PASSIVE EXPOSURE OF GUINEA PIGS TO CIGARETTE SMOKE INDUCES A SYSTEMIC ENDOTHELIAL DYSFUNCTION

LAVINIA NOVEANU[a], OVIDIU FIRĂ-MLĂDINESCU[b], VALENTIN ORDODI[a], FLORINA MIREA[a], GERMAINE SĂVOIU[b], DANINA MUNTEAN[b] and GEORGETA MIHALAŞ[a]

[a]University of Medicine and Pharmacy "Victor Babes" Timişoara, Department of Physiology
E – mail: lavinianoveanu@yahoo.com
[b]University of Medicine and Pharmacy "Victor Babes" Timişoara, Department of Physiopathology
P-ţa Eftimie Murgu nr.2, 300041, Timişoara, România

Received June 10, 2008

Systemic inflammation associated with chronic obstructive pulmonary disease (COPD) is a risk factor for the development of cardiovascular events. The aim of the present study performed in Guinea pigs chronically exposed to cigarette smoke was to assess the reactivity of rings of pulmonary artery and thoracic aorta isolated in organ bath. Twenty animals were randomly assigned to a control group (n = 10) and another one passively exposed to cigarette smoke for 12 weeks (n = 10). At the end of cigarette smoke exposure period significant decrease of vasodilator endothelial-dependent response to acetylcholine was found both in pulmonary artery (E_{max}% = 4.53 ± 3.09 *vs*. 19.28, $p < 0.0001$; EC_{50} –Log[M] = 6.45 ± 0.24 *vs*. 5.42 ± 2.24, $p < 0.0001$), and thoracic aorta (E_{max}% = 10.53 ± 5.69 *vs*. 19.28; $p < 0.001$, EC_{50} –Log[M] = 6.84 ± 1.72 *vs.* 5.06 ± 1.02, $p < 0.001$), whereas the vasodilator endothelial-independent response to sodium nitroprusside was not changed. The alteration of vasodilator endothelial-dependent response was associated with significant inflammatory changes in lungs and vascular walls. In conclusion, the experimental model we propose has proved viable for showing the systemic inflammatory response induced by passive exposure of guinea pigs to cigarette smoke.

Key words: Cigarette smoking; Inflammation; COPD; Endothelial dysfunction.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a condition characterized by chronic airways and lung inflammation and remodeling resulting in expiratory airflow obstruction, hyperinflation of lungs with loss of elastic recoil, and, ultimately, disturbances of gas exchange[1]. Although cigarette smoking is the most important cause of COPD, air pollution, chronic lung infection, and genetic factors are also considered potential contributors.

Epidemiologic studies have shown that COPD is associated with cardiovascular morbidity and mortality, even after taking into account smoking history[2,3]. The Lung Health Study reported that 10% decrement in lung function (FEV_1) among patients in the risk of deaths from cardiovascular diseases that include tromboembolic disease, arrythmias, heart failure, stroke, and sudden death[4]. The mechanisms whereby COPD adversely affects the cardiovascular system are not known, but the low–grade systemic inflammatory response associated with COPD may contribute to the atherotrombotic cardiovascular disease in these patients[1-4].

There is a huge body of experimental evidence describing the deleterious effects of cigarette smoke exposure effects on pulmonary and cardiovascular function in various animal species[5,6].

One of the frequently used animal species is the Guinea pig view several similarities with humans in

terms of the mechanisms involved in COPD development[7,8]. Accordingly, the present study performed in rings of pulmonary artery and thoracic aorta isolated from Guinea pigs chronically exposed to cigarette smoke was aimed at assessing the functional changes occurring on at the end of 12 weeks of exposure, assuming that pulmonary inflammation is responsible not only for the lung disease but also for the systemic endothelial dysfunction.

