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Acute pulmonary and hematological effects of two types of particle surrogates are influenced by their elemental composition ☆

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Abstract

Several epidemiological studies have consistently demonstrated significant associations between ambient levels of particulate matter and lung injury and cardiovascular events with increased morbidity and mortality. Particle surrogates (PS), such as residual oil fly ash (ROFA), have been widely used in experimental studies aimed at characterizing the mechanisms of particle toxicity. Since PS composition varies depending on its source, studies with different types of PS may provide clues about the relative toxicity of the components generated by high-temperature combustion process. In this work, we have studied the effects of nasal instillation of increasing doses of different PS in mice: saline, carbon, and two types of particle surrogates. PS type A (PSA) was the ROFA collected from the waste incinerator of our university hospital; PS type B (PSB) was collected from the electrostatic precipitator of a large steel company and thus had an elevated metal content. After 24 h, we analyzed hematological parameters, fibrinogen, bronchoalveolar lavage, bone marrow, and pulmonary histology. Nasal instillation of the two types of PS-induced leucopenia. PSB elicited a greater elevation of plasma fibrinogen levels. Bone marrow and pulmonary inflammatory changes were more intense for PSA. We concluded that the PS composition modulates acute inflammatory changes more significantly than the mass for these two types of PS.

Keywords: ROFA; Air pollution; Lung; Plasma fibrinogen; Bone marrow

1. Introduction

Over the last several years, a series of epidemiologic studies has demonstrated a statistical association between ambient air pollution particulate matter and mortality/morbidity among the exposed human population (Costa and Dreher, 1997).

Respiratory and cardiovascular events are those more strongly associated with particle pollution (Donaldson et al., 2001; Zareba et al., 2001). Inhalation of ambient particles can provoke a significant amount of pulmonary inflammation (Pope, 2000; Souza, 1998), alterations of the heart autonomic function, and changes in blood parameters (Dockery, 1994; Schwartz, 2001). There are important gaps in both the scientific evidence of causation and the scientific basis for the regulatory response. The most important is the inability to explain how fine particles (with a diameter of less than 2.5 μm) affect health (Ware, 2000).

The biological effects of the inhaled particles are determined by their size and by their physiochemical properties (Dockery and Pope, 1994). The chemical composition and amount of organic compounds contained in the particles modify their toxicity (Pope, 2000). Toxicological studies using particles with different compositions contribute to our understanding of the pathogenesis of particle-dependent health effects.

☆ The protocol of this animal model was approved by the Ethics Committee of the School of Medicine and was conducted in accordance with national guidelines for animal protection.

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Several works have reported significant effects of air pollution on hematological parameters. These events may contribute to the pathogenesis of pollution-related heart diseases (Baskurt et al., 1990; Gardner and Lehmann, 2000; Ghio et al., 2002; Pritchard et al., 1996; Schwartz, 2001; Vincent et al., 2001).

Surprisingly, little is known about acute bone marrow responses to inhaled particles. Since the number of circulating blood cells depends on their rate of production by the marrow, as well as their withdrawing from circulation by sequestration in the microcirculation, diapedesis, or apoptosis, evaluations of the effects of inhaled particles on bone marrow should be made as well.

We performed studies in mice exposed to two particle surrogates of different compositions and simultaneous analyses of the pulmonary tissue, circulating blood, and bone marrow. The objective was to verify how these organic compartments respond to increasing concentrations of particles of known elemental composition.

2. Materials and methods

2.1. Experimental groups

Male BALBc mice that were 60 days old and weighed 20 to 30 g were obtained from the *vivarium* of our school of medicine at Universidade de São Paulo and housed under controlled humidity and temperature and filtered air for 5 days before the experiments. Eight groups of 10 mice were submitted to the intranasal instillation of 10 μL of the following test solutions: sterile saline; 10 μg of carbon particles in sterile saline; 0.1, 1.0, and 10.0 μg of particle surrogates (A and B) suspended in sterile saline. The protocol was approved by the ethics committee of our institution.

2.2. Characterization of particles

Particle surrogate A (PSA) represented residual oil fly ash (ROFA) collected from the solid waste incinerator of the University Hospital of the Universidade de São Paulo, which is powered by combustible oil. Particle surrogate B (PSB) consisted of particles retained in the electrostatic precipitator installed in one of the chimneys of a large steel plant in Brazil. For controlling purposes, we used sterile saline and high-purity carbon powder.

