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Linking exposure to environmental pollutants with biological effects

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Abstract

Exposure to ambient air pollution has been associated with cancer. Ambient air contains a complex mixture of toxics, including particulate matter (PM) and benzene. Carcinogenic effects of PM may relate both to the content of PAH and to oxidative DNA damage generated by transition metals, benzene, metabolism and inflammation.

By means of personal monitoring and biomarkers of internal dose, biologically effective dose and susceptibility, it should be possible to characterize individual exposure and identify air pollution sources with relevant biological effects. In a series of studies, individual exposure to $PM_{2.5}$, nitrogen dioxide (NO_2) and benzene has been measured in groups of 40–50 subjects. Measured biomarkers included 1-hydroxypyrene, benzene metabolites (phenylmercapturic acid (PMA) and *trans-trans*-muconic acid (ttMA)), 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in urine, DNA strand breaks, base oxidation, 8-oxodG and PAH bulky adducts in lymphocytes, markers of oxidative stress in plasma and genotypes of glutathione transferases (GSTs) and NADPH:quinone reductase (NQO1).

With respect to benzene, the main result indicates that DNA base oxidation is correlated with PMA excretion. With respect to exposure to PM, biomarkers of oxidative damage showed significant positive association with the individual exposure. Thus, 8-oxodG in lymphocyte DNA and markers of oxidative damage to lipids and protein in plasma associated with $PM_{2.5}$ exposure. Several types of DNA damage showed seasonal variation. PAH adduct levels, DNA strand breaks and 8-oxodG in lymphocytes increased significantly in the summer period, requiring control of confounders. Similar seasonal effects on strand breaks and expression of the relevant DNA repair genes *ERCC1* and *OGG1* have been reported.

In the present setting, biological effects of air pollutants appear mainly related to oxidative stress via personal exposure and not to urban background levels. Future developments include personal time-resolved monitors for exposure to ultrafine PM and $PM_{2.5,}$ use of GPS, as well as genomics and proteomics based biomarkers.

Keywords: Environmental pollutants; Particulate matter; Biomarkers; Oxidative stress; PAH adducts; Benzene; 8-oxodG

Abbreviations: PM, particulate matter; NO_2, nitrogen dioxide; PMA, phenylmercapturic acid; ttMA, *trans-trans*-muconic acid; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; GST, glutathione transferase; NQO1, NADPH:quinone reductase; *CYP*, cytochrome P450; ETS, environmental tobacco smoke; 1-HP, hydroxypyrene; ROS, reactive oxygen species; DEP, diesel exhaust particles; AM, alveolar macrophages; SB, strand breaks; PLAAS, 2-aminoadipic semialdehyde in plasma proteins; MDA, malondialdehyde; FPG, formanidopyrimidine DNA glycosylase

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1. Introduction

Numerous epidemiological studies have shown an increased morbidity and mortality due to ambient air pollution [1,2]. Ambient air contains a complex mixture of toxics, including particulate matter (PM), irritant gases and benzene. PM is the component of air pollution believed to be responsible for many of these adverse health effects, and several studies have shown a positive association between overall daily mortality and ambient particle concentrations [1]. Long-term exposure to high particle levels increase risk of cancer, respiratory diseases and arteriosclerosis, whereas short-term exposure-peaks cause exacerbation of bronchitis, asthma and other respiratory diseases as well as changes in heart rate variability [2–4]. The size fraction of PM may have different effects, i.e. $PM_{2.5}$ and particularly ultrafine PM may be more potent than coarse PM. Carcinogenic effects of PM may relate both to the content of PAH and oxidative damage to DNA generated by transition metals, benzene, metabolism and/or inflammation. However, at low concentrations exposure assessment and establishing relationships with biological effects may be difficult. By means of monitors of individual exposure to air pollutants and of biomarkers of internal dose, biologically effective dose and early biologically effect as well as markers of individual susceptibility, such relationships may be documented (Fig. 1).

2. Measuring personal exposure to particles

Almost all studies of particle-related adverse health effects have relied upon urban background measurements as a surrogate for exposure of all individuals in a population. However, people spend around 90% of their time indoors [5], and it is widely recognized