MATERIAL AND METHODS

Study groups

Twenty female Guinea pigs of 4-8 weeks age and 250–300 g weight were randomly assigned into 2 groups: controls (n = 10) and "smokers" which were passively exposed to cigarette smoke (n = 10). At the end of the 12 weeks exposure period the animals were anesthetized by intra-peritoneal administration of 50 mg/kg body weight sodium thiopental and sacrificed by cervical dislocation. Pulmonary artery and thoracic aorta rings were harvested and subjected to: (i) functional studies of vascular reactivity within the organ bath and (ii) structural studies together with pulmonary resection fragments. The latter pathological studies were performed after fixation in 10% formaldehyde, wax inclusion followed by 4-5 µ serial section (4-5 sections/animal) and staining with hematoxyline-eosine (standard technique).

Protocol of cigarette smoke exposure

Guinea pigs were placed in a closed 0.56 m^3 glass box. The ceiling of the box was the "smoking device" consisting of a vacuum tube attached to a micro compressor (Fig. 1). The air flow generated by "smoking device" developed a pressure below the atmospheric one thus allowing the "smoking" of the cigarette attached to the system at the exterior side while directing the main stream smoke inside the exposure box. "Smoking" of a filter cigarette (tar 0.8 mg/cigarette and nicotine 0.6 mg/cigarette) is taking 5 minutes and was followed by a 20-minute period of continuous exposure to the smoke generated by this cigarette. Three cigarettes/hour, twice a day (morning and evening) were "smoked" with a total duration of exposure of 12 weeks.

Fig. 1. Smoke exposure box and cigarette "smoking device".

Organ bath studies

Vascular rings were introduced in 10 ml volume 2 organ baths, containing Tyrode solution with the following composition: NaCl 149.2 mM, KCl 2.7 mM, $NaHCO_3$ 11.9 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, NaH_2PO_4 0.4 mM and glucose 5.5, mM. For vascular reactivity study we used a FORT 10 model isometric force transducer (World Precision Instruments, WPI Inc.), connected to a data acquisition unit in computerized system BIOPAC MP100. Data interpretation and graphical render was performed using "AQKNOWLEDGE" 3.72 software.

Pure reagents provided by Sigma Chemicals Co. were used for vascular reactivity testing: (R)-(-)-Phenylephrine hydrochloride (PE), acetylcholine (ACh), indomethacin, and sodium nitroprusside dihydrate (SNP).

Isolated pulmonary artery and thoracic aorta rings pre-tensioned at 1.5 g force were equilibrated for one hour with the replacement of the Tyrode solution within the organ bath every 15 min.

Viability of smooth muscle preparations was primarily tested by obtaining 2 similar contractile responses to KCl 70 mmol/l. Subsequently, endothelial viability was assessed by measuring the vasodilator response to acetylcholine 10^{-5}M. A vasodilator response higher than 10-15% of the tension developed by KCl 70 mmol/l was found in normal preparations. The rings were washed and further incubated for 30 minutes in presence of 10^{-5} M indomethacin added to the organ bath for the entire duration of experiment.

Vasoconstrictor response was tested in presence of phenylephrine (PE) 10^{-5}M, until a stable plateau of the developed tension was obtained and expressed as absolute values (cN). Endothelial-dependent vasodilator response was assessed using cumulative doses of ACh 10^{-9} M – 10^{-4} M (Fig. 2).

Vasodilator response was expressed as percentage value of absolute pre-contraction values obtained to PE 10^{-5}M.

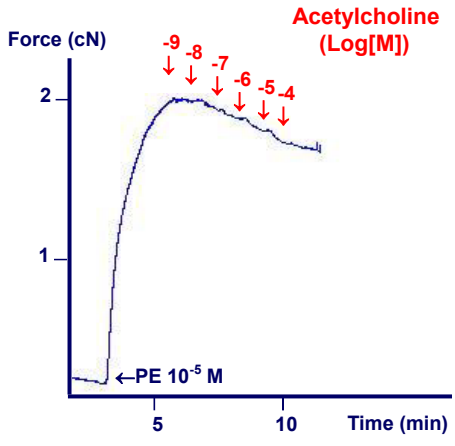

Fig. 2. Assessment of endothelium-dependent vasodilator response to ACh.