The element composition of PSA and PSB was determined by neutron activation analysis. Basically, the procedure involved short irradiations of 5 min for the determination of Cl, K, Mn, Na, and Sr using a pneumatic transfer system facility under a thermal neutron of $1.4 \times 10^{12}\,n\,cm^{-2}\,s^{-1}$. Longer irradiations of 16 h under a thermal neutron flux of about $10^{12}\,n\,cm^{-2}\,s^{-1}$ were performed for determinations of As, Ba, Br, Ca, Ce, Cr, Co, K, La, Mn, Na, Rb, Sb, Sc, Th, V, and Zn. After adequate decay times, the irradiated samples and standards were measured using a hyperpure Ge detector Model GX2020 coupled to Model 1510 (Perkin Elmer Ortec, Oak Ridge TN, USA) integrated signal processor, both from Canberra. Counting times from 200 to 50,000 s were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and γ-ray energies. The concentrations of the elements were calculated using a comparative method (Saiki et al., 1997).

Measurements of the diameters of the particles (largest diameter) were made using an integrating eyepiece with polarized light microscopy. Each line in the eyepiece represented a 10-μm length at a magnification of $1000\times$. We measured about 230 events of each particle (PSA, PSB, and carbon).

2.3. Administration of test solutions

The suspensions were prepared by dissolving 10 mg of the particle in 1.0 ml of saline; this mixture was progressively diluted until the required concentration was achieved. Before the instillation the suspensions were submitted to agitation by sonication for 5 min.

Test solutions were delivered by nasal instillation. Briefly, we instilled 10-μL of the solution in one of the nostrils of the awake animal with the aid of an automatic pipette. This instillation provokes a reflex of apnea followed by a deep inspiration that propels the liquid into the lungs.

After 24 h, the animals were deeply anesthetized with 3% sodium pentobarbital ($30\,mg\,kg^{-1}$ of weight for intraperitoneal injection) and submitted to laparotomy for the collection of blood from the abdominal aorta. After this, the animals were euthanized by section of the abdominal aorta and exsanguination. Immediately after that, the femur was removed for the collection of the bone marrow and lung fluid was collected by bronchoalveolar lavage. The lungs were removed and fixed by the intratracheal instillation of 10% buffered formalin solution at a constant pressure of $5\,cmH_2O$.

2.4. Hematological studies

Blood was collected in EDTA K3 vials for hemogram and reticulocyte counting. The analysis was performed with Pentra 120 equipment manufactured by ABX Diagnostics (Montpellier, France). The counting principle of red blood cells and platelets is based on an impedance variation generated by the passage of the cells through a calibrated microaperture. Each cell is classified in a distribution histogram according to its size, and the curve obtained is mathematically smoothed. The measuring principle of the white blood

cell counting is the impedance and light diffusion changes generated by each element according to its internal structure when this element passes through the flow cell. For the determination of the reticulocytes, the whole blood was diluted with a fluorescent stain (thiazol orange), which is specific to nucleic acids. The stain molecules enter through the cell membrane and fix the ribonucleic acid molecules; this binding gives an increase in fluorescence. After 25 s, the solution is measured with a laser optical bench. The equipment gives the percentage of total reticulocytes counted and the percentage of the low-, medium-, and high-fluorescence fractions. The fluorescence is proportional to the RNA content and therefore to its maturity grade.

The differential counting of leukocytes was performed by optical microscopy at a 1000× magnification in Romanowsky-stained slides.

Blood was collected in sodium citrate vials for the determination of fibrinogen. We used the von Clauss method, a functional assay for thrombin-clottable fibrinogen. Briefly, diluted plasma was warmed to 37°C and mixed with a thrombin solution, and the clotting time of the mixture was measured using a coagulation analyzer (Thrombolyzer Combi, bioMérieux, Marcus L'Etoiler, France). Fibrinogen concentration, which is inversely proportional to the clotting time, was determined from a standard curve (Gardner and Lehmann, 2000).

2.5. Bone marrow studies

Bone marrow was collected from the femur. We made a transverse section to expose the medulla. The specimen was obtained by aspiration and then smeared on a glass slide. After it dried, the material was stained with a Romanowsky stain.

For the counting procedure, the cells of the bone marrow were classified as young granulocytes (myeloblasts, promyelocyte, myelocyte, and metamyelocyte), mature granulocytes (bands and segmented), lymphocytes, and erythroblasts. The counting (200 cells per slide) was performed with an optical microscope at a magnification of 1000×.