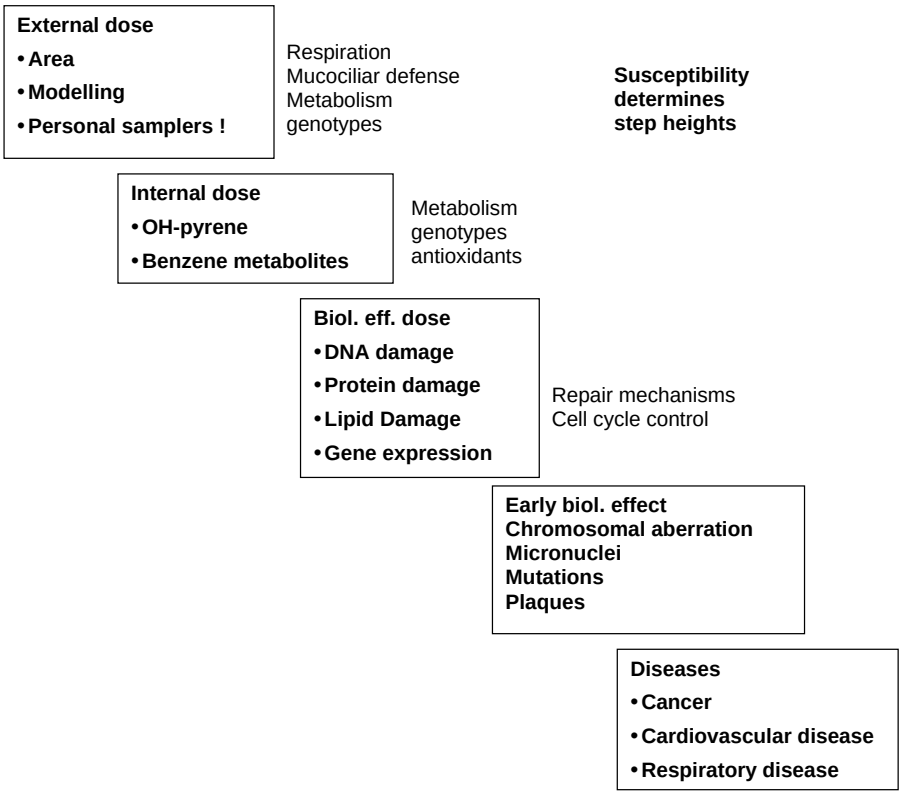

Fig. 1. Stairway of biomarkers of exposure related to air pollution.

that a significant proportion of personal exposure to particles occurs in indoor environments. Therefore, a more detailed and systematic knowledge of personal exposure will improve the ability to estimate personal exposure in future epidemiological studies.

Indoor particles consist of a combination of ambient particles that readily penetrate buildings and infiltrate indoor air [6,7], and non-ambient particles generated indoors during daily activities of home occupants. Several studies have investigated the generation of non-ambient particles in indoor environments, and various particle sources have been characterized [6,8–10]. Smoking has been found to be one of the major contributors to indoor particle concentrations [6], along with cooking [11,12] and other general activities involving combustion (e.g. burning candles), physical movement and ventilation [6,8,10,13].

During the last decade, several studies have investigated the relationship between ambient $PM_{2.5}$ concentrations and personal $PM_{2.5}$ exposure. Taken the above description of indoor sources of $PM_{2.5}$ into consideration, it is not surprising that only weak or no associations have been found [6,11,14–16]. Stronger associations are found between personal exposure and residence indoor $PM_{2.5}$ concentration. When comparing the actual concentrations of personal $PM_{2.5}$ exposure with indoor and outdoor concentrations, the personal exposure levels are found to be the highest: the ratios personal/indoor as well as personal/outdoor range are between 1 and 3 depending on e.g. smoking status, season and occupation [11,16,17]. The degree of association between personal exposure and outdoor $PM_{2.5}$ concentration varies notably in these studies. Interestingly, some of the stronger associations have been found in elderly study populations [15].

3. Biomarkers of internal dose and air pollutants

Measurement of personal exposure to particles and other ambient air toxics in relation to adverse health effect may be difficult. However, by means of biomarkers mechanistically related to the relevant health effect it may be possible to assess relevant exposure to particulate matter and the involved sources (Fig. 1). A biomarker should be sensitive and specific to only the source of exposure examined but unfortunately this is very rarely the case.

A biomarker of internal dose is an exposure compound or its metabolites, which is measurable in biological media, most commonly urine and blood. Identification of biomarkers of internal dose associated with PM is complicated because particles in contrast to many other pollutants are of complex chemical, physical and biological composition and because the causative constituents have not yet been fully determined. A relevant method is to measure a single compound or its metabolites known to be present in the particle fraction of interest.

3.1. PAH metabolites

Metabolites of PAHs have been proposed as biomarkers of recent exposure to particles [18]. PAHs are believed to be important compounds among the genotoxic agents present in urban air, where they are primarily associated with the respirable fraction of particles. They are formed by incomplete combustion of fuel and by pyrolysis of organic materials, during, e.g. roasting [19,20]. A recent review of internal dose biomarkers of PAH in environmental health suggested 1-hydroxypyrene (1-HP) in urine to be the most relevant parameter for estimating individual exposure to PAH [21]. 1-HP is a major metabolite of pyrene [22]. Since pyrene is always present in PAH-mixtures, 1-HP is commonly used as overall marker of PAH exposure [23]. It is excreted in urine either unbound or conjugated with glucoronic acid. The half-life for urinary 1-HP excretion has been found to range from 6 to 35 h in coke-oven workers [24]. Pyrene is not a specific marker for particle PAH as it is mostly found in the volatile fraction of diesel exhaust.