The endothelial-independent relaxing response was assessed using cumulative doses of SNP from 10^{-9} M – 10^{-4} M (Fig. 3).

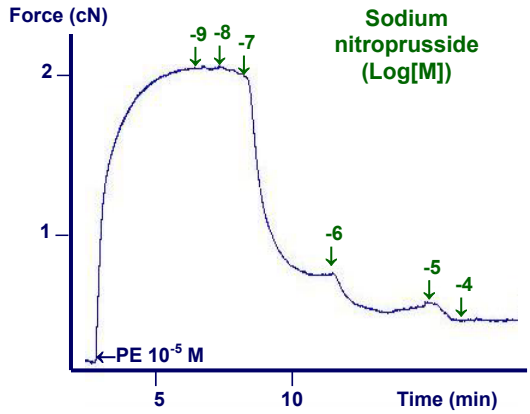

Fig. 3. Assessment of endothelium-independent vasodilator response to SNP.

Statistic analysis of data

After data acquisition, they were statistically analyzed using Excel Microsoft Office 2003 (Microsoft Corporation) and GraphPad Prism 4 (GraphPad Software, SUA) software.

Central tendencies of the variables obtained from *n* different rings were expressed as mean, while the dispersion ones as standard error (SE). The sigmoid Hill equation with 4 parameters was used to obtain a global evaluation of the vasomotor effect induced by different concentrations of vasodilator substances. Two parameters derived from the Hill equation were used: E_{max}(%) the percentage value of maximum vasodilator response, and EC_{50} (-Log[M]) the concentration of de vasodilator substances required for obtaining a 50% of maximum vasodilator response. The F test was used in order to compare dose – response curves estimated by the Hill equation and the "t" test was used comparison of the means. A value of $p < 0.05$ was considered of statistical significance.

RESULTS

The results obtained were assessed from the perspective of both changes of vascular reactivity, revealed within organ bath studies, as well as morphological anomalies identified at the level of lungs, pulmonary artery and thoracic aorta.

Vascular reactivity in organ bath

In order to assess the vascular reactivity the dose–response curves were compared using the Hill's parameters (E_{max}(%) and EC_{50}-Log[M] derived from the Hill equation). A significant reduction for the endothelial–dependent response to ACh was found in rings isolated from both in pulmonary artery (Table 1) and thoracic aorta (Table 2).

Table 1

Vascular reactivity of pulmonary artery rings estimated by Hill's parameters

Endothelial-dependent vasodilator response to acetylcholine		
Groups	**E_{max} (%)**	**EC_{50} (-Log[M])**
Control	19.28 ± 1.94	6.45 ± 0.24
Passive smoking	4.53 ± 3.09**	5.42 ± 2.24**
Endothelial-dependent vasodilator response to sodium nitroprusside		
Groups	**E_{max} (%)**	**EC_{50} (-Log[M])**
Control	98.81 ± 18.23	7.37 ± 0.07
Passive smoking	95.85 ± 13.45	6.08 ± 0.15

Note: results are expressed as mean ± SE; **p < 0.0001

Table 2

Vascular reactivity of thoracic artery rings estimated by Hill's parameters

Endothelial-dependent vasodilator response to acetylcholine		
Groups	**E_{max} (%)**	**EC_{50} (-Log[M])**
Control	19.28 ± 1.94	6.45 ± 0.24
Passive smoking	4.53 ± 3.09*	5.42 ± 2.24*
Endothelial-dependent vasodilator response to sodium nitroprusside		
Groups	**E_{max} (%)**	**EC_{50} (-Log[M])**
Control	97.88 ± 22.25	7.25 ± 0.72
Passive smoking	94.42 ± 14.37	7.76 ± 0.50

Note: Results are expressed as mean ± SE; *p < 0.001

The results of dose-dependent reactivity of pulmonary artery and thoracic aorta rings are presented in Table 3. The values of preconstriction with PE were not significantly different between groups. The endothelial – dependent relaxation to ACh was significantly reduced for all doses we used, both in pulmonary artery (Fig. 4) and thoracic aorta (Fig. 5). The endothelial – independent relaxation to SNP was approximately maximally in all groups.

