Toxicity of Inhaled Traffic Related Particulate Matter

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Abstract. Traffic generated ultrafine particulates may play a major role in the development of adverse health effects. However, little is known about harmful effects caused by recurring exposure. We hypothesized that repeated exposure to particulate matter results in adverse pulmonary and systemic toxic effects. Exposure to diesel engine exhaust resulted in signs of oxidative stress in the lung, impaired coagulation, and changes in the immune system. Pro-inflammatory cytokine levels were decreased in some regions of the brain but increased in the striatum implying that exposure to diesel engine exhaust may selectively aggravate neurological impairment. Data from these three studies suggest that exposure to traffic related PM can mediate changes in the vasculature and brain of healthy rats. To what extent these changes may contribute to chronic neurodegenerative or vascular diseases is at present unclear.

1. Introduction
Epidemiological studies have shown associations between both short-term and chronic exposure to particulate matter (PM) and shortening of life expectancy [1, 2]. In addition, several studies revealed that emissions from highways result in inflammation in the lung and cardiovascular changes [3, 4]. Ultrafine particulates (UF) which are part of diesel soot may enter the systemic circulation and might trigger systemic effects [5]. However, little is known about adverse effects from prolonged exposure. Therefore, we hypothesized that repeated exposure to PM results in exacerbation of adverse pulmonary effects which will lead to systemic toxic effects.

To test this hypothesis PM effects were studied in the presence of a mild inflammation status, induced by ozone. The assumption is that PM by itself is not causing (significant) health effects, but can induce or worsen them when some inflammation is present. Exposure to particulates could then result in oxidative stress which can not be handled adequately. As a result various processes may be initiated which eventually lead to negative consequences on overall health status.

2. Material and methods
Male Fischer F344/CrlBR rats of 15-16 weeks old were randomly allocated to either control or exposure groups (n=15/group). Three independent experiments were performed in which animals were exposed to different PM exposures in addition to HEPA-filtered air (clean air).
2.1. Exposure
All animals were exposed to 0.4 ppm ozone for 12 hours prior to (concentrated) PM exposure to induce a mild pulmonary inflammation at the onset of exposure. Thereafter, animals were exposed for 4 weeks, 5 days/week for 6 hours a day to clean air or the following various PM exposures:
   A) concentrated ambient particles (0.15-2.5 μm) at an urban background (CAPs background) using an Ambient Fine Particle Concentrator (AFPC);
   B) diluted diesel engine exhaust (DEE) obtained by mixing a small flow of freshly generated diesel engine exhaust taken from a stationary (1500 rpm) diesel engine (35 KVA Genset) into conditioned and purified air or;
   C) concentrated PM (<2.5 μm) near a freeway (CAPs freeway) generated by a Versatile Ambient Concentration Enrichment System (VACES).

2.2. Effect parameters
Different parameters were measured to assess the effect of PM exposure on inflammation, cell damage and oxidative stress. In addition, pathological and haematological changes were examined. Vascular function was assessed in arteries using the myograph system. Different brain sections were taken to investigate the inflammatory response in different brain regions. All effects were investigated 24 hours after termination of the exposure.

3. Results
3.1. Exposure
Particle mass levels ranged between 150 and 650 μg/m3 with the highest concentrations (and variation) observed for the freeway study. Particle number concentrations were relatively low for the urban background location (26,000 cm⁻³) and substantially higher for the DEE (430,000 cm⁻³) and the freeway (260,000 cm⁻³) exposures due to a larger contribution of freshly emitted particles.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Oxidative stress and inflammation measured in the bronchioalveolar lavage fluid (BALF) of rats exposed to different PM exposures or clean air. Data are expressed as percentages relative to clean air exposed animals (mean ± SEM), p ≥ 0.05.

3.2. Effects
There were indications of mild oxidative stress determined by a non-significant increase in the levels of glutathione (GSH) in bronchioalveolar lavage fluid (BALF) of rats exposed to DEE only (figure 1). Although a small non-significant increase in polymorphonuclear cells (PMNs) was detected in BALF after exposure to urban background PM (figure 1), levels of tumor necrosis factor-alpha (TNF-α) were significantly reduced (clean air 8.22 ± 1.26 versus CAPs background 6.06 ± 0.99; p < 0.001).
Exposure to DEE and freeway PM did not induce inflammation (figure 1). In addition, no significant changes were seen in markers related to cytotoxicity. Examination of the lung did not reveal any pathological effect after 4-week exposures.

Exposure to PM from an urban background and DEE resulted in mild increase in von Willebrand factor (vWF) after 1-week exposure and vWF was slightly reduced after 4-week exposure to DEE (figure 2). However, besides a reduction in vWF levels no major effect was detected on the coagulation cascade (fibrinogen, figure 2). Interestingly, white blood cell (WBC) numbers were decreased in animals exposed to DEE (figure 2).

![Figure 2](image.png)

Figure 2. Endothelial damage, immune modulation and blood coagulation measured in blood of rats exposed to different PM exposures or clean air. Data are expressed as percentages relative to clean air exposed animals (mean ± SEM), *p≤ 0.05.

No changes were observed in vascular function in arteries as measured by ex vivo responses to vasodilators in rat aortic rings (figure 3). No significant effect on responses to the endothelium-dependent vasodilator, acetylcholine (figure 3), the endothelium-independent vasodilator, sodium nitroprusside, or the NO-independent vasodilator, isoprenaline, was observed after exposure to different traffic related PM exposures.
A decrease in pro-inflammatory cytokine levels was noticed in some parts of the brain of animals exposed to urban background PM and DEE while these cytokines were enhanced in the striatum after DEE exposure (figure 4).

**Figure 3.** Ex vivo response to the endothelium-dependent vasodilator acetylcholine in rat aortic rings after exposure to different PM exposures or clean air. Vasodilator response is expressed as percentage of the maximal contraction to phenylephrine, $p \geq 0.05$.

**Figure 4.** Pro-inflammatory marker TNF-α in different brain regions of rats exposed to different PM exposures or clean air. Abbreviations: OB+T – olfactory bulb and tubercles; SC+M – spinal cord and medulla. Data are expressed as mean $\pm$ SEM, *$p \leq 0.05$.

### 4. Conclusions

The presented studies suggest that exposure to traffic related PM can mediate changes in the vasculature and brain of healthy rats. The fact that only very mild effects were detected may be a consequence of the relatively healthy animal model that has been used in these studies, despite the fact that pulmonary inflammation was induced at the onset of the PM exposures.

Prolonged exposure to PM (including gaseous co pollutants) mainly from traffic at levels roughly 10 times higher than ambient did not result in significant toxic effects in rats suggesting that a threshold for PM induced effects may exist. In general, no clear signs for a more harmful effect of DEE compared to ambient PM was noted, irrespective the contribution of any gaseous component. DEE seems to be a more powerful oxidant to which the defence system has been adapted.

Reduced numbers of white blood cells were observed after exposure to diesel engine exhaust suggesting that the immune system is affected and can not react adequately or these cells might adhere to the vessel wall. The increase in pro-inflammatory markers in the striatum implies that exposure to diesel engine exhaust may selectively aggravate neurological impairment. Data on CAPs freeway are not available yet. To what extent the changes in the vasculature and brain may contribute to chronic neurodegenerative or vascular diseases is at present unknown.
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