Several studies have investigated 1-HP excretion in individuals occupationally exposed to high levels of PAHs, and elevated 1-HP levels have been shown in, e.g. coke-oven workers and road pavers [25,26]. In non-occupationally exposed individuals, elevated 1-HP levels have been found in smokers and in subjects ingesting charbroiled meat [24,27,28]. Similarly, the relationship between ambient particle levels and 1-HP excretion have been investigated in non-occupationally exposed populations [18,29]. One study examined 1-HP excretion in non-smoking inhabitants from two Polish cities that showed marked differences in ambient particle concentrations [29]. Inhabitants from the city with highest PM_{10} lev-

els (>120 μg/m^3) excreted significantly higher 1-HP levels than inhabitants from the less polluted city (<70 μg/m^3) [29]. Another study found associations between excretion of glucoronid 1-HP and air particulate measures in six regions in South Korea [18]. This indicates that 1-HP may be useful as exposure marker of particles. However, other studies have compared subjects from urban areas with subjects from suburban areas and have found no significant differences in 1-HP levels [30,31]. Moreover, one study found that although personal exposure to benzo[a]pyrene in total suspended particulate (TSP) in non-smoking traffic police officers (Genoa, Italy) was many times higher than in the non-smoking reference group, there was no significant differences in 1-HP excretion [32].

Recently, personal exposure to $PM_{2.5}$ and black smoke was measured in 50 non-smoking students living and studying in central parts of Copenhagen [33]. The measurements were repeated four times in 1 year to cover seasonal variation. Particle measurements were carried out during 2-day periods for each subject. Twenty-four hour urine samples were collected at day 2 of each measuring period. No significant association between 1-HP excretion and personal exposure to $PM_{2.5}$ or black smoke was found [33]. There are several possible explanations for this. Firstly, these results were not adjusted for PAH in diet or other possible confounders. Secondly, the relative proportion of pyrene in particles is influenced by several factors such as the source of combustion and the weather conditions [24]. This weakens the association between the actual 1-HP source, pyrene, and the measured particle concentration. Only one other study known to us has investigated relationship between 1-HP excretion and actual measurements of personal exposure to particles in non-occupationally exposed subjects, and also here no associations were found [31]. This strongly suggests that 1-HP is not sensitive enough to be used as a biomarker of particle exposure in the non-occupational exposed population. Unfortunately, no other accepted biomarkers of exposure to particles in terms of internal dose are now in use.

3.2. Benzene metabolites

Identification of exposure markers is less complicated when exposure involves only one compound as in the case of benzene exposure. Benzene has been shown to be mutagenic and carcinogenic in animal experiments and epidemiological studies and is especially associated with bone marrow toxicity and leukemia [34,35]. TtMA and PMA are metabolites of benzene that are excreted into the urine. Especially PMA has been shown to be strongly correlated with external benzene exposure in occupational studies [36,37].

Recently, the relationship between urban benzene exposure and urinary excretion of ttMA and PMA was examined in 40 volunteers living and working in Copenhagen [38]. The measuring period was 5 days. No significant associations were found between benzene exposure and urinary excretion of ttMA or PMA, although after stratification for gender ttMA excretion was correlated to external exposure to benzene with borderline significance in men ($r = 0.45$, $P = 0.06$). It should be noted that the level of benzene exposure was relatively low, on average around 3 μg/m^3, as compared with reported occupational exposure of 1.88 mg/m^3 in road tanker drivers [36] or 99.2 mg/m^3 in Chinese factory workers [37]. Moreover, exposure was measured as cumulated exposure over 5 days whereas the biomarkers of internal dose were measured on only one of these days, which may have weakened the analysis [38]. These examples illustrate some of the difficulties in using exposure biomarkers in the non-occupationally exposed population, even with a more simple exposure source than particles.

4. Biomarkers of biologically effective dose and effects

The exact mechanisms whereby particles exert their toxic effects at the cellular level are not fully understood. Several hypotheses have been suggested, a schematic overview of which is presented in Fig. 2.

