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EXPERIMENTAL STUDY REGARDING THE INFLUENCE OF PARTICULATE AIR POLLUTANTS ON OXIDANTS/ANTIOXIDANTS BALANCE

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Abstract: Our study intends to explore the changes of oxidant/antioxidant (O/AO) balance generated by the exposure to particulate air pollutants (bentonite). Experiments were performed on white, male, Wistar rats, having an average weight about 150±20 g, divided in 6 experimental groups, as follows: C group (n = 10) – control group, without exposure to bentonite particle, housed in normal environmental conditions; 1st group (n = 10) – acute exposure (a single intranasal inoculation); 2nd group (n = 10) – acute exposure (a single intratracheal inoculation); 3rd group (n = 10) – chronic intranasal exposure (7 exposures); 4th group (n = 10) – chronic intranasal exposure (7 exposures), associated to vitamin E administration (7 intra peritoneal administrations), in order to evaluate the antioxidant effect of this product; 5th group (n = 10) – chronic exposure by ventilation (28 days). Acute exposure to air pollutants produced: an increase in malondialdehyde (MDA) level in serum for both groups, values being significantly higher for the 1st group as compared to control group; insignificant changes regarding carbonyl proteins (CP) level in serum for 1st group and a significant increase in the 2nd group; distinctly significant decrease in hydrogen donor (HD) ability of the serum for the 1st group and highly significant decrease for the 2nd group; highly significant decrease in thiol groups (SH) level in serum for both groups as compared to control group. Chronic exposure to air pollutants led to: a significant increase in MDA serum level regarding the 4th and the 5th group, and a distinct significant increase for the 3rd group as compared to control group; highly significant increase in CP serum level for all of three groups; highly significant decrease of the HD ability of the serum for the 3rd group and distinctly significant decrease in the 4th group and significant increase in the 5th group; insignificant decrease in SH groups serum level in the 3rd group, significant decrease in the 4th group and a distinctly significant increase for in the 5th group as compared to control group. Conclusions: 1. Particulate air pollutants caused O/AO balance disturbances, being unfavorable to AO. 2. Acute exposure determined an increase in oxidative stress parameters, especially in the group with intratracheal inoculation and a significant decrease in AO defense. 3. Chronic exposure led to a significant increase in oxidative stress markers and to a decrease in AO defense for all groups, excepting the group exposed by ventilation. 4. Chronic intranasal exposure to air pollutants associated to vitamin E administration caused different changes of the O/AO balance, namely increases in MDA and CP levels and decreases of HD and SH groups. These findings suggest the pro-oxidant effect of vitamin E in the dosage used in our experiment.

INTRODUCTION

Air pollution means the presence in atmosphere of certain substances which, depending on their concentration and/or their action time, generates changes of health state or alters the environment. These substances may differ from those in the normal air composition or may be usual compounds, such as ozone, carbon dioxide, radon, etc.

The most spread air pollutants are the irritant ones, generated by numerous pollution sources in present-day living environment. They include irritant gases (ozone, nitrogen dioxide, nitrogen oxides, sulfur dioxide, and sulfur oxides) and suspensions (particulate matter – PM).

In the last years, environmental air pollution reached alarming levels in industrialized countries. There is incontestable evidence which certifies wide implication of powders, nitrogen and sulfur oxides, combustion, and smoke gases in respiratory pathology. Recent epidemiological studies have shown a consistent association between ambient levels of inhalable particles (PM 10) and exacerbation of respiratory diseases as well as cardio-pulmonary morbidity and adult mortality, particularly in individuals with pre-existing disease. It has been proposed that the possible biological mechanisms of action of PM could be related to its radical activity and the induction of oxidative stress and lung inflammation (Balduzzi M 2003, Tao F 2003, Gonzales-Flecha B 2004). The physical characteristics and the chemical composition of PM play a key role in reactive oxygen species generation in vitro and in vivo (Xia T 2006).

Our study intends to follow the changes of oxidant/antioxidant (O/AO) balance generated by exposure to particulate air pollutants (bentonite).