No significant differences of endothelial – independent relaxation to SNP were observed between groups (Fig. 6 and Fig. 7).

The impairment of endothelial-dependent vasodilator response to ACh in the presence of a normal of endothelial-independent vasodilator response to SNP represents the hallmark of endothelial dysfunction induced by the passive exposure of Guinea pigs to cigarette smoke.

Table 3

Dose-dependent reactivity of pulmonary artery and thoracic aorta rings

Cumulative doses	Pulmonary artery			Thoracic aorta		
	Control group	Passive smoking group	p	Control group	Passive smoking group	p
Precontraction induced by PE (cN)						
10^{-5}M	1.28 ± 0.29	1,50 ± 0.63	0.33	1.28 ± 0.24	1.47 ± 0.50	0.36
Endothelial dependent relaxation induced by cumulative doses of ACh (%)						
10^{-9}M	3.95 ± 1.53	1.33 ± 0.27	< 0.001	0	0	
10^{-8}M	6.45 ± 3.04	2.90 ± 1.15	< 0.001	1.24 ± 0.39	0.66 ± 0.36	< 0.01
10^{-7}M	12.98 ± 9.67	4.05 ±1.41	< 0.001	5.27 ± 2.42	1.02 ± 0.98	< 0.01
10^{-6}M	27.28 ± 16.01	7.78 ± 2.76	< 0.001	13.02 ± 6.08	3.22 ± 1.43	< 0.01
10^{-5}M	39.77 ± 16.60	9.83 ± 4.36	< 0.001	19.02 ± 9.04	3.71 ± 2.81	< 0.01
10^{-4}M	40.99 ± 12.52	9.48 ± 1.10	< 0.001	18.38 ± 8.99	4.63 ± 2.47	< 0.01
Endothelial dependent relaxation induced by cumulative doses of SNP (%)						
10^{-9}M	3.89 ± 1.86	4.23 ± 1.31	0.55	0.56 ± 0.02	6.32 ± 1.97	0.44
10^{-8}M	30.40 ± 19.57	33.19 ± 5.23	0.56	24.59 ± 14.80	16.61 ± 0.89	0.51
10^{-7}M	66.16 ± 13.37	75.73 ± 11.3	0.16	66.02 ± 10.64	51.27 ± 19.66	0.32
10^{-6}M	80.02 ± 15.73	90.63 ± 6.20	0.08	91.90 ± 5.86	84.74 ± 18.03	0.08
10^{-5}M	94.38 ± 9.35	93.64 ± 5.99	0.56	99.72 ± 9.77	93.41 ± 6.93	0.08
10^{-4}M	96.38 ± 5.62	91.65 ± 5.62	0.10	100	100	

Note: Results are expressed as mean ± SE. PE: phenylephrine; ACh: acetylcholine; SNP: sodium nitroprusside.

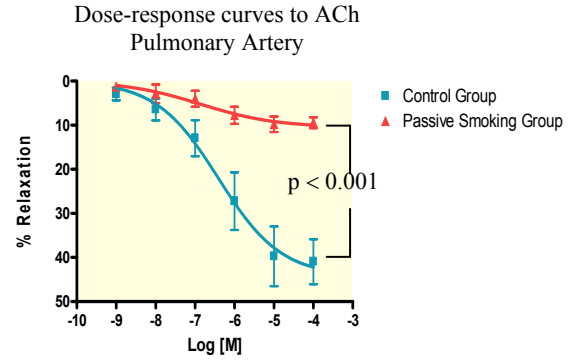

Fig. 4. Dose-response curves to ACh derived from sigmoid Hill equation, for pulmonary artery.