4.1. Oxidative stress

There is accumulating evidence that particles are capable of generating or inducing generation of free radicals in humans, thereby leading to an increase in oxidative stress. Several in vitro studies have demonstrated that diesel exhaust particles (DEP) induce production of superoxide and hydroxyl radicals without the presence of any biological activating systems

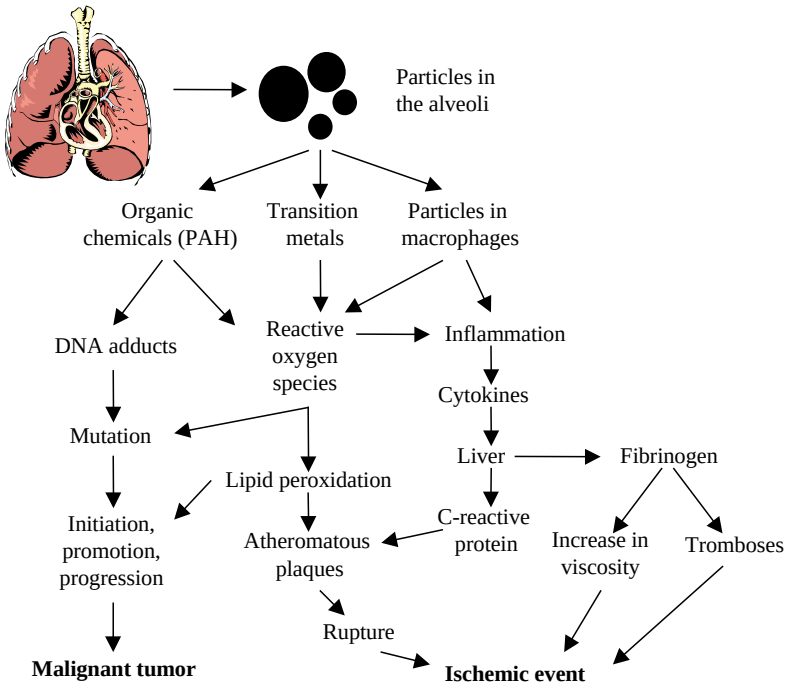

Fig. 2. Diagram of the hypothetical events leading from chronic exposure to particles to cancer and arteriosclerosis.

[39,40]. Currently, three theories of how particles induce oxidative stress have been suggested.

Firstly, particles contain several soluble transition metals on the surface such as iron and copper that can generate hydroxyl radicals through the Fenton reaction [41]. Several studies have reported that iron and other transition metals, such as manganese and nickel present in particles or on their surface induce generation of reactive oxygen species (ROS) in biological systems [42–44]. Metal-induced oxidative stress has been shown to subsequently affect the immune system, e.g. by causing neutrophilic lung injury and release of inflammatory mediators by several lung cell types [44,45]. It has even been suggested that metal-induced generation of oxidative stress is critical to a subsequent particle induction of inflammation [46].

The second mechanism involves reactions by alveolar macrophages (AM) and neutrophils. AMs and neutrophils are phagocytes known to ingest and remove inhaled particles from the lungs [47]. This leads to activation of these cells causing release of several cytokines as well as ROS through a “respiratory burst” [48,49]. It is unclear which components of ambient particles are responsible for phagocyte activation, as increased inflammation have been associated with several components such as metal content, ultrafine particles (surface area), organic compounds and endotoxins [48–51].

The third mechanism involves the organic fraction of particles, which consists of several redox-active quinones, e.g. 9,10-phenanthraquinone and 9,10-anthraquinone [40,52,53]. Quinones can also be generated during metabolism of several PAH, by *CYP1A1* in lung or lever tissue [54]. PAH have been found to induce the *CYP1A1* expression in several lung cell types as AM, Clara cells and type 2 cells [55]. Quinones produce ROS during their metabolism by redox cycling, unless they are catalysed by the phase II enzyme NQO1 [41]. A one-electron reduction of quinone yields a very unstable semiquinone that rapidly reduces O_2 to superoxide, which regenerates the quinone and thus completes one redox cycle. The organic fraction of particles has been shown to induce generation of ROS [56,57]. This induction seems to be

dependent of the presence of NADPH CYP reductase, which indicates an involvement of quinones [58].

4.2. Biomarkers of oxidative DNA damage

Almost all known biomarkers of oxidative damage belong to the category of biomarkers of biological effective dose (Fig. 1). From this follows that they occur early in the exposure-to-disease pathway, and that factors or processes acting at subsequent stages could alter the marker's ultimate impact on health, which would influence their relevance as measures of risk. On the other hand, several of these early damage biomarkers have been shown to be associated with increased risk of developing diseases as cancer and arteriosclerosis in animal models. They are therefore considered as important tools for investigating mechanisms behind exposure-induced adverse health effects.