MATERIAL AND METHODS

1. Experimental groups: Experiments were performed on white, male, Wistar rats, having an average weight about 150±20 g, divided in 6 experimental groups, as follows:

C group (n = 10) – control group, without exposure to bentonite particle, housed in normal environmental conditions

1st group (n = 10) – acute exposure (a single intranasal inoculation)

2nd group (n = 10) – acute exposure (a single intratracheal inoculation)

3rd group (n = 10) – chronic intranasal exposure (7 exposures)

4th group (n = 10) group – chronic intranasal exposure (7 exposures), associated to vitamin E administration (7 intra peritoneal administrations), in order to evaluate the antioxidant effect of this product (Tache S 2001)

5th group (n = 10) – chronic exposure by ventilation (28 days)

2. Methods:

Intranasal inoculation consisted in animals´ anesthesia, maintaining them in a vertical position, and instillation of the polluting agent (suspension of 500 µg bentonite in 100 µl physiological serum). The liquid is delivery in drops into the nostrils and the animal take over the whole quantity when inspire.

Intratracheal exposure consists in animals´ anesthesia and instillation of the particle suspension on the epiglottis surface, using a micropipette.

Exposure by ventilation was made in a special chamber where the bentonite powder is continuously recycled using a ventilator (axial type, 100 VKO, 105 m^3/hour, diameter 100 mm). Chamber volume is 0,023 m^3. The dose used for this type of exposure is 35 mg/m^3 of air/day. Dosage is made using a mechanism that release bentonite powder at certain periods. It is achieved the exposure of the entire body to the polluting agent.

Exposure to particulate air pollutants was made by the method used in the Toxicology Laboratory of the Faculty of Veterinary Medicine.

Exposure schedule was acute for the 1st and the 2nd group and chronic for the 3rd, 4th and 5th group.

Assessment of oxidants/antioxidants (O/AO) balance was performed in the Laboratory for Oxidative Stress Investigation at Physiology Department, University of Medicine and Pharmacy, Cluj-Napoca. After the exposure to air pollutants, venous blood was sampled from retroorbitar sinus and it was determined:

oxidative stress markers (***)

total malondialdehyde (MDA) by fluorimetric method (Conti method, 1991)
carbonyl proteins (CP) (Reznick method, 1994)
antioxidant activity (***)
hydrogen donor ability (HD) (Janazewska method, 2002)
content of total thiol groups (SH) (Hu method, 1994)

RESULTS

Acute exposure to air pollutants produced:

Increase in serical MDA level for both groups, values being significantly higher for the 1st group as compared to control group (figure 1).

Insignificant changes of CP in serum for the 1st group and significant increase in the 2nd group, as compared to control group (figure 2).

Distinctly significant decrease of the HD ability in serum for the 1st group and highly significant decrease for the 2nd group (figure 3).

Highly significant decrease of the SH in serum for both groups, as compared to control group (figure 4).

Chronic exposure to air pollutants determined:

Significant increase in serical MDA level for the 4th and the 5th groups, and distinct significant increase for the 3rd group, as compared to control group (figure 1).

Highly significant increase in serical CP level for all of the three groups, as compared to control group (figure 2).

Highly significant decrease of the HD ability in serum for the 3rd group and distinctly significant decrease for the 4th group; significant increase for the 5th group, as compared to control group (figure 3).

Insignificant decrease of SH groups in serum for the 3rd group, significant decrease for the 4th group and distinctly significant increase for the 5th group, as compared to control group (figure 4).

