Oil Fly Ash-Induced Elevation of Plasma Fibrinogen Levels in Rats

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Particulate matter air pollution (PM) has been associated with morbidity and mortality from ischemic heart disease and stroke in humans. It has been hypothesized that alveolar inflammation, resulting from exposure to PM, may induce a state of blood hypercoagulability, triggering cardiovascular events in susceptible individuals. Previous studies in our laboratory have demonstrated acute lung injury with alveolar inflammation in rats following exposure to residual oil fly ash (ROFA), an emission source particulate. In addition, increased mortality has been documented following exposure to ROFA in rats with preexistent cardiopulmonary disease. ROFA’s toxicity derives from its soluble metal content, which appears also to drive the toxicity of ambient PM. The present study was conducted to test the hypothesis that exposure of rats to a toxic PM, like ROFA, would adversely alter hemostatic parameters and cardiovascular risk factors thought to be involved in human epidemiologic findings. Sixty-day-old male Sprague-Dawley rats were exposed by intratracheal instillation (IT) to varying doses (0.3, 1.7, or 8.3 mg/kg) of ROFA, 8.3 mg/kg Mt. Saint Helen’s volcanic ash (MSH, control particle), or 0.3 ml saline (SAL, control). At 24 h post-IT, activated partial thromboplastin time (APTT), prothrombin time (PT), plasma fibrinogen (PF), plasma viscosity (PV), and complete blood count (CBC) were performed on venous blood samples. No differences from control were detected in APTT and PT in ROFA-exposed rats; however, ROFA exposure did result in elevated PF, at 8.3 mg/kg only. In addition, PV values were elevated in both ROFA and MSH-exposed rats relative to SAL-control rats, but not significantly. Although no changes were detected in APTT and PT, alteration of important hematologic parameters (notably fibrinogen) through PM induction of an inflammatory response may serve as biomarkers of cardiovascular risk in susceptible individuals.

Key Words: particle; air pollution; lung; plasma fibrinogen; plasma viscosity; coagulation; particulate matter; residual oil fly ash; Mt. Saint Helen’s volcanic ash; alveolitis; coagulation.

Epidemiological associations between levels of ambient particulate matter and morbidity and mortality (Burnett et al., 1995; Committee of Departmental Officers and Expert Advisers appointed by the Minister of Health, 1954; Poloniecki et al., 1997; Schwartz and Morris, 1995; Wichmann et al., 1989) and mortality (Pope et al., 1992; Schwartz, 1994; Schwartz and Dockery, 1992; Wordley et al., 1997) from cardiovascular diseases in humans have been reported. Studies that have investigated specific underlying causes of cardiovascular disease have suggested that both ischemic heart disease (Schwartz et al., 1996; Schwartz and Morris, 1995; Wichmann et al., 1989) and cerebrovascular disease (Schwartz, 1994; Wordley et al., 1997) are involved. The mechanism(s) responsible for these observed health effects are not understood; however, it has been hypothesized that alveolar inflammation, induced by exposure to particulate air pollution, may result in increased blood coagulability, triggering cardiovascular events in susceptible individuals (Seaton et al., 1995). Interestingly, a recent report found that plasma viscosity, a risk factor for ischemic heart disease (Low et al., 1997; Sweetnam et al., 1996; Yarnell et al., 1991) and cerebrovascular disease (Low et al., 1997) was elevated in men and women, and also associated with particulate matter (PM), during an air pollution episode (Peters et al., 1997). Although not specifically examined in this study (Peters et al., 1997) fibrinogen, an important contributor to plasma viscosity (Harkness, 1981), has also been associated with ischemic heart disease (Ernst and Resch, 1993; Meade et al., 1993; Sweetnam et al., 1996; Thompson et al., 1995) and cerebrovascular disease (Ernst and Resch, 1993; Wilhelmsen et al., 1984).