In relation to cancer, biomarkers of oxidative damage DNA are considered most important in this context. Nearly 100 different oxidative DNA modifications have been identified in vitro ranging from modified bases to single and double strand breaks, although less of these have been detected as stable lesions in tissues of humans or animals [59–62]. One of the most abundant base modifications is C8 hydroxylation of guanine, producing 8-oxo-7,8-dihydro-2′-guanine (8-oxoGua), which is believed to be a major product of hydroxyl radical attack on DNA [63]. It is repaired by base excision repair, which involves the DNA glycosylase 8-oxodG glycosylase (*OGG1*) in humans, nucleotide excision repair, or mismatch repair proteins. Measurements of 8-oxoGua or its corresponding nucleoside 8-oxodG are among the most widely used markers of oxidative DNA damage. The most frequently used analyzing method for this lesion is HPLC with electrochemical detection of either DNA hydrolyzed from tissue samples or urine samples and the comet assay with measurement of FPG sensitive sites [64]. The 8-oxodG level in DNA isolated from tissue is believed to illustrate the steady-state damage of DNA being a result of damage and repair, while 8-oxodG excreted in urine is believed to be an estimate of total DNA excision repair within an organism. As it is assumed that DNA repair under normal circumstances is almost complete, 8-oxodG excretion is also a marker of the rate of total DNA damage [65]. However, 8-oxodG measured in urine may also originate sanitization of oxidations in the cellular pool of GTP by the MTH1 enzyme and from turnover of mitochondria and cells [65].

An increased 8-oxodG level after in vitro and in vivo exposure to PM has been shown [66–72]. For example, extracts of $PM_{2.5}$ filters collected from urban background in five American cities increased strand breaks (SB) measured by comet assay [40]. Several studies have investigated effects of PM, generally DEP, on oxidative DNA damage in experimental animals. These studies have repeatedly found that 8-oxodG increased in lung tissue following intratracheal DEP installation [69,71–73]. This increase appeared not to be related to organic DEP fractions, as methanol, acetone, benzene or hexane extractions did not increase 8-oxodG in mice lungs, whereas both DEP and the residues after extraction did [70]. Effects were reduced by treating DEP with catalase, ethanol, mannitol and desferoxamine suggesting that oxidative damage was dependent on hydroxyl radicals, partly generated by Fenton type reactions [70].

4.3. Air pollution exposure and biomarkers of oxidative DNA damage

Most in vivo exposure experiments use particle concentrations many times higher than those found in urban air, and it is unclear whether increased DNA damage actually occurs at realistic exposure levels. Only few studies have investigated effects of urban particle exposure on oxidative DNA damage in humans. In Copenhagen, 24 h urine excretion of 8-oxodG from 57 non-smoking bus drivers was examined. The bus drivers were studied on a workday and on a day off work. Comparisons were made between drivers from central and rural/suburban areas of Copenhagen. On the workday, 8-oxodG excretion was significantly higher in bus drivers from central areas compared with bus drivers from suburban/rural areas of Copenhagen [74]. Another study measured 8-oxodG (by immunohistochemical method) in nasal biopsies from 87 children living in Mexico City and 12 controls from low-polluted coastal towns [75]. 8-OxodG levels were increased more than two-fold in the children from Mexico City compared with the controls. In addition, SB in the nasal biopsies were also increased in the children from Mexico City. The same study found that SB in nasal cells increased two-fold

from week 1 to week 2 in young men who moved to Mexico City [76]. In contrast to these studies, a Greek study measured SB in lymphocytes in 40 subjects from urban areas (central Athens) and 40 subjects from rural areas [77]. Twenty subjects in each group were smokers. The lymphocytes were also incubated with hydrogen peroxide to test for antioxidant capacity. No significant effects of living area (urban versus rural) on either base level SB or hydrogen peroxide induced SB were found. Smoking, however, significantly increased both base level SB and hydrogen peroxide-induced SB, indicating higher DNA damage and lower antioxidant capacity in smokers. In addition, a large study from the Czech Republic did not find any difference in the level of SB in mononuclear blood cells of inhabitants of a highly polluted area (Teplice) compared with a low polluted area [78].

Smoking and exposure to environmental tobacco smoke (ETS) have been found to be predictors of personal particle exposure in various studies [15]. Several other studies have investigated the effect of tobacco smoking on oxidative DNA damage [65,79,80]. Increased levels of urinary 8-oxodG excretion have repeatedly been found in smokers whereas an increased 8-oxodG levels in lymphocyte DNA has been a less consistent finding [80,81]. Diverse results have been obtained with regard to the level of SB in mononuclear blood cells among smokers and non smokers, probably reflecting types of cigarettes smoked in different parts of the world [82]. Although the biomarkers mentioned above could be influenced by a number of other factors such as diet and age, there appears to be some effect of urban air exposure on DNA damage in humans.