2.6. Bronchoalveolar lavage

Immediately after the euthanasia of each animal, the trachea was sectioned and one fine catheter was introduced and fixed with cotton thread, and 1.5 mL of phosphate buffer was injected in three series of 0.5 mL, followed by aspiration. The rate of recovery of the injected solution was approximately 80%. The material was conditioned in Eppendorf plastic tubes and transported to the laboratory for analysis.

The total cell count was determined using a hemocytometer. After the total counting, the material was cytocentrifuged at 1000 rpm for 10 min and the slides for the differential count were stained with a Romanowsky stain (Leishman). The counting (100 cells per slide) was performed with an optical microscope at a magnification of 630× (Seaton et al., 1999).

2.7. Protein electrophoresis

After the hematological analysis, the blood was centrifuged and the plasma obtained was used for the protein electrophoresis. We utilized the classical method; 5 μL of undiluted plasma was applied in wells to the cellulose acetate gel, 200 μm thick (Chemegel, Chemetron Chimica, Rozzono, Milan, Italy), embedded in barbital buffer of pH 8.8. Each end of the gel was then immersed in separate chambers filled with the same buffer in which electrodes were mounted. A voltage of 250 was applied between the electrodes, generating a current that passed through the gel for a period of 30 min to achieve the desired resolution (Jeppsson et al., 1979). (The ionic strength of the buffer determines the amount of current and the movement of the proteins for a fixed voltage.)

Following electrophoresis, the gel was treated with acetic acid that precipitated the proteins at the positions to which they had migrated. They were then stained with Ponceau S, and the gel was dried and cleared. A Cliniscan densitometric scanner (Helena Laboratories, Beaumont, TX, USA) was used to generate tracings and quantitate the relative percentages of protein in each fraction. The control was made utilizing human reference serum (Trilab P, Diasys Diagnostic Systems, Germany). The percentages were analyzed using statistical methods. The given fractions were albumin, $\alpha 1$, $\alpha 2$, β, and γ globulin.

2.8. Pulmonary histology

All lobes were sampled for histological analysis. The samples were embedded in paraffin and processed using routine histological procedures. Five-micrometer-thick slides were stained with hematoxylin and eosin. Descriptive histology and the determination of the numerical density of inflammatory cells (neutrophils, eosinophils, macrophages, and lymphocytes) were determined in the alveolar septum and perivascular connective area using an integrating eyepiece at 1000× (Saldiva et al., 2002). The number of fields evaluated was enough to keep the coefficient of error (standard error/mean) below 10%.

2.9. Statistical analysis

Descriptive statistics were calculated for the size of the particles, blood parameters, bone marrow, BAL data, and tissue measurements. The values were tested for

normality; those that were not normally distributed were log-transformed. For each parameter (dependent variable), the differences between the groups were determined using analysis of variance (ANOVA) considering terms for treatment (saline, carbon, PSA, and PSB), dose, and an interaction term (dose × treatment). When a significant difference was detected in ANOVA, it could be present in any one of the three terms. In such a case, post hoc Bonferroni and Student–Newman–Keuls were employed to localize the difference.

The significance level was set at 5%. The statistical program employed was the Statistical Package for the Social Sciences (SPSS) 8.0 (Chicago, IL, USA).

3. Results

3.1. Particles

Table 1 shows the chemical elements of PSA and PSB with the highest concentrations. Particle surrogate type A presented greater concentrations of Ce, Co, La, and V, while 44.6% of the composition of particle surrogate type B was iron. The other elements found in high concentrations were Br, Cr, Mn, Rb, Se, and Zn.

Almost all particles had a diameter of less than 10 μm; the means and standard deviations were 1.7 ± 2.56 μm for carbon, 1.2 ± 2.18 μm for PSA, and 1.2 ± 2.24 μm for PSB.

About 78% of the carbon particles and 98% of the PSA and PSB particles had a diameter of less than 2.5 μm.

3.2. Blood analysis

In comparison to the controls (saline) and carbon group, at the three doses of PSA the leukocyte count decreased (saline: $P = 0.007$ for 0.1 μg and $P < 0.001$ for both 1.0 and 10.0 μg, carbon: $P = 0.037$ for 0.1 μg, $P = 0.009$ for 1.0 μg, and $P = 0.001$ for 10.0 μg). For PSB, there was a decrease in the leukocyte count at the doses of 0.1 and 1.0 μg in comparison to the saline ($P = 0.010$ and 0.001, respectively) and carbon groups ($P = 0.048$ and 0.006, respectively) (Fig. 1A). No statistical difference was observed in the leukocyte differential counting among the groups.

