Effects of air pollution on haemostasis and atherosclerosis

Summary

Air pollution, as a part of urban life, consists of gases (carbon monoxide; CO, nitrogen oxide; NO, nitrogen dioxide; NO_2, and ozone; O_3) and small particles (particulate matter; PM) varying in aerodynamic diameter (< 10 μm; PM10, <2.5 μm; PM2.5 and < 0.1 μm; ultrafine particles; UFPs) and chemical composition. Exposure to air pollution has been linked with changes in cardiovascular health, including enhanced systemic inflammation, blood coagulation, thrombosis and atherosclerosis. Although the most responsible components of air pollution with regard to adverse health effects are not fully understood, several studies focus on PM. The effects of PM (based on their aerodynamic diameter) on blood coagulation, platelet function and thrombosis have been summarized in **chapter 1 part I**. In general, PM may enhance blood coagulation through different mechanisms and may contribute to the risk of developing cardiovascular diseases. Given the close link between inflammation and blood coagulation, PM may trigger procoagulant responses via inflammatory cytokines such as interleukin -6 (IL-6). In addition to this indirect effect of PM on blood coagulation, UFPs may directly initiate blood coagulation since direct translocation of these small particles from the lungs into circulation has been suggested by several studies. Induced platelet activation, venous and arterial thrombosis have also been associated with exposure to PM.

In addition to procoagulant and protrombotic effects, in long term, PM may also contribute to the progression of atherosclerosis via distinct pathways, which are addressed in **chapter 1 part II**. There is clear evidence from experimental and epidemiological studies that PM affect progression of atherosclerosis at different stages from the initiation phase to advanced atherosclerosis. A mechanistic outlook suggests that proapoptotic, prooxidative and inflammatory effects of PM modulate the proatherogenic events. PM induced oxidative stress and DNA damage ultimately mediates endothelial dysfunction directly (UFPs) or via systemic inflammation. Endothelial dysfunction and following accumulation of low density lipoprotein (LDL) and induced oxidation of LDL (oxLDL) are the first events related to initiation phase of atherosclerosis. At this early stage of atherosclerosis, adhesion and activation of monocytes and migration of T lymphocytes into the subendothelial space are known events. Uptake of oxLDL by macrophages leads to the formation of foam cells, a characteristic cell constituent of atherosclerotic lesions. It has been shown that PM activates leukocytes particularly monocytes and induces foam cell formation which sustains the proinflammatory responses within the vasculature. Since atherosclerosis accepted as an ongoing inflammatory disease in life time, continuous exposure to PM is leading to advance atherosclerotic plaque formation and cardiovascular complications.

In **chapter 2** of this thesis, the effects of exposure to PM10 and corresponding gas pollutants CO, NO, NO_2 and O_3 on inflammation and blood coagulation were determined in a population study with 40 healthy subjects. The time lags within 24h before blood sampling were considered to represent direct effects of air pollution whereas time lags within 24-96h before blood sampling represented indirect effects. Light-transmittance platelet aggregometry, thrombin generation (tissue factor dependent), fibrinogen, and C reactive protein (CRP) were measured in blood samples collected consecutively 13 times within a 1-year period from study subjects. Air pollution (except O3) was associated with indirect effects on platelet aggregation and thrombin generation, but not with the inflammatory markers fibrinogen and CRP.

The gaseous air pollutants, especially NO2 and CO but not PM10 are associated with direct effects on thrombin generation. Since the gas pollutants can be considered markers for motor vehicle traffic and have been shown to be highly correlated with PM2.5 and UFPs, it is mainly this subset of PM2.5 and UFPs from the overall PM air pollution that has an effect on thrombin generation.

In chapter 3, an animal exposure study is presented. In addition to human volunteer study in which the procoagulant effects of PM10 and gas pollutants are shown in **chapter 2**, PM2.5 subset of air pollution is the focus of this chapter. Fisher F344 rats, with a mild pulmonary inflammation (O_3 exposure) at the onset of exposure, were exposed for 4 weeks, 5 days/week for 6 hours a day to diluted diesel engine exhaust (PM_{DEE}), or near roadside PM (PM2.5). Changes in vascular function and tissue factor dependent blood coagulation measured by thrombin generation and tissue factor activity in the lungs were investigated in extent to pulmonary and cardiovascular effects of traffic related PM2.5 and diesel exhaust in rat. The observed changes were a reduction in white blood cell numbers, diminished levels of von Willebrand Factor (vWF) protein, and reduced lung tissue thrombin generation and tissue factor activity. Consistent with these findings, other studies also showed decreased vWF in patients with pulmonary disease and metabolic syndrome after exposure to PM. The results may be explained to either desensitization to repeated exposure or the increased expression of anticoagulant proteins (such as Thrombomodulin) as a protective mechanism against inflammation due to O_3 exposure at the onset of experiment.