A recent study was the first to relate personal particle exposure to oxidative DNA damage in non-occupationally exposed subjects [33]. Personal exposure to $PM_{2.5}$ and black smoke was measured four times in 50 students living and studying in Copenhagen. 8-OxodG was measured in lymphocyte DNA and in 24 h urine by HPLC, and SB and FPG sensitive sites were measured in lymphocytes by comet assay. Personal $PM_{2.5}$ exposure was found to be a predictor of 8-oxodG concentrations in lymphocyte DNA causing an 11% increase in 8-oxodG per 10 $\mu g/m^3$ increase in personal $PM_{2.5}$ exposure ($P = 0.007$) [33]. There was no relationship with the particle concentrations in urban background.

In the Copenhagen study on $PM_{2.5}$, the effect of exposure to particles was also assessed on protein oxidation as 2-aminoadipic semialdehyde (AAS) in plasma proteins (PLAAS) and lipid peroxidation in plasma as malondialdehyde (MDA) [83]. There was a significant relationship between personal black smoke exposure and PLAAS as well as a borderline significant association ($P = 0.068$) between personal $PM_{2.5}$ exposure and PLAAS. Similarly, personal $PM_{2.5}$ exposure was significantly related to lipid peroxidation in terms of plasma MDA in women [83]. AAS is an oxidation product of lysine and reflects changes in damage over a few days up to several weeks [84,85]. These results are in agreement with an earlier study of Copenhagen bus drivers, which found PLAAS and MDA levels to be significantly higher in bus drivers from central Copenhagen compared with both bus drivers from rural/suburban areas and Copenhagen postal workers [86]. Although PLAAS concentrations in blood can also be influenced by a number of other factors such as diet and age [84,87], this indicates an effect of inhaled particles on protein oxidation in peripheral blood. Oxidation of proteins and lipids may also be important in the pathogenesis of atherosclerosis (Fig. 2).

The Copenhagen study suggest that even at low exposure concentrations particulate matter may induce systemic oxidative stress with effects on DNA as well as other biomolecules [33,83]. There was no significant relationship between background $PM_{2.5}$ concentration and 8-oxodG levels in lymphocytes. This implies that, compared with background $PM_{2.5}$ concentration, personal exposure is more directly related to the component(s) inducing oxidative stress. In the Copenhagen study on $PM_{2.5}$, no relationships were found between exposure and 8-oxodG in urine, SB or FPG sensitive sites.

Epidemiological data show that chronic exposure to particles is associated with increased risk of cancer [88]. 8-OxoGua can base pair with adenine during replication causing a G:C to T:A mutation and is thus hypothesized to be a promutagenic lesion in DEP induced carcinogenesis [69]. The finding of a significant correlation between 8-oxodG induced by DEP and lung tumor incidence in mice strongly supports this hypothesis [89]. Another study examined mutation frequency caused by DEP using *lambda/lacI* transgenic rats (Big Blue system) [72]. After 4 weeks of inhaling DEP, the mutation frequency and also 8-oxodG levels

in lung tissue were significantly increased. Examining the mutations revealed that one of the major mutations was G:C to T:A. This again supports the theory that DEP induced oxidative DNA damage could play a role in the observed increase in cancer.

4.4. PAH and genotoxicity

PAHs comprise a group of organic compounds with two or more fused aromatic rings. Typically, 10–40% of DEP mass consists of organic compounds including PAHs, and PAHs constitute one of the major classes of airborne carcinogens. IARC stated in 1989 that PAHs are among the most potent mutagens and carcinogens present in diesel exhaust [90]. PAHs include several reactive compounds such as quinones, which are believed to be mutagenic through generation of ROS and adduct formation. In addition, several PAHs found to be mutagenic in biological systems are activated into reactive derivatives by metabolizing enzymes within cells. Most often this involves *CYP1A1*-catalyzed formation of epoxides and diolepoxides, which have in numerous studies been shown to form covalent adducts to DNA and protein [91]. PAH-adducts in the DNA can lead to mutations, and a high adduct level is associated with an increased risk of developing cancer [92].

Several biomarkers of PAH exposure are currently available. Besides measurements of internal dose by urinary metabolites, these include biomarkers of biological effective dose such as adducts to DNA and protein (hemoglobin and albumin) [93,94], and circulating anti-BP DNA adduct antibodies [95]. DNA PAH-adducts measured by ^{32}P-postlabeling is frequently the biomarker of choice [78]. For simplicity, adducts measured by ^{32}P-postlabeling will be referred to as DNA PAH-adducts.