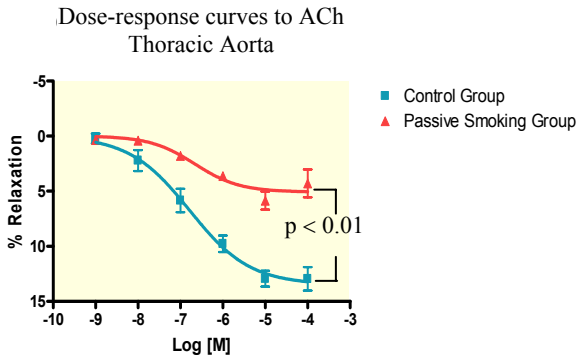

Fig. 5. Dose-response curves to ACh derived from sigmoid Hill equation, for thoracic aorta.

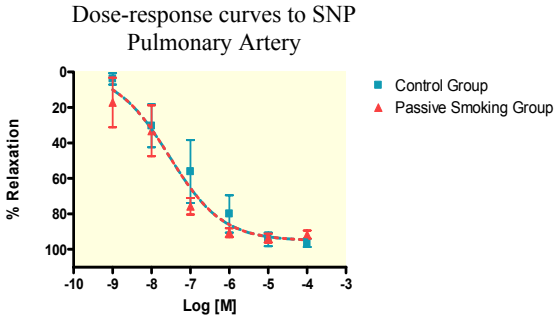

Fig. 6. Dose-response curves to SNP derived from sigmoid Hill equation, for pulmonary artery.

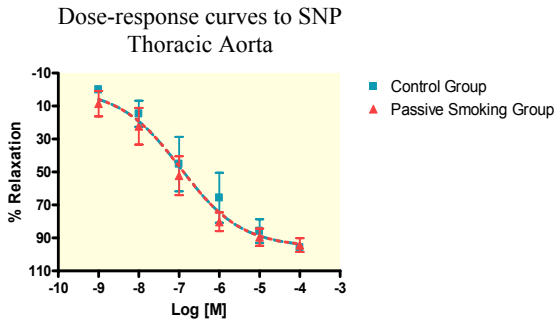

Fig. 7. Dose-response curves to SNP derived from sigmoid Hill equation, for thoracic aorta.

Morphological changes

Morphologic examination of lungs identified the presence of an inflammatory infiltrate of the pulmonary vascular wall and the development of characteristic lesions for pulmonary emphysema. At the end of the exposure period a large inflammatory infiltrate surrounding the adventitia significant for lesions of pulmonary vasculitis (Fig. 8) together with important thickening of vascular walls were found (Fig. 9).

The morphological changes described in both pulmonary artery and thoracic aorta were rather discrete and consisted of inflammatory thickening of vascular adventitia and media occurring together with areas of media mucoid degeneration (Fig. 10). Moreover, inflammatory thickening of vascular walls contained by vasa vasorum were identified (Fig. 11).

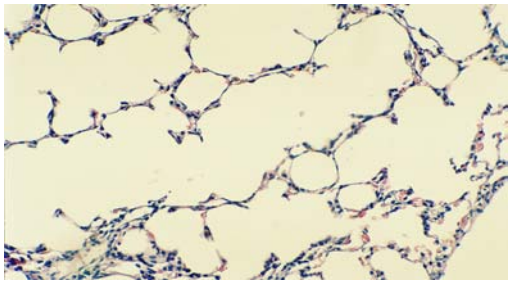

Fig. 8. *Lungs section*: Panacinous pulmonary emphysema; thin or ruptured alveolar septum (HE × 400).

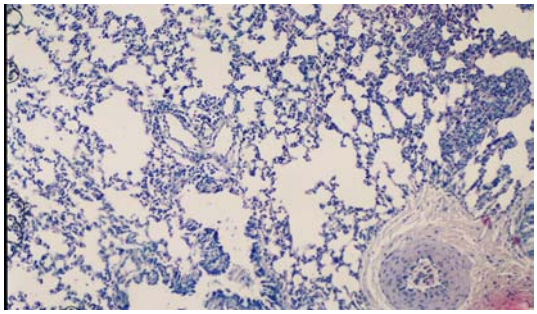

Fig. 9. *Lungs section*: emphysema areas combined with colapsed alveoli, thick walls vessels (HE × 100).

