Study Protocol

Title of Research Study: Effects on the Olfactory Epithelium and the Olfactory Nerve from Exposure to Diesel Exhaust

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Scientific Research Question

The overall goal of this research project is to study and deepen our understanding of the health effects associated with exposure to air pollution generated by the combustion of diesel, focusing on the nervous system.

Specific aim:

- To study the oxidative and inflammatory effects of exposure to diesel exhaust on the respiratory epithelium and the olfactory nerve in healthy individuals. Additionally, the study aims to determine whether these exposures result in measurable effects on the sense of smell and cognitive ability.

Background

UNICEF recently published a report highlighting the health effects of air pollution from a global perspective, with a particular focus on the situation for children. It is estimated that air pollution causes approximately 7 million deaths each year globally, with around 600,000 of these deaths being children under 5 years old. Indoor and outdoor air pollution are the 3rd and 9th leading causes of death, respectively, contributing to about 3.5 million premature deaths in 2010.

The Swedish Environmental Research Institute IVL has estimated the Swedish healthcare and societal costs due to the negative impacts of air pollution on heart attacks, strokes, asthma, chronic obstructive pulmonary disease (COPD), and pneumonia at 42 billion SEK annually.

Living in areas with high levels of air pollution is a risk factor for diseases of the central nervous system, including Alzheimer's disease (AD) and cognitive impairment in the elderly, Parkinson's disease, and stroke.

It has also been demonstrated in people living in highly polluted urban areas that fine particles (PM2.5) have been found in the brain, showing that iron particles (magnetite) can be translocated to the brain. The entry point is the olfactory nerve, where particles can enter directly without having to penetrate the blood-brain barrier to reach the brain. It is primarily ultrafine particles (with an aerodynamic diameter <100-200 nm) that can enter through the olfactory nerve via inhalation. Particles of this size range originate from both motor exhausts and biomass combustion, depending on the combustion technique.

The olfactory nerve area is thus an important region of interest for understanding how particulate matter (PM) damages the brain. Interestingly, olfactory dysfunction has been reported in elderly women exposed to high levels of PM (19). It is critical that this possible entry route into the central nervous system is studied in detail to better understand the health effects of PM exposure in humans and to identify potential new biomarkers for air pollution effects.

Dementia is a major cause of morbidity and mortality worldwide. By 2050, the number of Alzheimer's disease (AD) patients is expected to rise to over 130 million, up from 47 million today. In a study from northern Sweden, researchers (Forsberg et al.) demonstrated that exposure to traffic-related air pollutants may be a risk factor for the development of vascular dementia and Alzheimer's disease. Alzheimer's disease is the most common neurodegenerative disease, and there is currently no cure. Current medications only alleviate symptoms. Neuropathological changes in AD include the deposition of protein aggregates, neuroinflammation, and oxidative stress. Despite the clinical evidence that olfactory dysfunction precedes AD-associated cognitive decline by several decades, the significance of air pollution effects on the olfactory area has not been studied in human trials.

Lipophilic particles such as diesel exhaust have been shown in animal studies to penetrate the brain via the olfactory epithelium and the olfactory nerve, causing neurological effects. Particles can, depending on the hydrocarbon profile on their surface and protein interactions, be transported along neurons, leading to post-translational neurogenic effects. This is likely not unique to the central nervous system but may also occur in the airways. We have previously demonstrated CNS effects using quantitative EEG analysis, which is consistent with increasing epidemiological reports of neurogenic impacts of air pollution.

Our colleagues in Finland have established a new physiologically relevant human model of olfactory epithelium from healthy non-demented controls and Alzheimer's patients that can be used to study the effects of air pollution. By examining the acute effects on the olfactory nerve following exposure to diesel exhaust, this is a crucial step in advancing knowledge about the health effects of these two major contributors to PM.

Inclusion Criteria and Population

Healthy non-smoking participants aged 20-70 years with an equal gender distribution will be recruited.

The participants will be examined regarding clinical status, lung function, prick test, ECG, blood status, bleeding status, Na+, K+, and creatinine.

Exclusion Criteria

1. Allergic rhinitis
2. Asthma
3. Diabetes mellitus
4. Renal or liver failure
5. Angina or previous ventricular arrhythmia
6. Systolic blood pressure >190 mmHg or <100 mmHg
7. Previous blood disorders
8. Blood donation within the past three months

9. Previous or ongoing tobacco smoking and exposure to air pollution in the workplace
10. Lack of informed consent

Project Description

The studies will be conducted using our well-established techniques and methods. Exposure to controlled levels of motor exhaust from diesel fuel has been conducted for more than two decades, and in recent years we have also developed a collaboration with Associate Professor Christoffer Boman's group at the Thermochemical Energy Conversion Laboratory (TEC-Lab) at Umeå University. This offers a unique opportunity to study under different combustion conditions.

Hypothesis: Diesel exhaust cause cell dysfunction in the olfactory system secondary to mitochondrial dysfunction and oxidative stress. Additionally, we hypothesize that these exposures result in measurable effects on the sense of smell and cognitive ability.

Project Description

The studies will be conducted using our well-established techniques and methods. Exposure to controlled levels of diesel fuel exhaust has been studied for over two decades.

Hypothesis:
Diesel exhaust gases cause cell dysfunction in the olfactory system secondary to mitochondrial dysfunction and oxidative stress. The hypothesis also includes studying whether exposures lead to measurable effects on the sense of smell and cognitive abilities.

Study Design

Forty people will be exposed to diesel exhaust gases and filtered air. Exposures will be blinded and randomized. Both groups will undergo mucosal biopsies of the olfactory epithelium after local anesthesia following exposure.