Previous studies in our laboratory have demonstrated acute lung injury with alveolar inflammation in rats, following exposure to residual oil fly ash (ROFA), an emission source particulate (Dreher et al., 1997; Kodavanti et al., 1997a,b). These studies have demonstrated that ROFA-induced lung injury occurs in a dose-dependent manner with dose-independent peak-inflammatory and acute-injury phases at 18–24 h, which then slowly subside, but remain significantly elevated at 96 h. The primary toxic component of ROFA comprises the bioavailable metal fraction. When ambient PM are instilled into the rat lung, the temporal pattern of inflammation and injury follows that of ROFA, and like ROFA, the severity of
response is proportional to the bioavailable metal content (Costa and Dreher, 1997; Ghio et al., 1996). Moreover, rats with preexistent cardiopulmonary disease succumb to instilled ROFA in part through exacerbated oxidant stress to the lung consistent with the induction of metal-induced oxidants (Costa and Dreher, 1997; Kodavanti et al., 1999; Watkinson et al., 1998). Thus, while ROFA, as a model PM, is about 10-fold more potent than most ambient PM, it nevertheless can serve as a tool to test or screen hypotheses that relate to the toxic potential for ambient PM. The present study was conducted as a preliminary examination of selected hemostatic changes and cardiovascular risk factors in healthy rats, following intratracheal instillation of ROFA to address the hypothesis that exposure to PM can modulate important hematologic parameters.

MATERIALS AND METHODS

Animals. Sixty-day-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC) were housed in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Food (Purina Rodent Lab Chow, St. Louis, MO) and water were available ad libitum.

Particles. ROFA had been previously collected using a Teflon-coated fiber filter at a temperature of 204°C by Southern Research Institute downstream from the cyclone of a power plant in Florida, which burned low sulfur No. 6 residual oil. The mass mean aerodynamic diameter of ROFA was 1.95 μm and the geometric mean was 2.19 (Dreher et al., 1997). ROFA was suspended in sterile 0.9% sodium chloride solution (saline, Fujisawa USA Inc., Deerfield, IL) at concentrations of 0.3, 1.7, and 8.3 mg/ml prior to exposure. Mt. Saint Helen’s volcanic ash (MSH) was collected from an open field near Ritzfield, Washington on June 8, 1980 and fractionated (Hatch et al., 1985). The median count diameter of MSH was 1.4 μm. MSH was suspended in sterile saline at a concentration of 8.3 mg/ml prior to exposure. Both PM samples have been tested and found to be free of endotoxin (Cape Cod Assoc., MA).

Exposure. Intratracheal instillations were performed as previously described (Dreher et al., 1997). Experiment 1: ROFA suspension (8.3 mg/kg) or saline (control) was instilled intratracheally (1 ml/kg) in rats (n = 6). Experiments 2 and 3: ROFA suspension (0.3, 1.7, or 8.3 mg/kg) or MSH suspension (8.3 mg/kg or saline (1 ml/kg) was instilled intratracheally in rats (n = 6).

Blood collection. Twenty-four h after exposure (previously found to be the time point of peak alveolar inflammation), the rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Blood was collected from each rat by cardiac puncture, using a vacutainer needle directly into (1) a 2.7-ml sodium citrate vacutainer tube for measurement of plasma fibrinogen, activated partial thromboplastin time (APTT), and prothrombin time (PT); and a 3.0-ml ethylenediaminetetraacetic acid (EDTA) vacutainer tube for complete blood count (CBC) analysis for Experiment 1; (2) a 2.7-ml sodium citrate vacutainer tube for measurement of plasma fibrinogen and a 2.0-ml EDTA vacutainer tube for CBC analysis for Experiment 2; (3) a 5.0-ml EDTA vacutainer tube for measurement of plasma viscosity for Experiment 3. Rats were then sacrificed by administration of an overdose of sodium pentobarbital intraperitoneally (150 mg/kg).

Complete blood count. Complete blood counts were performed on a Technicon H-2 hematology analyzer (Bayer Corporation, Tarrytown, NY) using Bayer Technicon reagents.

Fibrinogen. An imidazole buffer was used to make 3 dilutions of each plasma sample. Samples were warmed to 37°C, warmed thrombin (MDA Fibriquik, Organon Teknika, Durham, NC) was added, and the time required for clot formation was measured using a coagulation analyzer (MDA 180, Organon Teknika). A reference curve was generated using reference plasma (MDA Verify Reference Plasma, Organon Teknika). Sample results were calculated to a concentration (mg/dl) by interpolation from the reference curve, and the concentration was multiplied by a dilution factor. Control plasma samples were run concurrently (Organon Teknika Corp., 1996).