The platelet number in the PSA group decreased at the doses of 1.0 and 10.0 μg in relation to the saline group ($P = 0.005$ and 0.030, respectively) and at all three doses in relation to the carbon group ($P = 0.005$, 0.001, and 0.009, respectively). For PSB the platelet count decreased at the doses of 0.1 and 1.0 μg in relation to the saline ($P = 0.049$ and < 0.001, respectively) and carbon ($P = 0.018$ and < 0.001, respectively) groups (Fig. 1B). The hemoglobin levels and erythrocyte counts did not present any differences among the groups.

There was no significant difference among the groups either in the counting of total reticulocytes or in the medium- and low-fluorescence reticulocytes. However, the values of high-fluorescence reticulocytes showed increases only in the PSA group in relation to the saline ($P = 0.003$ for all doses) and carbon ($P = 0.009$ for 0.1 and 1.0 μg and $P = 0.008$ for 10.0 μg) groups.

We observed an increase in the fibrinogen levels for the PSA and PSB groups. In the PSA group there was an increase at the doses of 0.1 and 1.0 μg in relation to the saline ($P = 0.011$ and 0.045, respectively) and carbon ($P = 0.001$ and 0.008, respectively) groups. In the PSB group there was an increase at the three doses in relation to the saline and carbon groups ($P < 0.001$ for all doses) (Fig. 1C). PSB presented higher values than PSA, with a significance level of $P < 0.001$.

The results of protein electrophoresis did not show statistically significant differences among the groups. The levels of α1 globulin were slightly elevated in the PSA and PSB groups, but these increases were not statistically significant.

Table 1
Chemical element concentrations in particle surrogates types A and B determined by neutron activation analysis are shown as the means and corresponding standard deviations (Fe is given as a percentage)

Chemical element	PSA	PSB
Br ($\mu g\,g^{-1}$)	8.7 ± 0.6	1.482 ± 19
Ce ($\mu g\,g^{-1}$)	51.1 ± 0.4	16.3 ± 0.3
Co ($\mu g\,g^{-1}$)	122.9 ± 3.1	9.90 ± 0.25
Cr ($\mu g\,g^{-1}$)	32.4 ± 0.4	107.7 ± 1.4
Fe (%)	3.28 ± 0.07	44.6 ± 0.1
La ($\mu g\,g^{-1}$)	972 ± 12	10.3 ± 0.1
Mn ($\mu g\,g^{-1}$)	1977 ± 14	3884 ± 24
Rb ($\mu g\,g^{-1}$)	11.4 ± 1.1	719.7 ± 1.0
Sb ($\mu g\,g^{-1}$)	39.8 ± 0.7	2.27 ± 0.09
Sa ($\mu g\,g^{-1}$)	20.5 ± 0.2	154.4 ± 0.8
V ($\mu g\,g^{-1}$)	1816 ± 220	35 ± 4
Zn ($\mu g\,g^{-1}$)	115.7 ± 1.5	491.9 ± 3.1

3.3. Bone marrow analysis

The erythroblasts were the only cells in the bone marrow that presented significant differences among the groups. The PSA group showed an increase in the erythroblast number at the three doses in relation to the saline group ($P < 0.001$, $= 0.001$, and $= 0.048$, respectively) and at the doses of 0.1 and 1.0 μg in relation to the carbon group ($P < 0.001$ and $= 0.007$, respectively). In the PSB group we observed an increase in the erythroblast number at the doses of 0.1 and 10.0 μg in relation to the saline group ($P = 0.004$ and 0.006, respectively) and in relation to the carbon group ($P = 0.019$ and 0.027, respectively) (Fig. 1D).

