is accepted that the unnatural character of aggression, that does not permit the natural evolution of the inflammatory phenomenon, limits these models and tissue remodeling that take place in COPD patients. It is well known that cigarette smoking is a major cause of COPD. There have been reported some cases of COPD in never smoking patients exposed to air pollutants. We aimed to create an experimental model of COPD in rat through exposure to smoke resulted from solid combustibles burn for the same period and in the same conditions of cigarette smoke exposure and to compare the pulmonary morphological changes. 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 (SCS). Apart from the control group, these were treated with solid combustibles smoke (SCS group) or cigarette smoke (CS group) for six months. Morphological and morphometry studies have been assessed. We have established a rat COPD model based on natural cigarette smoke exposure versus solid combustible burn resulted smoke, usable for a further approach in human non-smoker COPD investigation. Our procedures resulted in clear pulmonary morphological lesions that are characteristic for COPD. The achieved data support the idea that solid combustible burn resulted smoke determines emphysematous parenchyma lesions that are similar, but with an attenuated morphological appearance when comparing to the cigarette smoke exposure.

Keywords: emphysema, rat experimental model, COPD in non-smokers.

Introduction
According to the GOLD 2006 [1] definition, COPD is a preventable and treatable pathological situation characterized by the partially reversible airflow limitation. A variable proportion from person to person mixture of small airways disease (obliterative bronchiolitis) and parenchyma destruction (emphysema) determines the chronic airflow limitation. Parenchyma destruction is also a result of the inflammatory mechanisms that lead to the bronchiolo-alveolar attachments loss and secondary the pulmonary elastic recoil reduction with the decrease of the capability of open remaining during the expire of the air ways.

Tobacco smoke is the major risk factor that is incriminated in the COPD genesis. The fact that only 15% of heavy smokers develop airway obstruction and COPD suggests the implication of other factors in the COPD pathogeny. There are, on the other hand, epidemiological studies showed that 5–12% of the COPD patients never exposed to cigarette smoke. There are many questions concerning the mechanisms of the constant airflow limitation in these patients.

A major impediment in the study of the COPD is represented by the fact the fundamental morphological changes that determine the major pulmonary dysfunction take place in the small, peripheral, airways, at the bronchiolo-alveolar attachments. More, the COPD patients have progressive respiratory dysfunction that limits the fiber optic endoscopies and surgical indication. That is why the experimental model of COPD developed progressively to the transgenic mouse.

There are many experimental studies on the animal models that have obtained emphysema rapidly through intratraheal instillation of elastasis or bronchitis/bronchiolitis through intratraheal instillation of particles. It is accepted that the unnatural character of
aggression that does not permit the natural evolution of the inflammatory phenomenon limits these models and tissue remodeling that take place in COPD patients. The majority of the study groups that have used the natural exposure method have obtained increase of the aerial space in volume that is small but significant and demonstrated the decrease of the pulmonary elastic recoil. On the other hand, even the cigarette smoke is considered the main factor responsible for COPD other factors including genetic predisposition, particles [2] or gas pollutants and smoke resulted from solid combustibles burn may explain the COPD cases in never smoking patients.

We aimed to create an experimental model of COPD in rat through exposure to smoke resulted from solid combustibles burn for the same period and in the same conditions of cigarette smoke exposure and to compare the pulmonary morphological changes.

Material and Methods

Study population

Study population consisted in 30 Wistar male rats, adults 3-month-old with medium weight of 250 g. Biobasis conditions were standard. The whole study populations have been investigated during the same period in the Biobasis of the “Gr. T. Popa” University of Medicine and Pharmacy, Iassy, according to the Somerset West Amendment (1996).

Experimental method

Pollutants exposure

The 30 Wistar adult male rats were divided in three lots of 10 rats each:
- the CS (cigarette smoke) lot consisting of 10 Wistar male rats having weight between 214 and 262 g was daily exposed to the smoke resulted from burning of 10 cigarettes;
- the SCS (solid combustible smoke) lot consisting of 10 Wistar male rats having weight between 212 and 280 g was exposed to the smoke resulted from the burning of solid combustible (SRBSC), 300 g of wood and coal;
- the third lot, C (control), consisting of 10 Wistar male rats having weight between 280 and 308 g was unexposed to pollutants.

The exposure was made in one 50/50/50 cm ventilated with a flow rate at 0.1 m³/hour precinct, 60 minutes daily. The pollutant exposure of the CS and SCS lots was initial progressive concerning the burned material mass and the exposure time in order to permit the biological adaptation of the animals and to avoid accidents such as smoke intoxication that could determine death of rats.