Activated partial thromboplastin time. Phospholipid (Ortho Thrombosil APTT reagent, Ortho Diagnostics, Raritan, NJ) was added to plasma samples, which were recalculated, and the time required for clot formation was measured using a centrifugal coagulation analyzer (ACL300+, Instrumentation Laboratories, Lexington, MA). Control plasma samples were run concurrently. (Instrumentation Laboratories, 1996)

Prothrombin time. Warmed tissue thromboplastin (MDA Simplastin L reagent, Organon Teknika) was added to warmed plasma samples (37°C), and the time required for clot formation was measured using a coagulation analyzer (MDA 180, Organon Teknika). Control plasma samples (high, normal, low) (MDA Verify 1,2,3, Organon Teknika) were run concurrently (Organon Teknika, 1996a).

Plasma viscosity. Plasma viscosity measurements were performed on a viscometer (Viscometer II, Coulter Electronics Ltd., Luton, England). Control plasma samples were run concurrently.

Statistical analysis. Results are expressed as means ± standard deviation (SD). Experiment 1: Data were analyzed (SAS, Version 6, 1990; Cary, NC) by the Kruskal-Wallis test. Experiments 2 and 3: Data were analyzed by analysis of variance. Pairwise comparisons were performed as subsets of the overall analysis of variance. The significance level for all tests was set at 0.05.

RESULTS

Experiment 1

An initial exploratory study was conducted to ascertain whether the toxic PM, ROFA, would affect hematologic and hemostatic parameters. Table 1 lists the mean ± SD for these various parameters in rats at 24 h after saline or 8.3 mg/kg ROFA instillation. The blood leukocyte count in the ROFA-exposed rats was significantly elevated compared to controls; however, the mean values of both groups fell within what is considered the normal range for male rats of this age and strain (Schalm et al., 1986). Erythrocyte, hematocrit, and hemoglobin values were unaffected. The APTT and PT were also unaltered by ROFA, although plasma fibrinogen was significantly elevated (86%) compared to control levels.

Experiment 2

Table 2 lists confirmatory dose-related data (mean ± SD) for hematologic parameters in rats at 24 h after saline, inert MSH (8.3 mg/kg), and ROFA (8.3, 1.7, and 0.3 mg/kg) instillations. The APTT and PT were omitted, since there was no evidence of impact at the highest ROFA dose. Plasma fibrinogen was significantly elevated (~45%) in rats exposed to 8.3 mg/kg ROFA compared to rats exposed to saline, the MSH, and the lower doses of ROFA. There were no significant differences among the ROFA-exposed, MSH-exposed, or saline-exposed groups in any of the other hemostatic parameters.

Experiment 3

We ascertained plasma viscosity in a separate replicate of Experiment 2 because of the volume of plasma needed for
The present study was conducted to evaluate selected hemostatic changes and cardiovascular risk factors in healthy rats following intratracheal instillation of residual oil fly ash (ROFA), a toxic component of PM rich in bioavailable metal sulfates (Dreher et al., 1997). Initially, rats were exposed to a dose of ROFA (8.3 mg/kg), previously demonstrated in our laboratory to cause acute lung injury and alveolar inflammation (Dreher et al., 1997; Kodavanti, et al., 1997a,b). This dose of ROFA, while admittedly high relative to potentially inhaled ambient PM (~500× the PM10 [particulate matter with an aerodynamic diameter of 10 µm or less] daily National Ambient Air Quality Standards [NAAQS]), is only ~5× the estimated dose to a worker exposed at the occupational limit for fly ash. In addition, we have compared ROFA instilled intratracheally into rats at the same dose as that which was deposited after a 6-h, 12-mg/m³ exposure and found virtually identical inflammatory cell profiles and injury kinetics over a 96-h postexposure period (unpublished data). Thus, we felt that this upper limit challenge with ROFA would represent a definitive test of the feasibility of the hypothesis and its merit for further analysis.