The increased numbers of high-fluorescence reticulocytes in the blood and erythroblasts in the bone marrow

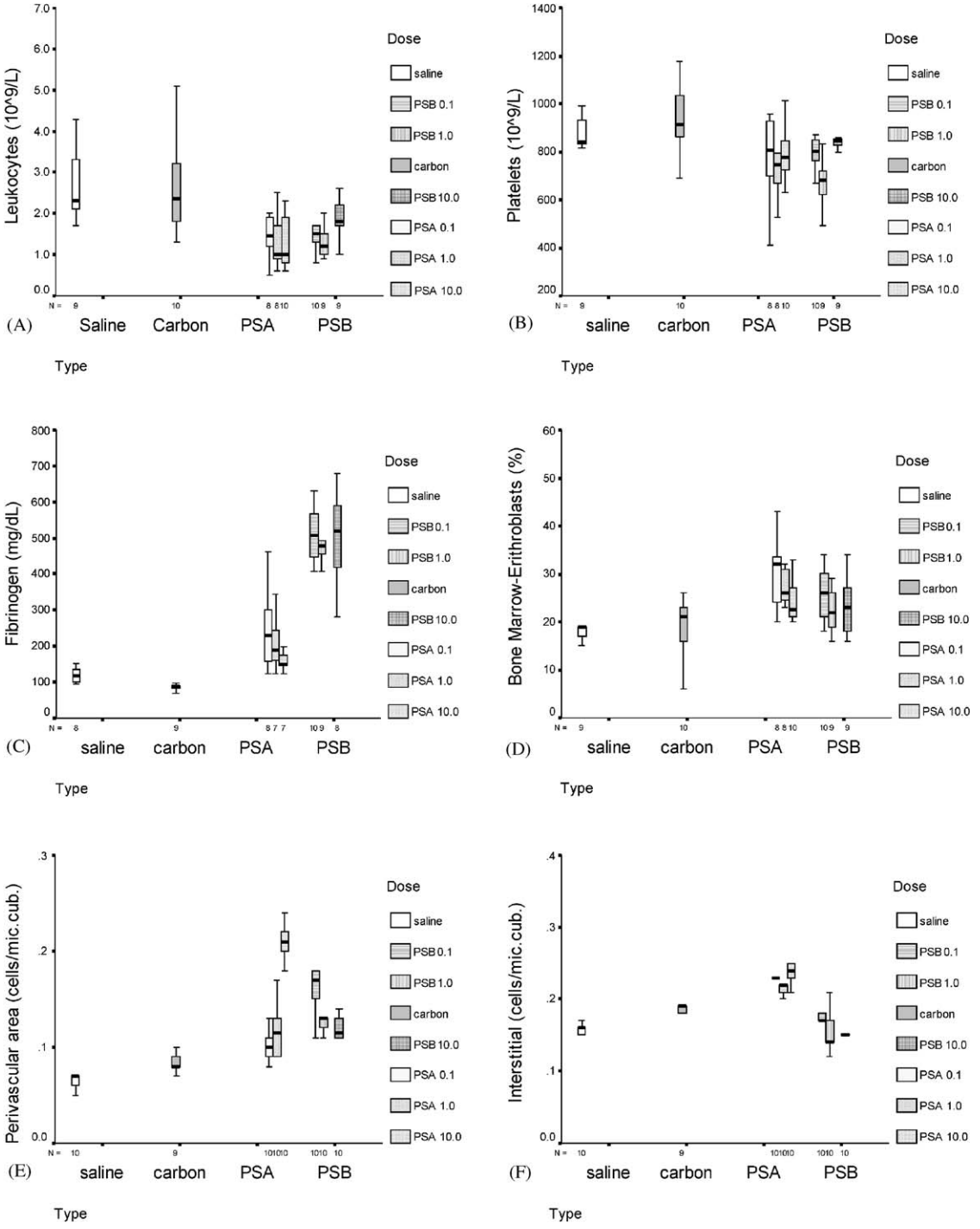

Fig. 1. A graphic representation of the parameters studied in relation to the experimental groups and doses in box-plot format. The vertical line extending up from each box represents the 75th percentile, the line extending down represents the 25th percentile, and the horizontal line inside each box represents the arithmetic mean. (A) Leukocyte values, with a decrease in the PSA and PSB groups. (B) Platelet values, with a decrease in the PSA and PSB groups. (C) Fibrinogen values, with an increase in the PSA group and a greater increase in the PSB group. (D) The erythroblasts in the bone marrow analysis showing an increase in both the PSA and the PSB groups. (E,F) The numerical density of inflammatory cells performed in lung histology. (E) Representation in the perivascular area, with an increase in the PSA and PSB groups: a large increase in the inhaled dose of 10.0 μg of PSA. (F) Representation in the alveolar interstitial area, with an increase only in the PSA group. (PSA, particle surrogate type A; PSB, particle surrogate type B.)

suggest that PSA inhalation provoked a stimulus in the bone marrow that accelerated the red cell production.