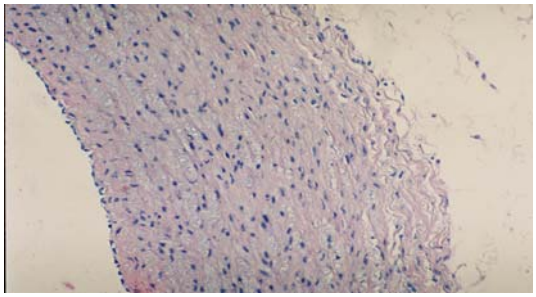

Fig. 10. *Aorta section*: thickened media and mucoid degeneration areas (HE × 400).

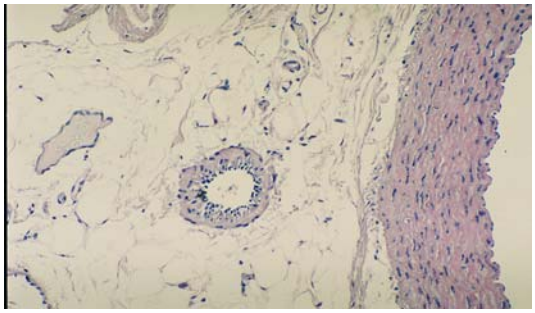

Fig. 11. *Pulmonary artery section*: thickened adventice, lax aspect, edema, including arterioles having thickened walls (HE × 200).

DISCUSSIONS

We have shown that chronic exposure to cigarette smoke in Guinea pigs induced not only a local inflammatory response of pulmonary vasculature but also an important degree of systemic inflammation in aortic wall suggestive for the occurrence of systemic endothelial dysfunction. This supports the hypothesis that exposure to cigarette smoke or to ambient particulate matter triggers a pulmonary inflammation that may be responsible for the exacerbations of COPD and asthma, and also may initiate the vascular effects in COPD[1,9,10]. Van Eeden *et al.* (2005) proposed a mechanism for the lung inflammation induced vascular disease. Inhaled particles of cigarette smoke are processed by alveolar macrophages and lung epithelial cells. These cells produce proinflammatory mediators such as cytokines (IL-6, IL-1, TNF-alpha, GM-CSF) that promote a local inflammatory response contributing to the exacerbation of COPD and induce a systemic inflammatory response. This response includes stimulation of the bone marrow to release leukocytes and platelets, activation of the clotting factors, and activation of endothelial cells' response.

According to this theory, in early phases, these endothelial effects could be responsible for the onset of endothelial dysfunction, which was also demonstrated in our study. However, in later stages, chronic inflammatory response underlying the COPD evolution could induce destabilization of atherosclerotic plaques, thus developing an acute cardiovascular event, such as coronary artery thrombosis, arrhythmia or cardiac failure[1-4].

We must underline the fact that histological studies showed that Guinea pigs exposure to cigarette smoke relatively rapidly induced the occurrence of pulmonary emphysema. There is

consistent experimental evidence suggesting that repetitive exposure of guinea pigs to cigarette smoke triggers pulmonary accumulation of macrophages and neutrophils[11], increases production of pro-inflammatory cytokines[12], but also increases proteases activity, such as serin-elastase[13] and interstitial collagenase[11], which can induce in their turn alterations of pulmonary structure and increase air flow resistance[7].

Moreover, many authors consider that inflammatory changes revealed in Guinea pigs at the pulmonary level, after exposure to cigarette smoke, are similar with those noticed in human smoker subjects with or without COPD[11-13].

Literature data suggest there is a basic degree of similarity among a wide variety of animal models of cigarette smoke – induced lung disease[8], although the severity and the types of abnormalities described are different according to the length of exposure time and protocols used to induce pulmonary lesions[7,8,14].