Interestingly, fibrinogen was elevated in the high-dose ROFA rats. No changes in other blood and hemostatic parameters were observed. The subsequent dose-response study was performed to confirm and expand this observation at 2 lower doses of ROFA, shown in our laboratory to cause substantially less lung injury and inflammation. Included was a negative control PM (MSH), a particle having virtually no water-leachable transition metal (Hatch, et al., 1985). MSH at 8.3 mg/kg would control, in part, for the mass dose of particle matrix instilled at the highest dose of ROFA. Previous study has shown MSH to cause only a mild inflammatory response when inhaled or instilled into the lungs of rats (Ghio et al., 1996; Raub, et al., 1985). It should be noted, however, that the soluble metal sulfate content of ROFA directly implicated in its toxicity comprises 92% of the mass of the particle (Dreher, et al., 1997; Kodavanti, et al., 1997a). Thus the insoluble matrix of MSH was ~12 times that of ROFA, yet it did not elevate plasma fibrinogen levels in rats significantly when compared to saline-exposed rats. Likewise, it had no significant effect on plasma viscosity. The highest dose of ROFA, on the other hand, clearly elevated plasma fibrinogen levels compared to saline- or MSH-exposed rats, but also had no significant effect on plasma viscosity. The cross-dose and cross-PM increase of 4%, however, may suggest an ill-defined, nonspecific particle effect not associated with composition, but this difference was not statistically significant. No clinical or other parameter seemed to correlate with this trend.

Fibrinogen is both an acute phase reactant that is increased

### TABLE 1

<table>
<thead>
<tr>
<th>Hematologic parameter</th>
<th>Saline</th>
<th>ROFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10³/ml)</td>
<td>3.78 ± 0.45</td>
<td>6.61 ± 2.50*</td>
</tr>
<tr>
<td>RBC (×10³/ml)</td>
<td>6.43 ± 0.31</td>
<td>6.57 ± 0.37</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12.6 ± 0.43</td>
<td>12.9 ± 0.61</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>39.8 ± 1.59</td>
<td>41.2 ± 1.75</td>
</tr>
<tr>
<td>PLT (×10³/ml)</td>
<td>1089 ± 64</td>
<td>1036 ± 115</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>245.7 ± 16.0</td>
<td>458.1 ± 158.5*</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>15.6 ± 2.6</td>
<td>16.0 ± 1.3</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>18.2 ± 1.1</td>
<td>19.2 ± 2.3</td>
</tr>
</tbody>
</table>

*Significant compared to saline.

**Note.** Mean ± SD values for hematologic parameters from rats 24 h after IT of ROFA (8.3 mg/kg) or saline (1ml/kg). WBC (white blood cell count); RBC (red blood cell count); HGB (hemoglobin); HCT (hematocrit); PLT (platelet count); APTT (activated partial thromboplastin time); PT (prothrombin time).

### TABLE 2

<table>
<thead>
<tr>
<th>Hematologic parameter</th>
<th>MSH (8.3 mg/kg)</th>
<th>ROFA (0.3 mg/kg)</th>
<th>ROFA (1.7 mg/kg)</th>
<th>ROFA (8.3 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10³/ml)</td>
<td>9.77 ± 3.39</td>
<td>6.72 ± 2.41</td>
<td>6.24 ± 1.59</td>
<td>6.39 ± 1.88</td>
</tr>
<tr>
<td>RBC (×10³/ml)</td>
<td>6.06 ± 0.45</td>
<td>6.32 ± 0.28</td>
<td>6.41 ± 0.45</td>
<td>6.33 ± 0.29</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12.6 ± 0.6</td>
<td>13.4 ± 0.5</td>
<td>13.3 ± 0.9</td>
<td>13.2 ± 0.6</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37.6 ± 1.9</td>
<td>39.4 ± 1.5</td>
<td>39.0 ± 2.6</td>
<td>38.6 ± 1.1</td>
</tr>
<tr>
<td>PLT (×10³/ml)</td>
<td>1134 ± 120</td>
<td>1103 ± 69</td>
<td>1117 ± 165</td>
<td>1059 ± 63</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>237.2 ± 25.3</td>
<td>263.7 ± 36.6</td>
<td>242.2 ± 28.9</td>
<td>240.0 ± 21.9</td>
</tr>
</tbody>
</table>

*Significant compared to other groups.