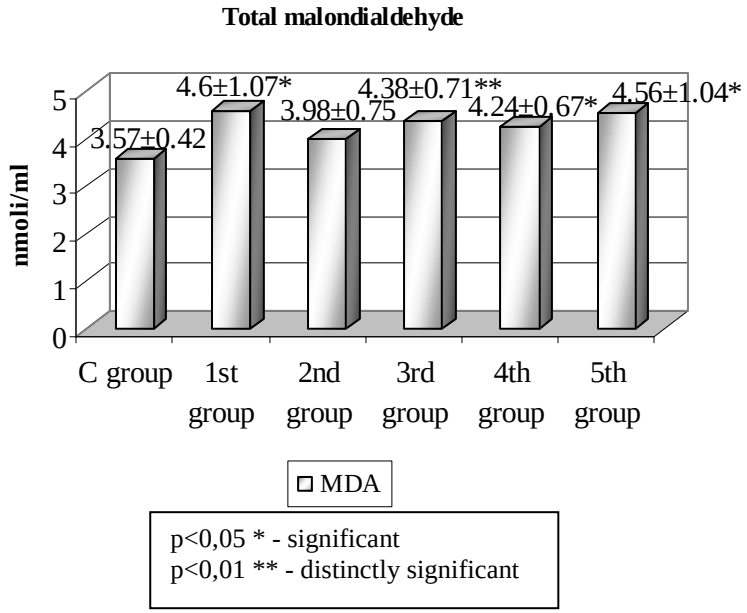

Figure 1. Total malondialdehyde values of the experimental groups

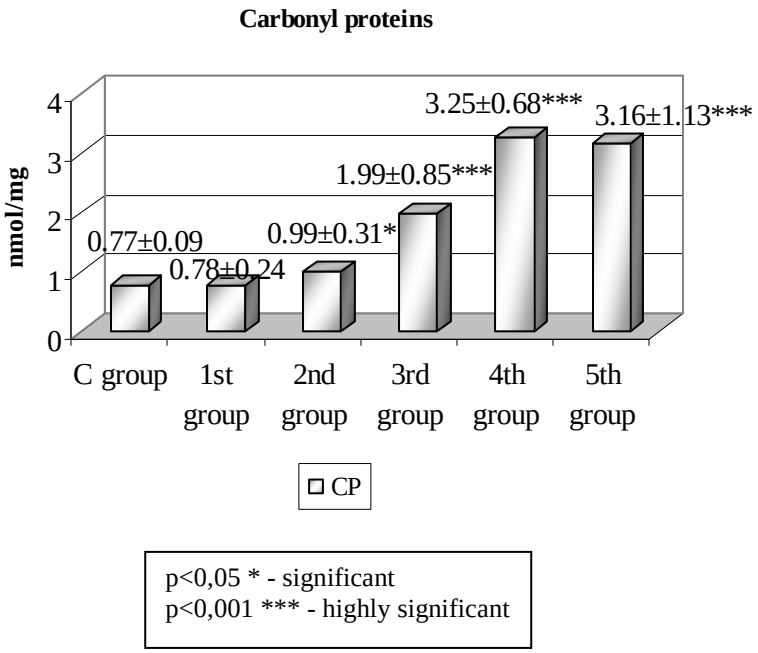

Figure 2. Carbonyl proteins values of the experimental groups

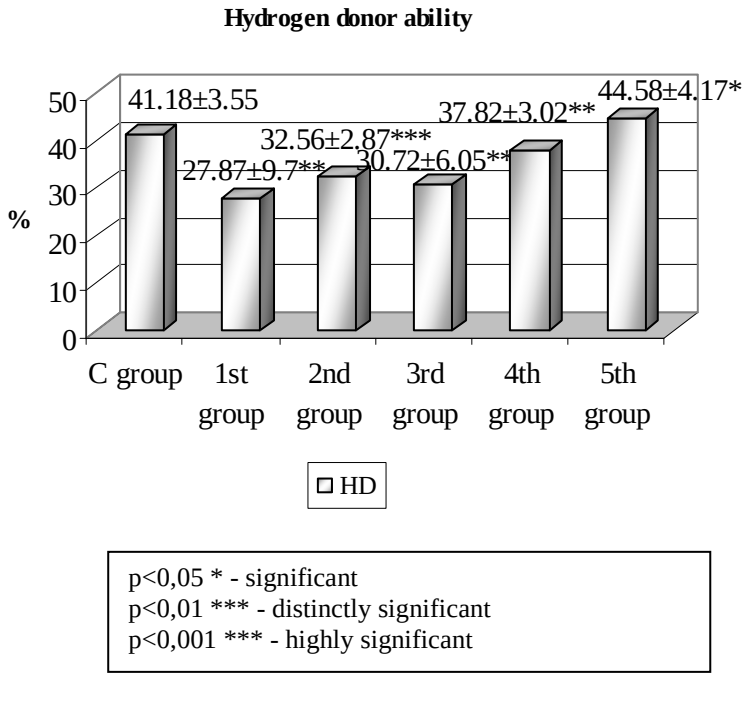

Figure 3. Hydrogen donor ability of the experimental groups

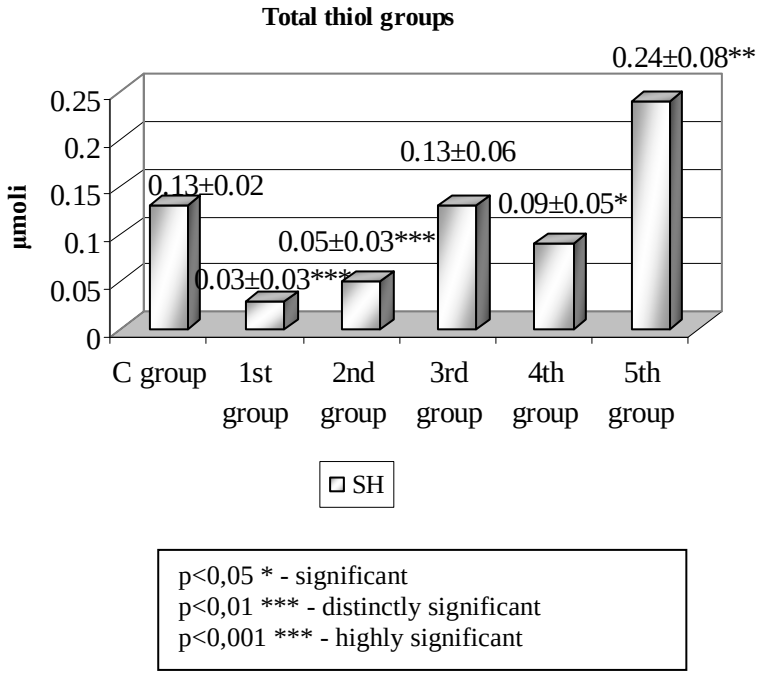

Figure 4. Total thiol groups of the experimental groups

DISCUSIONS

Total MDA shows the degree of lipid peroxidation; after exposure, the values were statistically significant or distinctly significant increased than those of the control group for all five experimental groups. These findings suggest an increase in lipid peroxidation in animals exposed to particulate air pollutants. Animals who received vitamin E registered MDA values close to those in the group exposed to the same air pollutants but without vitamin E administration, showing that vitamin E, in this dose, had no antioxidant effect.

CP represent the oxidized proteins; our results showed a highly significant increase in all experimental groups chronically exposed to bentonite powders, as compared to control group or to animals subjected to acute exposure. CP reached maximum levels following vitamin E administration, as compared to the other batches.

HD ability represents the antioxidant activity of the plasmatic compounds with small molecular weight, except the enzymes (vitamins, glutathione); it was highly decreased in animals following the acute or chronic exposure to powders, as compared to control group, except those exposed by ventilation, where the values were distinctly significant increased after exposure. Regarding the acute exposure, decreases were higher for exposure by intranasal inoculation, as compared to intratracheal administration. Vitamin E administration caused a decrease in antioxidant defense as compared to control group and a moderate increase as compared to the 3rd group.