The slaughter of the animals was performed two days after the last exposure, according the EC rules. Blood and tissues were removed. After the cardio-pulmonary block was removed, 1.8–2 ml 10% formalin solution was instilled into the lungs in order to maintain the pulmonary morphology and to avoid the alveolar collapse, according Valença SS et al. [3].

Lungs were immediately immersed in 10% formalin. Lung tissue was processed by usual histopathological technique: 10% formalin-fixed, paraffin-embedded, Hematoxylin–Eosin and van Gieson stained.

Study methodology

We have adopted the experimental method of Cendon SP et al. [4] that mimics the natural exposure conditions. Many studies recognize the limitations of rapidly obtained experimental models of emphysema through intratracheal instillation of proteases or bronchitis/bronchiolitis through intratracheal instillation of particles [5, 6] that does not permit the natural evolution of the inflammatory and tissue remodeling events that evolve in human COPD patients.

On the other hand, the long exposure period is disadvantageous because of higher costs and the risk of death of the animals. The 6 month 10 cigarettes per day smoke exposure is necessary and sufficient to obtain pulmonary lesions corresponding for human COPD and avoid the changes caused by aging [4, 7–9].

Though cigarette smoke is considered the main risk factor for COPD, other factors including genetic predisposition, particulate and pollutant gases as biomass combustion smoke, viral or bacterial infections may be involved in COPD pathogenesis [2, 10].

The quantity of solid combustible to burn, 300 g per day, have been established through tests that have semi quantitative appreciated the volume of the smoke thus the overexposure with toxic effects determining death of the animals to be avoided.

The analysis of the data resulted after the two types of pulmonary inhalator aggressive factors have been made considering as reference lot the rat group exposed to the cigarette smoke because of the impossibility of pulmonary function evaluation. The control lot was instituted in conditions of biological imperfect animal use, in order to establish the basal biological status and to eliminate the natural factors such as respiratory infections that could impinge the analysis.

The pulmonary tissue preparation made for morphometric study considered the pulmonary anisotropy. Assessment of the areas, the structures thickness and cell number in the pulmonary tissue was made on serial isotropic randomly sections, according to Valença SS et al. [3].

Serial, classical stained (HE, van Gieson, Orcein, reticulin fibers) and immunohistochemical (SMA) sections were used. Lungs were cut in longitudinally axe and so each section contains hilum, mediopulmonary respectively peripheral pulmonary area having the alveolar and aerial ducts distribution compatible with the Weibel–Gomez [11] system of structures counting on randomized sections.

Image acquisition

Unbiased histopathological specimens were scanned in same lighting conditions with the Zeiss Observer Z1 Tissue Gnostics system (Tissue Faxes), under a 20× objective, acquiring digital image fields of view (FOVs) at 200×, for a final resolution of 1280/1024 color pixels.
Morphometrical and morphological study

Morphometrical and morphological study was performed on the digital images archived in the database on the same PC, by the same observer, in the same lighting conditions.

Counting methodology

Dilated alveolar spaces, morphological normal alveoli, alveolar blunts morphological identified were counted on 1000 of 200× FOV (field of view) for each group. Alveoli are defined as alveolar lumens and septae, excluding terminal and alveolar bronchioles and alveolar ducts.

Method for assessment of the elastic fibers with Orcein stain

Ten representatives for elastic fibers assessment 200× FOVs were chosen from 30 studied for each study group and printed. On each of them, we have established the score types hierarchised from 0 to 3 with clear examples: 0 – absence of the marker; 1 – minimum target, 1–0.8 µm thickness red wire; 2 – more than 0.8 µm thickness red wire; 3 – maximum marker, more than 0.8 µm thickness red wires, intense stained. Reference images have been stored in special folder (Figure 1).

Figure 1 – Reference images for the Orcein score types: (a) Orcein score 0; (b) Orcein score 1; (c) Orcein score 2; (d) Orcein score 3

Four field corners placed alveoli (up-left corner, up-right corner, right-down corner, left-down corner) and centered one were assessed for each FOV. The length of each segment of the alveolar perimeter having particular Orcein score has been measured, on the circumference. The investigated alveolar areas were measured. All the data have been written in the database. An comparison between algorithm that restrict the four parameters, three for Orcein stain intensity and alveolar perimeter to one statistical parameter, weight per alveolar perimeter, have been elaborated. The obtained data have been statistically analyzed.