Finally, there appear to be species differences and strain differences that must be taken into account when selecting an appropriate model. For instance, it appears that Guinea pigs will acquire lung parenchyma and vascular alterations with a relatively short period of smoke exposure that are not found in other animal models within the same delay of time.

CONCLUSIONS

Our results suggest that in Guinea pigs chronic exposure to cigarette smoke induced a series of inflammatory events focused on lungs. Pulmonary inflammation and inflammatory changes at the level of a systemic artery vascular wall were associated with statistically significant endothelial dysfunction, which was already present at the end of cigarette smoke exposure period.

Data emerging from this study also suggest that this experimental model can be considered a useful tool for assessment of pathogenic mechanisms involved in association of COPD with athero-sclerotic disease. However, each animal model has its own strengths and weaknesses, and further investigations in other species are required in order to obtain consistent results which might be extrapolated in humans.

Several epidemiologic studies showed the relationship between COPD and increased mortality and morbidity by cardiovascular disease. Pathophysiological mechanisms responsible for this association are numerous and partially elucidated so far. However, a key role is attributed to pulmonary inflammation that may further induce a systemic inflammation, which in turn is responsible for endothelium functional changes. Accordingly, the vascular atherosclerotic changes may be further aggravated with the evolution of COPD.

REFERENCES

1. van Eeden S.F., Yeung A., Quinlam K., Hogg J.C., *Systemic response to ambient particulate matter. Relevance to chronic obstructive pulmonary disease, Proc Am Thorac Soc,* **2005**, 2, 61–67.
2. Mishaud C.M., Murray C.J., Bloom B.R., *Burden of disease - implications for future research, JAMA,* **2001**, 285, 535–539.
3. Coultas D.B., Mapel D., Gagnon R., Lydick E., *The health impact of undiagnosed airflow obstruction in a national sample of United States adults, Am J Respir Crit Care Med,* **2001**, 64, 372–377.
4. Anthonisen N.R., Connett J.E., Kiley J.P., *Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of the decline of FEV1: the Lung Health Study, JAMA* **1994**, 272, 1497–1505.
5. March T.H., Green F.H., Hahn F.F., Nikula K.J., *Animal models of emphysema and their relevance to studies of particle – induced disease, Inhal Toxico,l* **2000**; 12(suppl.4), 155–187.
6. Shapiro S.D., *Animal models for COPD*, *Chest,* **2000**, 121 (Suppl.5), 223S-227S.
7. Wright J.L., Churg A., *Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig, Am Rew Respir Dis,* **1990**, 142, 1422–1428.
8. Wright J.L., Churg A., *A model of tobacco smoke – induced airflow obstruction in the guinea pig, Chest,* **2002**, 121, (Suppl.5), 188S–191S.
9. Seaton A., MacNee W., Donald K., *Particulate air pollution and acute healts effects, Lancet,* **1995**, 345, 176–178.
10. Sunyer J., Basagana X., *Particles, and not gases, are associated with the risk of death in patient with chronic obstructive pulmonary disease, Int J Epidemiol,* **2001**, 30, 1138–1140.
11. Selman M., Montano M., Ramos C., *Tobacco smoke – induced lung emphysema in guinea pigs is associated with increased interstitial collagenase, Am J Physiol,* **1996**; 271: L734–743.
12. Kubo S., Kobayashi M., Masunaga Y., Ishii H., Hirano Y., Takahashi K., Shimizu Y., *Cytokine and chemokine expression in cigarette smoke – induced lung injury in guinea pigs, Eur Respi,r* **2005** 26: 993–1001.
13. Wright J.L., Farmer S.G., Churg A., *Synthetic serine elastase inhibitor reduces cigarette smoke – induced emphysema in guinea pigs, Am J Respir Crit Care Med,* **2002**; 166: 954–960.
14. Wright J.L., *The importance of ultramicroscopic emphysema in cigarette smoke induced lung disease, Lung,* **2001**, 179, 71–81.