3.4. Pulmonary studies

No statistically significant differences among the groups were observed in the bronchoalveolar lavage cell counts. Macrophages with carbon pigment were detected in all animal groups, indicating that particles reached the alveolar territory. The quantitative cellular counts (cells per μ^2) for the perivascular area of the lung showed an increase in the PSA and PSB groups at the three doses in comparison to the saline group. The levels of significance for the PSA group were 0.050 for 0.1 μg and less than 0.001 for 1.0 and 10.0 μg; for PSB the levels of significance were 0.001 for 0.1 and 1.0 μg and 0.003 for 10.0 μg. In comparison to the carbon group there was an increase of inflammatory cells in the PSA group at the doses of 1.0 and 10.0 μg ($P = 0.006$ and <0.001, respectively) and at the doses of 0.1 and 1.0 μg for the PSB group ($P<0.001$ and $= 0.018$, respectively). We observed a greater intensity in the PSA group at a concentration of 10 μg (Fig. 1E).

In the alveolar septum area, we observed an increase in inflammatory cells only in the PSA group at the three doses in relation to the saline group ($P<0.001$ for all) and at the doses of 0.1 and 10.0 μg in relation to the carbon group ($P = 0.017$ and 0.001, respectively). The distribution is demonstrated in Fig. 1F.

Fig. 2 shows images from lung histology at a magnification of 400× and hematoxylin and eosin staining for all groups. Fig. 2A shows normal parenchyma of the saline group and Fig. 2B shows the parenchyma of the carbon group with few inflammatory cells (not statistically significant). Figs. 2C and 2D are from the PSA group: Fig. 2C shows the perivascular inflammatory process and Fig. 2D shows an interstitial inflammatory process. Figs. 2E and 2F are from the PSB