Alveolar walls cellularity assessment methodology

Ten representatives for alveolar walls cellularity assessment 200× FOVs were chosen from 30 studied for each study group and printed. On each of them, we have established the score types hierarchised from 0 to 3 with clear examples: 0 – lots of nuclei are visible; 1 – medium number of visible nuclei; 2 – few nuclei visible; 3 – no nuclei visible. Reference images have been stored in special folder (Figure 2).

Figure 2 – Alveolar wall cellularity: (a) score 0; (b) score 1; (c) score 2; (d) score 3

In order to avoid the misconsideration of the terminal or respiratory bronchioles as enlarged alveolar spaces, the analysis have been made under the control of the respective region resulted from the digital stitch of the FOVs (Figure 3).

Figure 3 – Image of the region obtained through the digital stitch of the 200× FOV, HE (* – enlarged alveolar space; ↓ – terminal bronchiole; ↓ – respiratory bronchiole)

Results

Total number of pulmonary alveoli on the 200× FOV is significant greater in the control group than in the CS and SCS groups. There is no significant difference between the medium values of the total number of pulmonary alveoli on the 200× FOV in the smoke exposed groups (p = 0.007268).

The analysis of the standard deviation indicates the unhomogeneity in the control group because of the variability that is normal present in the pulmonary parenchyma: there are alveoli, respiratory ducts, large and peripheral, small airways.

The close standard deviations for the CS and SCS groups and smaller than in the control group indicate the homogeneity of the data, explicable by the tissular reshuffle determined by the aggression.

Concerning the number of normally pulmonary alveoli on the 200× FOV there is statistical significant difference between the smoke exposed groups, respectively between each of them and the control group.

The distribution curve indicates that there are more normal alveoli on the FOV in SCS group than in CS one; this is an aspect that sustains the idea that emphysematous destruction exists in the SCS group but is inferior to that from the CS group (Figure 4).
Figure 4 – Distribution of normal alveoli per 200× FOV, comparing CS/SCS groups (C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group)

From statistical point of view, the $t$-Student test asserts significant differences of the medium number of enlarged alveolar spaces on the 200× FOV between smokes exposed and C groups (Table 1).

Table 1 – Medium number of enlarged alveolar spaces on the 200× FOV

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>$t = 63.17$; GL = 1905; $p &lt; 0.001$</td>
<td>–</td>
</tr>
<tr>
<td>SCS</td>
<td>$t = 87.50$; GL = 1912; $t = 7.59$; GL = 2399; $p &lt; 0.001$; $p &lt; 0.001$</td>
<td></td>
</tr>
</tbody>
</table>

C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group.

There are no enlarged alveolar spaces in control group and that indicates we have correctly chosen the emphysematous enlarged alveolar spaces criteria. Although the medium values for CS and SCS groups are close, the Kruskal–Wallis test indicates significant statistical difference between them. The groups’ homogeneity is pronounced and that indicates that the alveolar destructions are uniformly distributed in the pulmonary parenchyma (Figure 5).

Analyzing data we note that the control group presents medium 60 alveoli per 200× FOV (59.93 ± 28.16), all of them typical; CS group presents 6.08 enlarged alveolar spaces per 200× FOV in 20.59 ± 11.53 medium total number of alveoli per 200× FOV, representing 29%. For the SCS group, medium number of enlarged alveolar spaces per 200× FOV is 5.22, representing 24.39% of medium total alveoli number per 200× FOV (21.40 ± 8.63) (Figure 6).

We found out the presence of bronchiolo-alveolar attachment destruction in both CS and SCS groups, less frequent in SCS group (Figure 7).

Figure 5 – Distribution of enlarged alveolar spaces per 200× FOV, comparing the CS/SCS groups (C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group)

Figure 6 – Weight of enlarged alveolar spaces per typical alveoli in the total alveoli number per 200× FOV (C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group)

Figure 7 – (a) C group. Normal bronchiolo-alveolar attachment (*), HE, 200×; (b) CS group. Destroyed bronchiolo-alveolar attachment (arrows), HE, 200×; (c) SCS group. Destroyed bronchiolo-alveolar attachment, IHC, 200× (C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group)
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Alveolar destructive index was assessed using the formula DI% = \([D/(D+N)] \times 100\%\) where D – destroyed (enlarged alveolar spaces), and N – normal (normal morphology alveoli) according to Robbesom AA et al. [12].