All samples collected during the study will be coded, and data will be processed in a blinded manner.

ResearchParticipants:
Forty healthy, non-smoking individuals aged 20-70 years will be included. Strict inclusion and exclusion criteria will be applied based on a medical assessment as outlined above.

Exposure to Diesel Exhaust

Diesel exhaust will be generated using a Volvo diesel engine. More than 90% of the exhaust will be shunted, with the remaining portion mixed with filtered air (HEPA filter) and directed into the exposure chamber at a concentration of 300 µg/m³ using techniques established over decades. The exposure will last for 1 hour, blinded and randomized. During the exposure, participants will alternate between 15-minute periods of rest and exercise on an ergometer cycle with a load that ensures a minute ventilation of 20L/min/m² body surface area. Symptoms will be recorded every 30 minutes based on a modified Borg scale.

Olfactory Epithelium Biopsy

After local anesthesia with Xylocain following exposure, a small biopsy of the nasal mucosa will be taken by a specialist doctor. Material from the nasal biopsies will be used both for culture experiments and for later analyses, including PCR analysis after freezing at -80°C, and will be sent to our collaborators, Dr. Katja Kanninen and Dr. Pasi Jalava at the University of Eastern Finland. Nasal mucosa biopsies have been performed previously at the ENT department at Umeå University following ethical approval. Complication risks are virtually nonexistent, but there may be minor discomfort or slight nasal bleeding, which can be easily stopped using a compress applied to the biopsy site. ENT doctors are experienced in managing nasal bleeding. Biopsy sampling will be randomized between the left and right sides across two exposure sessions, with the contralateral biopsy taken after the second exposure.

In-Vitro Studies and Biomarker Analysis

Effects at the cellular level of exposure to air pollution will be studied in olfactory epithelial cells using in-vitro tests at the University of Kuopio, Finland.
RNA sequencing and DNA methylation will be conducted to identify biomarkers for acute exposure to air pollutants at the Institute of Experimental Medicine, Czech Academy of Science, Czech Republic.

Olfactory Testing

The Sniffin' Sticks test (Hummel et al., 1997), a commercially available test, will be used to determine the participant's odor detection threshold. The odorant (n-butanol) is presented in marker pens filled either with n-butanol or a blank (no odor). The test uses 16 concentrations of n-butanol, from the weakest concentration (1.2 ppm n-butanol) to the strongest (4% n-butanol). In a "triple-forced-choice paradigm," the detection threshold is determined using a staircase method (Doty, 1991). Three pens are presented in a randomized order—two with blank stimuli and one with the odorant at a given concentration. The participant's task is to identify which pen contains the odorant.

Participants wear eye masks to prevent visual identification of the pens. The pen is placed approximately 2 cm below the nostrils for about 3 seconds. The test starts at the 8th concentration level, and if the participant correctly identifies the odorant four times in a row,

the test continues with higher concentrations. If the participant fails to do so, the test proceeds to lower concentrations.

The test has good test-retest reliability (Albrecht et al., 2008) and validity (Ribeiro et al., 2016). The exposure levels of n-butanol are much lower than the occupational exposure limit of 15 ppm (45 mg/m³), so no toxic effects or discomfort risks are expected.

Cognitive Testing

Cognitive ability will be assessed using the Letter Digit Substitution Test, a paper-and-pencil test that evaluates working memory, processing speed, and attention. A key at the top of the page provides the correct mapping for the numbers 1 to 9, which are paired with specific letters. Below the key, there are 120 letters (9 different ones), each connected to a blank square. The participant's task is to write as many numbers as possible in the empty squares under a 60-second time limit according to the letter-number mapping provided in the key.

The test has good test-retest reliability (Hoex et al., 2002) and validity (Natu & Agarwal, 1995). There is no risk of pain or discomfort associated with the test.

Blood Tests

Blood samples will be taken before exposure and before olfactory epithelium biopsies. These will be analyzed for cell count, inflammation markers, and lymphocyte subtyping using flow cytometry. A maximum of 100 ml of blood will be drawn during each exposure.

The effects of exposure to air pollution at the cellular level will be studied in monocytes from peripheral blood using in-vitro tests. Further analyses, including PCR (polymerase chain reaction), will be conducted for the APOE gene, a genetic factor that can influence sensitivity to air pollution. These analyses will be performed at Kuopio University, Finland.

RNA sequencing and DNA methylation will also be conducted to identify biomarkers for acute exposure to air pollution at the Institute of Experimental Medicine, Czech Academy of Science, Czech Republic.

COVID-19 Testing

Participants will be tested for SARS-CoV-2 before each exposure session.

Significance

Air pollution causes significant health effects on the lungs, cardiovascular system, and nervous system, which affects many patients and places a heavy burden on healthcare systems, with societal costs exceeding 40 billion SEK annually in Sweden. Our research aims to deepen the understanding of the multifaceted health effects of air pollution, which impacts various organ systems. This is especially important as we have previously shown that current medications do not prevent the full extent of the health effects of air pollution. Advanced research will lead to a better understanding and the potential development of more effective interventions, which is crucial for health as well as for industries and authorities.

we are one of the few research groups with the knowledge and resources to conduct human exposure studies to examine this specific type of combustion and subsequently investigate its health effects using unique and advanced methods. Therefore, this project is highly significant in increasing our knowledge of how diesel exhaust negatively affects the olfactory nerve, which is crucial for understanding the mechanisms behind the health effects of air pollution, such as the development of cognitive decline and dementia. The findings may have important implications for public health policies and strategies to mitigate the impact of air pollution on cognitive health.