Several studies of short-term and long-term DEP exposure in rats have investigated the levels of DNA PAH-adducts following exposure. Many of these studies have suggested a role for PAHs in rat lung tumor response. Firstly, because higher DNA PAH-adduct levels have been found in the lungs of rats exposed to high concentrations of diesel exhaust compared with controls [72,96,97]. Secondly, because the highest adduct levels have been found in peripheral lung tissue where the tumors arise [97]. However, not all studies have confirmed this increase in DNA PAH-adduct levels in diesel emission exposed rats [98]. In addition, inhalation of TiO_2 and carbon black particles, which are associated with only small concentrations of adsorbed organic matter, and diesel particles with over 30% solvent-extractable adsorbed organic matter have in rats been observed to result in similar tumor responses [98,99]. This suggests that organic compounds in diesel exhaust do not play a significant role in the rat carcinogenic response to high concentrations of diesel exhaust.

Many studies have investigated concentrations of DNA PAH-adducts in individuals occupationally exposed to high concentrations of airborne PAH, e.g. foundry workers, and they have generally demonstrated an increase in DNA PAH-adducts with increasing exposure [100]. This indicates that airborne PAHs at high concentrations induce genotoxic effects in humans. In addition, some studies have attempted to address the question whether the low to moderate concentrations of PAH on particles in urban environments can cause genotoxic effects. While higher levels of DNA PAH-adducts were observed in bus drivers working in central parts of Copenhagen compared with a similar group driving in suburban areas [101], DNA PAH-adducts were not elevated in bus drivers of central Stockholm relative to a less exposed control group [102] or in street vendors in Milan working in areas of high versus low traffic density [103]. In the recent Copenhagen study, no relationships were found between personal $PM_{2.5}$ exposure and PAH-adducts in lymphocytes [33]. However, there was no specific information on the actual exposure to PAHs. A recent, similar study investigated personal $PM_{2.5}$ and PAH exposures in 194 non-smoking students living either in the city of Athens or in Halkida in rural surroundings with minimal urban air pollution [104]. Surprisingly, significantly higher levels of DNA PAH-adducts were found in the Halkida subjects than in the subjects from Athens, although the Athens subjects were exposed to significantly higher concentrations of particle-bound PAHs [104]. There were no associations between PAH exposure from $PM_{2.5}$ particles and adduct formation. Data on exposure to ETS and urine cotinine concentrations indicated that exposure to ETS could be a determinant of the DNA PAH-adducts [104]. In the Greek study, biomarkers of effects in terms of HPRT-mutations, sister chromatid exchanges and chromosomal aberrations where also measured without finding relationships with expo-

sure. This strongly indicates that for cohorts exposed to low to moderate particle-bound PAHs no simple correlation with biomarkers of genotoxicity can be detected, possibly due to contributions to the overall genotoxic burden by additional factors [31,33].

Several studies have investigated the risk of cancer in populations occupationally exposed to PAHs. Increased risk of lung and bladder cancer has been demonstrated in populations exposed to levels of PAH orders of magnitude higher than those commonly found in urban air [105]. However, epidemiological studies have failed to provide conclusive evidence of increased cancer risk in study populations such as bus and truck drivers who, due to occupational reasons, were exposed to elevated urban air pollution [105]. In conclusion, it is still unclear whether the presence of PAHs in urban particles is involved in the particle induced cancer risk observed in epidemiological studies.

5. Susceptibility

A considerable inter-individual variability in response to many xenobiotics, has been found and in inhalation toxicology there is a growing interest in the importance of susceptibility, especially related to particles. In epidemiological studies investigating exposure to particulate matter, findings have been driven largely by effects observed in presumed susceptible subpopulations including the aged, especially those with underlying cardiopulmonary diseases, and children with asthma [2]. Besides such acquired characteristics, susceptibility to the harmful action of toxic air pollutants may also be the result of genetic characteristics of individuals. The effect of genetic variability in response to air pollutants has been examined by the use of susceptibility biomarkers. Biomarkers of susceptibility are indicators of sensitivity towards exposure to xenobiotics and they can modify several events in the progression from exposure to disease (Fig. 1).

5.1. Cytochrome P450

Variations in activity of the CYPs may influence adverse toxic effects, such as the ability to generate ROS or mutations, of a number of xenobiotics. Ideally, to estimate the relevance of a CYP genotype in relation to a certain xenobiotic, information on both exposure and effect is necessary. As previously described, identification of exposure and effect biomarkers specific to only PM involves several complications because of the complex composition of particles. Focusing on one compound class such as PAH partially solves this problem. Many PAHs are metabolized by *CYP1A1*, which can result in, e.g. generation of 1-HP and in metabolic activation with subsequent formation of PAH-adducts in DNA. The *CYP1A1* gene is polymorphically expressed in humans, and nine alleles different from the wild-type have been identified [106]. Information on the catalytic activity of different *CYP1A1* alleles relative to the wild-type is sparse. The *CYP1A1*2C* allele protein (allele nomenclature [106]) were found not to differ in vitro in 3-hydroxylation of benzo[a]pyrene compared with the wild-type [107]. Another in vitro study found that when comparing the wild-type (*CYP1A1*1*) with *CYP1A1*2B* and *CYP1A1*4* the wild-type was most active in metabolizing benzo [a]pyrene, with activities relative to the wildtype of 40 and 60%, respectively [108].