Following data resulted (Table 2):

<table>
<thead>
<tr>
<th>DI%</th>
<th>C</th>
<th>CS</th>
<th>SCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>710</td>
<td>100</td>
<td>197</td>
</tr>
<tr>
<td>1–20</td>
<td>–</td>
<td>–</td>
<td>415</td>
</tr>
<tr>
<td>21–40</td>
<td>–</td>
<td>663</td>
<td>614</td>
</tr>
<tr>
<td>41–60</td>
<td>–</td>
<td>216</td>
<td>145</td>
</tr>
<tr>
<td>61–80</td>
<td>–</td>
<td>62</td>
<td>18</td>
</tr>
<tr>
<td>81–99</td>
<td>–</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>710</td>
<td>1197</td>
<td>1204</td>
</tr>
</tbody>
</table>

C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group.

Data analysis revealed that the DI% was 0% in control group. For the CS group, DI% was between 21–40% for 55.39% of 200× FOV and 100% for 3.17% of 200× FOV. For the SCS group, DI% was between 21–40% for 51% of 200× FOV and 100% for 0.58% of 200× FOV (Figure 8).

There are significant differences between the medium values of the DI% both for smoke exposed groups and for control group. Medium DI% value is larger in CS group than in SCS group.

We have named alveolar blunts the remnant fragments of alveolar walls present in the emphysematous destructed alveolar spaces. They correspond to the so-called “drummer sticks” and are part of the diagnostic criteria for emphysema (Figure 9).

There is no significant difference between the medium values of the blunt number per 200× FOV for the smoke exposed groups but this difference exists relative to control group (Table 3).

The blunt homogenous distribution in smoke exposed groups positive correlates with same manner of distribution of the alveolar macrophages (unpublished data). Alveolar macrophage is considered responsible for apoptotic events that dictate the emphysematous alveolar destruction (Figure 10).

There is a direct correlation (57%) between the number of blunts per 200× FOV for the enlarged alveoli number per 200× FOV and a slight indirect correlation \((r = -0.22)\) with DI% in CS group.

For the SCS group, there is a slight direct correlation between the number of blunts per 200× FOV and the enlarged alveolar spaces number per 200× FOV \((r = 0.37)\); comparing with the DI%, variables are independent \((r = -0.09)\).
Elastic fibers are fine, delicate and distributed in uniform manner along the alveolar perimeter in the control group (Figure 11).

In smoke exposed groups, the distribution of elastic fibers per perimeter is uneven. There are numerous unstained alveolar wall segments indicating the absence of the elastic fibers (Figures 12 and 13).

Analyzing resulted data (Table 4) we note that in the control group 13.35% alveolar perimeter presents no elastic fibers, 55.86% presents medium quantity of elastic fibers, respectively 5.94% with large elastin amount; 56.78% alveolar perimeter presented no elastic fibers and 39.15% showed small elastic amount in CS group; 64.61% alveoli perimeter presented no elastic fibers while 28.80% alveoli perimeter presented small quantity of elastic fibers in SCS group.

All three groups presented small weight per alveolar perimeter of the intense Orcein stain (5.94% for C group, 0.12% for CS group, 0.25% for SCS group) (Figure 14).

$t$-Student test stands out significant differences between the medium alveolar perimeter in the three studied groups. It can be observed also that the medium alveolar perimeter value is significant greater in CS group than in SCS group (Table 5).

<table>
<thead>
<tr>
<th>Group</th>
<th>0 ± σ</th>
<th>%</th>
<th>1 ± σ</th>
<th>%</th>
<th>2 ± σ</th>
<th>%</th>
<th>3 ± σ</th>
<th>%</th>
<th>Total perimeter ± σ</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n = 995)</td>
<td>43.63 ± 52.35</td>
<td>13.35</td>
<td>182.53 ± 80.99</td>
<td>55.86</td>
<td>81.22 ± 67.73</td>
<td>24.85</td>
<td>19.40 ± 38.61</td>
<td>5.94</td>
<td>326.77 ± 72.46</td>
<td>100.00</td>
</tr>
<tr>
<td>CS (n = 1001)</td>
<td>292.65 ± 198.87</td>
<td>56.78</td>
<td>201.82 ± 142.37</td>
<td>39.15</td>
<td>20.04 ± 51.42</td>
<td>3.89</td>
<td>0.62 ± 9.35</td>
<td>0.12</td>
<td>515.44 ± 272.99</td>
<td>100.00</td>
</tr>
<tr>
<td>SCS (n = 1000)</td>
<td>255.50 ± 144.36</td>
<td>64.61</td>
<td>113.87 ± 85.40</td>
<td>28.80</td>
<td>25.06 ± 42.61</td>
<td>6.34</td>
<td>1.00 ± 6.04</td>
<td>0.25</td>
<td>395.43 ± 141.52</td>
<td>100.00</td>
</tr>
</tbody>
</table>

C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group; 0–3 – Orcein score.
The large weight of the alveolar walls without elastic fibers in smoke exposed groups is statistically confirmed (Table 6).