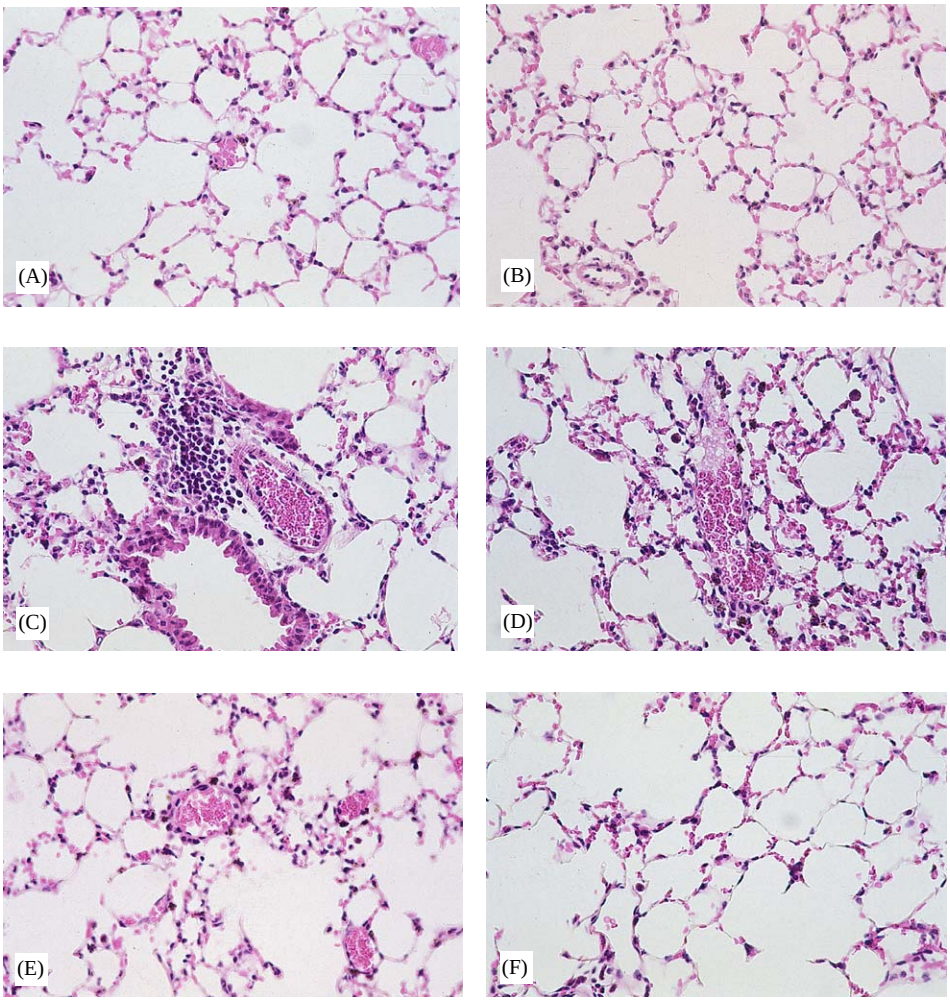

Fig. 2. Images of hematoxylin and eosin-stained lung parenchyma at a magnification of 400×. (A) Saline group without inflammatory cells. (B) Carbon group with few inflammatory cells. (C) PSA group showing the inflammatory process in the perivascular area. (D) PSA group with inflammatory infiltration in the interstitial area. (E) PSB group showing inflammatory cells in the perivascular area. (F) PSB group without the inflammatory process in the interstitial area. (PSA, particle surrogate type A; PSB, particle surrogate type B.)

group: Fig. 2E shows the perivascular inflammatory process and Fig. 2F shows lung parenchyma without the inflammatory process. The lack of inflammatory cells in the alveolar space is compatible with normal bronchoalveolar lavage cytology.

4. Discussion

The present study demonstrated significant acute inflammatory and hematological alterations in mice challenged with a single dose of PS. The particle surrogates utilized should be considered fine particles with the ability to reach the alveolar territory, since the diameter measurements of 98% of them were less than 2.5 μm. Significant differences among the types of particles studied were observed: some exhibited dose-dependent alterations type, whereas others exhibited an "on–off" behavior. In our experimental model, we tried to use low doses of particles (in the range of 0.1 to 1.0 μg). These levels were closer to those that, theoretically, would be deposited into the lungs in a period of 24 h in a town with the characteristics of São Paulo, which exhibits a mean annual concentration of $PM_{2.5}$ of about 20 $\mu g\,m^{-3}$ at the site of our medical school. We focused on the capacity of particles exhibiting different compositions to induce pulmonary and hematological alterations. For control purposes, we employed saline and inert carbon particles. The test particles were selected based on their different sources which, ultimately, affect their composition, as demonstrated in Table 1.

Our main findings can be summarized as follows: (1) both types of PS presented significant effects in comparison to saline and carbon particles, but there were differences between the two test particles; (2) there were reductions of leukocyte and platelet counts for both PS; (3) there was an increase in reticulocytes in the peripheral blood, accompanied by an increased production of erythroblasts in the bone marrow that was more intense for PSA; (4) the increase in levels of plasma fibrinogen was more intense for PSB; and (5) we saw histological pulmonary inflammation in the perivascular area for both types of PS and in the interstitial area for PSA only.

In our study, we observed a decrease in leukocyte counts in the animals exposed to toxic PS, whereas other studies have reported an increase in leukocytes in humans (Schwartz, 2001), dogs (Clarke et al., 2000), and rabbits (Mujae et al., 2001) exposed to ambient particles. It is important to note that we evaluated one challenge with particles at a single time point. Thus, the decrease of leukocytes in our study could reflect the trapping of these cells in the marginal compartment, which would not have been fully compensated by the bone marrow. In fact, the most conspicuous response of the bone marrow to the PS studied in our case was that of red cells. Bone marrow analysis and the studies considering circulating reticulocytes indicated that red cell production and delivery to the circulating blood pool were enhanced after exposure to toxic particles. Such a response has not been to our knowledge, observed previously, although Seaton et al. (1999) have already reported that repeated exposure to particulate pollution caused significant decreases of the red cell count in humans, but without a bone marrow reference. Again, differences between an acute single exposure and repeated noxious stimuli may explain this difference. The release of reticulocytes in the circulation is a well-defined response to several types of stress (Fallon and Bishop, 2002; Noble et al., 1990). Stress-induced changes in erythropoiesis seem to be dependent on glucocorticoid receptors (Bauer et al., 1999) and are controlled by a delicate balance between inhibitory mediators (TGF-β) and stimulatory (IL-3) cytokines (Croizat and Nagel, 2002). Stress reticulocytes positive for CD36 exhibit a greater adhesion to endothelium, which is thrombospondin-mediated (Browne and Hebbel, 1996). Although much of the aforementioned arguments are speculative in our case, our data support the evidence that erythroblasts in the bone marrow received a stimulus provoked by inhaled toxic particles without evidence of a hemolytic process or red cell loss.