**Note.** Mean ± SD values for hematologic parameters from rats 24 h after IT of ROFA (0.3, 1.7, 8.3 mg/kg) or MSH (8.3 mg/kg) or saline (1ml/kg). WBC (white blood cell count); RBC (red blood cell count); HGB (hemoglobin); HCT (hematocrit); PLT (platelet count).
in inflammatory processes (Dinarello, 1988) and a clotting factor. It has been shown to be a risk factor for ischemic heart disease (Ernst and Resch, 1993; Meade et al., 1993; Sweetnam et al., 1996; Thompson et al., 1995) and cerebrovascular disease (Ernst and Resch, 1993; Wilhelmson et al., 1984). Plasma levels of fibrinogen correlate with the presence and severity of coronary artery disease (Bolibrk et al., 1993) and carotid artery disease (Wu et al., 1992). Furthermore, elevated fibrinogen levels are a risk factor for re-occlusion after grafting and angioplasty (Hicks et al., 1995; Montalescot et al., 1995).

The pathogenetic mechanism(s) whereby fibrinogen acts as a cardiovascular risk factor are likely to be multifactorial. Proposed mechanisms include fibrinogen’s effects on blood rheology, platelet aggregation, hemostasis, and endothelial function (Ernst, 1993). Fibrinogen contributes to plasma viscosity (Ernst and Resch, 1993) and induces erythrocyte aggregation (Pearson and Rampling, 1994), both of which decrease the fluidity of blood, potentially leading to slowing of capillary blood flow and decreased tissue oxygenation (Lowe, 1986). Plasma fibrinogen levels are associated with platelet aggregability (Meade et al., 1985), important in the genesis of a vascular lesion (Ross et al., 1974). Fibrinogen has been detected in atherosclerotic lesions in the aorta (Smith and Staples, 1981) and cerebral arteries (Sadoshima and Tanaka, 1979), where it is converted to fibrin, binds low density lipoproteins, and sequesters more fibrinogen. In addition, a kinetic model has been reported that predicts a direct relationship between plasma fibrinogen levels and the quantity of fibrin generated during clot formation (Naski and Shafer, 1991). In rabbits, after induction of the acute phase response, plasma fibrinogen concentrations were associated with the extent of induced thrombi (Chooi and Gallus, 1989). Furthermore, newly generated fibrinogen, which would occur during an acute inflammatory response, is highly thrombogenic, and the resulting thrombi typically have a tight, rigid, space-filling network (Reganone et al., 1993).

Fibrinogen also contributes to plasma viscosity (Harkness, 1981), which is another risk factor for ischemic heart (Lowe et al., 1997; Sweetnam et al., 1996; Yarnell et al., 1991) and cerebrovascular disease (Lowe et al., 1997). We found an elevation in plasma fibrinogen levels in rats exposed to ROFA, and Peters et al. (1997) reported that, during an air pollution episode, plasma viscosity associated with PM was elevated in men and women. Based on these findings, we measured plasma viscosity in rats, following exposure to 3 doses of ROFA in concentrations that would induce a spectrum of injury (minimal to severe), as well as to MSH ash, which is largely inert. The mean plasma viscosity values for all ash-exposed rats (both ROFA and MSH) were higher than those of the saline-exposed rats, but none of the differences between the individual groups was statistically significant, due to the variability in responses within groups. Variability in plasma viscosity responses in men and women was noted in the Peters et al. (1997) study as well; however, their population sample size was very large in comparison to ours.