Alveolar perimeter without elastic fibers correlates with the DI% (Table 7).

The medium value of the alveolar area is significantly larger in CS group than in the SCS group (Table 8).

We also have evaluated the alveolar walls cellularity (Figure 15).

Table 5 – Statistical differences between the alveolar perimeter on the study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Orcein score</td>
<td>43.63 ± 52.35 (n = 995)</td>
<td>292.65 ± 198.87 (n = 1001)</td>
</tr>
<tr>
<td>Alveolar destructive index</td>
<td>0 ± 0 (n = 710)</td>
<td>34.89 ± 18.57 (n = 1197)</td>
</tr>
<tr>
<td>t-Student statistical significance</td>
<td>t = 25.80; GL = 1703; p&lt;0.001</td>
<td>t = 40.86; GL = 2196; p&lt;0.001</td>
</tr>
</tbody>
</table>

C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group.

Table 6 – Statistical differences of the elastinless segments of the alveolar perimeter

<table>
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</tr>
<tr>
<td>t-Student statistical significance</td>
<td>t = 4.78; GL = 2202; p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

C – Control group, air exposed; CS – Cigarette smoke exposed group; SCS – SRBSC exposed group.

Discussion

We aimed to analyze the alveolar walls destruction degree, comparatively, in the two smoke exposed groups and applied the Weibel–Gomez [11] principle to the 200× FOV digital images. The systematic scanning of the histopathological slides and the digital images analysis permitted us the systematical, unbiased study of a great number of FOVs of each smoke exposed group.

The enlarged alveolar spaces have been identified according to the morphological criteria: at least two alveolar walls defects, presence of the alveolar walls blunts, so called “drummer stick”, enlarged aerial spaces with abnormal morphology or classical emphysematous aspects [12].

The consistent alveolar destruction in smoke exposed rats somewhat surprised us considering the literature data [6] suggest that the rats are the most emphysema resistant animals.

Pulmonary alveoli have sacs like form and open in the alveolar duct. The alveolar wall, also named alveolar septa, is discontinuous being perforated by numerous pores of Kohn. It consists of capillary endothelium lining the intertwining network of anastomosing capillaries, basement membrane and surrounding interstitial tissue separating the endothelial cells from the alveolar lining epithelial cells, alveolar epithelium. Loosely attached to the epithelial cells or lying free within the alveolar spaces are the alveolar macrophages. The alveolar epithelium comprises a continuous layer of two principal cell types: flattened, plate-like pavement type I pneumocytes (membranous pneumocytes) and rounded type II pneumocytes. The alveolar epithelium lays on the basement membrane, which is continuous, thin, and evident only in electronic microscopy. In thin portions of the alveolar septum, the basement membranes of epithelium and endothelium are fused, whereas in thicker portions they are separated by an interstitial space (the pulmonary interstitium) containing fine elastic fibers, small bundles of collagen, a few fibroblast-like interstitial cells, smooth muscle cells, mast cells, and rarely lymphocytes and monocytes [13].
Analyzing data we note that the control group presents medium 60 alveoli per 200× FOV (59.93 ± 28.16), all of them typical; CS group presents 6.08 enlarged alveolar spaces per 200× FOV in 20.59 ±11.53 medium total number of alveoli per 200× FOV, representing 29%. For the SCS group, medium number of enlarged alveolar spaces per 200× FOV is 5.22, representing 24.39% of medium total alveoli number per 200× FOV (21.40 ± 8.63).

Emphysema contributes to airflow limitation through parenchyma elastic recoil decrease due to parenchyma destruction and alveoli dimension increase, and also through aerial waves applied elastic forces decrease because of the bronchiole-alveolar attachment destruction. Although there are increasing evidences of large airways inflammation involvement in COPD patients, this does not directly contribute to airflow limitation. Many studies demonstrated that the bronchiole-alveolar attachment destruction degree is major and positive correlated with the peripheral airways inflammation intensity in cigarette smokers. It is possible that the inflammatory mediators secreted by the inflammatory cells situated in the bronchiolar walls to interact with alveolar tissue especially in the bronchiolo-alveolar junction point where the mechanical stress is probably maximum. The significance of this aspect as responsible mechanism for the airflow limitation is hold up by the fact that the alveolar attachment loss degree correlates with peripheral airways inflammation and mainly with the lung elastic recoil decrease and bronchial obstruction degree in cigarette smokers. Obviously progressive airflow limitation in COPD results from the complex interaction between emphysema and airways anomalies, not only by totaling up [14–17].