SH groups explore the glutathione-type antioxidants; they decreased highly significant after exposure as compared to control group, for animals subjected to acute exposure to bentonite powders, the decrease being higher for intranasal inoculation comparing with the intratracheal administration. Also, decreases of SH groups were more pronounced in chronic exposure when intranasal inoculation was associated to vitamin E, comparing with the group subjected to the same exposure, but without vitamin E.

Our research is in accordance to literature data regarding the biochemical mechanism by which PM acts on the organism. The oxidative stress mediated by PM may arise from direct generation of reactive oxygen species from the surface of particles, soluble compounds such as transition metals or organic compounds, altered function of mitochondria or NADPH-oxidase, and activation of inflammatory cells capable of generating reactive species (Risom L 2005). Studies in cellular and animal models suggest a variety of possible mechanisms including direct effects of particle components on the intracellular sources of reactive oxygen species (ROS), indirect effects due to pro-inflammatory mediators released from PM-stimulated macrophages, and neural stimulation after particle deposition in the lungs (Gonzalez-Flecha B, 2004). PM contain redox-active chemicals and transition metals which generate ROS. Excessive ROS can induce oxidative stress, which proceeds in hierarchical fashion to generate cellular responses (Li N, 2006).

CONCLUSIONS

Particulate air pollutants cause disturbance of O/AO balance, unfavorable to AO.

Acute exposure causes an increase in oxidative stress markers, more pronounced in the group with intratracheal administration and a significant decrease in AO defense.

Chronic exposure causes a significant increase in oxidative stress markers and a decrease in AO defense for all experimental groups, excepting the group exposed by ventilation.

Chronic intranasal exposure to air pollutants associated to vitamin E administration causes different changes of the O/AO balance, namely increases of MDA and CP and decreases of HD and SH groups, which plead for the pro-oxidant effect of vitamin E in the dosage used in the experiment.

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