The changes observed in the plasma fibrinogen levels were an expressive finding in this study. There was no relationship between the dose of the pollutant particles inhaled and the degree of increase in the fibrinogen. PSB, the composition of which is rich in metals, promoted the greatest increase. Since soluble transition metals may elicit oxidative stress and secretion of cytokines by alveolar macrophages (Dye et al., 1999; Lindroos et al., 1997; Stringer and Kobzik, 1998; Van Eeden et al., 2001; Zhao et al., 1997), it is tempting to propose that the inflammation triggered at the alveolar level stimulated the liver to increase the production of fibrinogen, probably mediated by IL-6 (Seaton et al., 1995). The composition of PSB was iron, an important metal involved in free radical generation (Lay et al., 1999).

Levels of ambient particulate matter have been associated with mortality and morbidity from both ischemic heart disease (Schwartz et al., 1996) and stroke (Schwartz, 1994) in humans. The rise in cardiovascular events is probably associated with increased blood coagulability resulting from alveolar inflammation induced by particle exposure (Seaton et al., 1995). Nevertheless, as fibrinogen is a risk factor for ischemic vascular diseases, the alteration of important hematologic parameters secondary to an inflammatory response to particle inhalation may initiate a cascade whereby cardiovascular events might be triggered in susceptible individuals at relatively low exposures.

In the two indicative parameters of pulmonary inflammation—bronchoalveolar lavage and lung histology—bronchoalveolar lavage was not sensitive enough to detect the inflammatory changes 24 h after the nasal instillation. Gavett et al. (1999) in their study in mice after intratracheal instillation of ROFA observed inflammatory alterations in bronchoalveolar lavage using doses larger than those in our study.

The best information regarding this aspect was given by the quantitative morphologic studies of pulmonary parenchyma. PSA (derived from oil combustion) was responsible for the highest degree of pulmonary inflammation.

It appears that the induction of injury by the particle surrogates is determined primarily by constituent metals and their bioavailability. Based on studies with fly ash, the earliest phases of the response appear to be driven by the individual metals (Dreher et al., 1997), but the persistence of the response might reflect the complexity of the bioavailable metal mix or the unique qualities of one metal compared to those of another (Costa and Dreher, 1997). The mechanism of lung injury after exposure to particulate matter is unknown. It may be mediated by metal-catalyzed oxidant generation, which in turn may be modulated by specific redox changes, by metal ion dysregulation of phosphotyrosine metabolism, or possibly by elements of both mechanisms. These events would result in the activation of specific transcription factors such as nuclear factor κB and AP-1, an increased expression of proinflammatory proteins, the genes of which have binding sites for these transcription factors in their promoter regions, and finally inflammatory injury to the lung (Ghio et al., 2002).

Respiratory epithelial cells exposed to vanadium, but not iron or nickel, showed increased messenger RNA and protein expression of numerous cytokines, including interleukin and tumor necrosis factor (Carter et al., 1997). In our study, PSA had a high vanadium concentration, which may explain the high lung inflammatory response. There is a vast amount of literature on lung inflammatory injury and vanadium (Ghio et al., 2002).

Zinc can induce lung injury and hematological changes following acute and long-term exposures in rats (Kodavanti et al., 2002). Prieditis and Adamson (2002), in their study, showed that zinc and copper are more likely to cause lung injury and inflammation than metals such as iron and vanadium. Particle surrogate types A and B had large amounts of zinc. This may have been another cause of the inflammatory alterations observed in our study. Saldiva et al. (2002) encountered a strong association between pulmonary inflammation and vanadium, bromine, lead, and organic carbon in his study in rats. These findings were consistent with those observed in canines exposed to CAPs (Clarke et al., 2000).

It is difficult to identify from our study how exposure to particle surrogates initiates pulmonary inflammation and the systemic effects. Further studies are necessary to determine the potential of injury for each metal component in the inhaled solutions and/or the interaction of the metal mix.

In conclusion, this study supports the concept that composition is critical in determining the biological response to inhaled particles. Test particles derived from oil combustion elicited a more intense pulmonary inflammation, whereas those rich in metals were responsible for a larger increase in plasma fibrinogen. In some aspects (induction of leucopenia, for instance), both particles exhibited similar behavior. We postulated that erythropoiesis might represent a significant target for inhaled particles. Inert carbon particles did not promote a significant inflammatory response, at either the pulmonary or the systemic level.

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