Considering the small size of UFPs and their ability to translocate into the circulation, study presented in **chapter 4** is conducted. PM in different size was tested in human plasma for thrombin generation. All PM materials increased thrombin generation in human plasma via activation of factor XII (FXII) and this finding is confirmed in FXII and FXI deficient plasmas with observation of flat thrombin generation curve. PM induced thrombin generation in human plasma could be abolished by addition of corn trypsin inhibitor (CTI; a specific inhibitor for activated FXII). *In vitro* studies are also suggested that the effect of PM on activation of FXII in plasma can be modulated with both chemical composition and size of PM.

In addition to our *in vitro* findings, UFPs were intratracheally instilled in wild-type (WT) and FXII deficient ($FXII^{-/-}$) mice and plasma thrombin generation was analyzed in plasma from treated mice at 4 and 20 h post exposure. UFPs induced a transient increase in tissue factor driven thrombin formation at 4h post instillation in WT mice compared to saline instillation. Intratracheal instillation of UFPs resulted in a procoagulant response in WT mice plasma at 20 h, whereas it was entirely suppressed in $FXII^{-/-}$ mice. Based on these findings, the study is first established a role for FXII and contact activation in the sustained procoagulant response to PM shown in mice and *in vitro.* In part, this finding enlightens our understanding for PM mediated thrombus formation and clot stability.

In respect to the effects of UFPs and gas pollutants derived from diesel exhaust on tissue factor dependent thrombin generation in healthy human subjects, the study was conducted and the results are presented in **Chapter 5.** Sixteen healthy volunteers were exposed to (i) dilute diesel exhaust, (ii) pure carbon nanoparticulate, (iii) filtered diesel exhaust, or (iv) filtered air, in a

randomized double blind cross-over study. Following each exposure at 2, 6 and 24 hours, blood samples were collected and plasma thrombin generation assessed in the presence or absence of 1 pM tissue factor. In consistent with our earlier experimental study in which we demonstrated no tissue factor mediated blood coagulation in mice at 4 and 20 hrs, the study results also did not suggest any effect of the particulate or gaseous components of diesel engine exhaust on the extrinsic blood coagulation in humans. However, activation of intrinsic pathway of coagulation should be the focus of future studies in respect to prothrombotic effects of diesel exhaust.

In Chapter 6, the effects of exposure to diesel and biodiesel (an alternative to traditional petroleum diesel) particles on progression of atherosclerosis was studied. $LDL^{-/-}$ mice were intratracheally instilled to comparable concentrations of diesel and biodiesel particles for once a week, 5 times during 6 weeks and fed with high fat diet. A collar was placed on the left carotid arteries of mice on 3^{th} week. Left carotid arteries were embedded, sectioned and the atherosclerotic plaques were investigated by immunohistochemical analysis. There were no differences in plaque burden, intima/media ratio, total vessel lumen and macrophage content after both diesel and biodiesel administration compared to saline. On the other hand, exposure to biodiesel particles resulted in more vulnerable atherosclerotic plaques characterized by loss of smooth muscle cells, bigger necrotic areas, thinner fibrous cap and less collagen content than diesel particles. Additionally, atherosclerotic lesions were more proapoptotic and prooxidative after exposure to biodiesel particles compared to atherosclerotic plaques after diesel particles treatment. Although biodiesel is considered to be less hazardous, our results suggested a greater vulnerable atherosclerotic plaque formation with biodiesel particles. These effects should be considered before replacement of petroleum diesel with biodiesel.

In **chapter 7**, based on the main findings of this thesis, the effects of air pollution including PM and gas pollutants on hemostasis and atherosclerosis are discussed. Exposure to particulate air pollution contributes in the long-term to the development and progression of atherosclerosis and, in the frame of short-term exposures, triggers hypercoagulation, thrombosis. Taken together, presented studies bring a mechanistic outlook for better understanding of air pollution induced cardiovascular diseases.