We found out the presence of bronchiole-alveolar attachment destruction in both CS and SCS groups, less frequent in SCS group. This histopathological aspect together with pulmonary parenchyma emphysematous destruction, airways inflammatory infiltrate and smooth muscle hypertrophy represent the morphological support of the airflow decrease.

As it was expected, there were no alveolar blunts identified in control group. The irrelevant difference between the two smoke exposed groups concerning the medium values of alveolar blunts per 200× FOV and the close values of the standard deviation indicate the homogenous distribution of the alveolar blunts in both smoke exposed groups.

This aspect surprised us considering the statistical significant differences perceived analyzing the number of the emphysematous enlarged alveolar spaces and the normal alveoli number on the 200× FOV. Under those circumstances, number and the distribution of alveolar blunts on 200× FOV denote the advanced phase of evolution of the emphysematous destruction in CS group, close followed by the SCS group.

Elastin fibers amount and alveolar area have been obtained same results using 1981 Weibel’s “test points for the second type pneumocytes absence (Figure 2).
The attributed score varied from 0 to 3, 3 representing no nucleus visible; these having 3 score alveolar walls segments are going to be destroyed.

Comparing the three studied groups resulted that 9% of assessed probes presented score 3 in CS group while 25.5% of probes in SCS group were in the same situation; score 2, with rare nuclei, was present with frequency almost equal in the two smoke exposed groups, 29.9% in CS group, respectively 28.1% in SCS group; score 1 (medium number of nuclei), presents the highest frequency in SCS group, 19.9% and, finally, score 0 is registered in 100% probes in control group, 51% in CS group, respectively in 26.5% probes in SCS group.

One can observe that the medium score was significantly higher in both smoke exposed groups comparing control group, respectively in SCS group comparing with CS group.

The “hypo-/acellular” aspect of the alveolar wall, in fact hypo-/anuclear aspect, denotes these segments consist of type I pneumocytes exclusively. The “hypercellular” aspect, in fact hypernucleolated aspect is due to the presence of both types of pneumocytes.

Type I pneumocytes (membranous pneumocytes) are thin, flattened cells, difficult to be observed in optical microscopy. They present cytoplasmic prolongations that may traverse the pores of Kohn, and pass from an alveolus to another and connect with neighboring type I pneumocytes. The type II pneumocytes (gobular or granular) are cubical, large in size cells, in contact with basement membrane through their basal pole that are prominent into the pulmonary alveoli lumen. The type II pneumocytes are involved in the repair of the alveolar epithelium after the destruction of type I pneumocytes and control the alveolar inflammatory activity through the anti-inflammatory cytokines and antioxidants (superoxid dismutase, nitric oxide) that is induced by the proinflammatory cytokines, γ-Interferon and TNF-α [13, 19].

Cytodinamic studies revealed that under the normal circumstances the type II pneumocytes turnover takes place in 28–35 days.

Absence of type II pneumocytes denotes the destruction candidate status of the respective alveolar walls’ segments (Figure 16).

There is no fibrosis in the emphysematous enlarged alveolar spaces areas in both pollutants exposed groups (Figure 17).

![Figure 16 – (a) CS; (b) SCS. HE, 200× (CS – Cigarette smoke exposed group; SCS – SRBSC exposed group)](image-url)
Conclusions

We have established a rat COPD model based on natural cigarette smoke exposure versus solid combustible burn resulted smoke, usable for a further approach in human non-smoker COPD investigation. Our procedures resulted in clear pulmonary morphological lesions that are characteristic for COPD.

CS (rat cigarette smoke exposed) and SCS (rat solid combustible smoke exposed) groups have alveolar enlarged spaces (weight between 24 and 30% of the total alveoli number per 200× FOV) referred to non-exposed individuals. Even if the total alveoli number per 200× FOV counts did not differ for the two smoke exposed groups, the medium number of enlarged alveolar spaces per 200× FOV and the alveolar destructive index registered powerful statistical differences.

These data support the idea that solid combustible burn resulted smoke determines emphysematous parenchyma lesions that are similar, but with an attenuated morphological appearance when comparing to the cigarette smoke exposure.

References


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