The APTT and PT assays were performed on the high-dose ROFA-treated rats as non-specific screening tests of the hematostatic system, in order to ascertain whether this toxic PM might activate intravascular coagulation. The APTT reflects the activity of the intrinsic (thought to link to endothelial damage) and common pathways of coagulation, including Factors I, II, V, VIII, IX, X, XI, and XII (Duncan and Prasse, 1986), where the PT relates the activity of the extrinsic (initiated by the release of tissue thromboplastin from injured cells) and common pathways of coagulation, including Factors I, II, V, VII, and X (Duncan and Prasse, 1986). Despite the severity of the ROFA-induced lung injury, no differences were detected in either the APTT or PT indices when compared to controls. Had increases in either PT and/or APTT been observed, one might suspect ongoing intravascular coagulation due to diminished availability of one or more coagulation factors within the cascades. A decrease in circulating platelets as they aggregate, and a decrease in plasma fibrinogen as it is converted to fibrin might also have been expected. However, plasma fibrinogen increased, and platelet counts and clotting times did not change, thus leading to the conclusion that while risk of a “cardiovascular event” may be increased based on the associations found in human population fibrinogen data, intravascular coagulation itself is apparently not activated by ROFA, by its constituent sulfated metals, or by the degree of lung injury induced. The finding that plasma viscosity did not change in spite of the elevated fibrinogen indicates that viscosity and fibrinogen, as indices of cardiovascular risk, are likely interlinked in a complex manner. Since the tests employed in this study were conducted in vitro with ex vivo samples collected postexposure, they could underestimate coagulation in vivo (Bauer and Weitz, 1994), however, we have no evidence to suggest or refute this possibility.

While other indices of coagulation activation in plasma exist, we selected these end points of hemostasis and hematol-
logy, along with counts of formed blood elements, as well-documented and standardized procedures that could be most readily interpreted clinically. Recently, Nadziejk and coworkers (1999) undertook studies to assess coagulation after exposure of rats to concentrated New York City PM, using related indices of intravascular coagulation: an ELISA assay of thrombin-antithrombin III (ATT) complex as an index of prothrombin activation and potential intravascular thrombosis, and tissue plasminogen activator (T-PA)/plasminogen activator inhibitor-2 (PAI) activities as indices of the fibrinolytic capacity of plasma. These measures focus on the overall coagulation process, and to date, as we have found with ROFA, no significant thrombogenic activity associated with ambient PM has been revealed (personal communication). Interestingly, Donaldson et al. (1998) showed a significant increase in Factor VII of the extrinsic coagulation pathway in rats for up to 168 h after a 7-h exposure to ultrafine carbon black (1 mg/m³). Increased levels of Factor VII have been associated with ischemic heart disease in humans (Meade et al., 1986).

Levels of ambient PM have been associated with morbidity and mortality from both ischemic heart disease (Schwartz et al., 1996; Schwartz and Morris, 1995; Wichmann et al., 1989) and stroke (Schwartz, 1994; Wordley et al., 1997) in humans. Seaton et al. (1995) have postulated that the rise in cardiovascular events associated with particulate air pollution is due to increased blood coagulability, resulting from alveolar inflammation induced by particle exposure. Peters et al. (1997) have added support to this hypothesis by documenting an elevation in plasma viscosity, a risk factor for ischemic heart disease (Lowe et al., 1997; Sweetnam et al., 1996; Yarnell et al., 1991) and stroke (Lowe et al., 1997), in men and women during an air pollution episode. Peters et al. (1997) also reported a direct association between plasma viscosity and PM concentrations. While the present study may appear to give some credence to the hypothesis, the dose of ROFA to the lung at which elevated fibrinogen was observed was substantially higher than a likely human PM exposure dose. The associated high-dose ROFA injury and inflammation obviously did not affect the APIT or PT either, but fibrinogen was indeed elevated. In contrast, the fibrinogen response was not observed at the lower doses of ROFA. It is unclear to what extent the fibrinogen response reflected an acute phase reaction to the lung injury or whether repeated exposures could induce a cumulative response of elevated fibrinogen. Nevertheless, as fibrinogen is a risk factor for ischemic heart disease and stroke, alteration of important hematologic parameters secondary to an inflammatory response to PM may initiate a cascade whereby cardiovascular events might be triggered in susceptible individuals at relatively low exposure doses. However, at present only qualitative evidence exists to provide a feasible link between ambient PM-mediated alterations of plasma viscosity and elevated plasma fibrinogen in the human epidemiology and analogous responses in rats exposed to a toxic emission source PM.

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