*CYP1A1*2A* seems associated with increased risk of lung cancer in smokers, such as squamous cell carcinoma, which is a tumor thought to be related to PAHs, indicating that individuals with *CYP1A1*2A* could have a greater capacity to activate PAHs [109]. However, the influence of *CYP1A1* polymorphisms on metabolic conversion of PAHs into carcinogenic species is not fully understood. Some studies have investigated the effect of *CYP1A1* genotypes in subpopulations exposed to high levels of airborne PAH. In smokers [110] and foundry workers [111], a significant higher DNA PAH-adduct level in lung and blood cells was found in individuals homozygote for *CYP1A1*2A* relative to individuals having at least one wild-type allele. In addition, some studies have explored the association between *CYP1A1*2A* and 1-HP excretion. In smokers and coke-oven workers, 1-HP concentrations were higher in individuals homozygote for *CYP1A1*2A* compared with those with the wild-type allele [112,113]. In conclusion, evidence exists that the *CYP1A1*2A* allele can enhance the toxicity of PAHs through increased metabolic activation relative to the wild-type. However, a number of studies have failed to demonstrate an effect of *CYP1A1*2A* and other CYP polymorphisms on DNA PAH-adducts or 1-HP in smokers or occupational exposed workers. Thus, finding associations in non-occupationally ex-

posed humans, e.g. as a result of urban air pollution, seems somewhat unlikely.

5.2. Phase II enzymes

Metabolism of reactive PAH metabolites generated by CYP generally involves detoxification through glutathione conjugation by GSTs. GSTs are key phase II enzymes. In humans, there are eight distinct families of cytosolic GSTs. Polymorphism has been described in many of these genes, though most attention has focused on allelism in mu (GSTM1), theta (GSTT1) and pi (GSTP1) families [114,115]. For GSTM1, a gene deletion resulting in a null genotype without any catalytic GSTM1 activity is the most studied genotype. The frequency of homozygous GSTM1 null type carriers is very high (20–50%) in most populations studied to date. Epidemiological studies suggest that individuals who are homozygous null type carriers for GSTM1 have an increased risk of developing cancer in a number of sites such as lung [116] and bladder [117]. As GSTM1 participate in detoxifying reactive PAH species, individuals with GSTM1 null should theoretically have higher DNA PAH-adduct levels than individuals with GSTM1 wild-type, presuming similar exposure PAH. However, results obtained in this area are unclear. A study of foundry workers exposed to high concentrations of airborne PAHs found no effect of GSTM1 null on DNA PAH-adducts levels and neither did a Polish study examining exposure to high concentrations of air pollutants [29,111]. This is in contrast to a study that investigated the effect of GSTM1 genotype on DNA PAH-adduct levels in subjects living in high and low polluted areas. That study found that subjects with GSTM1 null had the highest DNA PAH-adduct levels. Notably, this effect of GSTM1 was significant in both the high and low polluted areas [118]. Similarly, an effect of carrying GSTM1 null on DNA PAH-adduct level has been found in smokers [119]. The effect of the GST genotype seems to depend on the analytical procedure to detect DNA adducts and the level of exposure, as it apparently is having the greatest effect at low dose exposure. GSTM1 null genotype has in addition been found to predict DNA PAH-adduct levels in both malignant and non-malignant breast tissue from women with breast cancer [120].

The GSTT1 genotype also exhibits null polymorphism, and the frequency of homozygous GSTT1 null type carriers is 16–40% depending on the population studied. Epidemiological studies do not show a clear association between GSTT1 null genotype and cancer development [121,122]. Some evidence exists of an additive effect of the combination GSTT1 null and GSTM1 null for the risk of developing some types of cancers, although inconsistent results have been reported here also [121,123]. For GSTP1 a common polymorphism (allele frequency of 30–35%) involving an amino acid substitution (isoleucine to valine) has attracted most attention, as it has been associated with higher risk of several types of cancers [124]. Moreover, higher DNA PAH-adduct levels have been observed in lung cancer patients homozygous for the valine substitution [125]. In the Copenhagen study on $PM_{2.5}$ exposure and biomarkers in non-smoking, non-occupationally exposed subjects, genotypes of GSTM1, GSTT1 and GSTP1 were not found to be predictors of the PAH-adduct level in lymphocyte DNA, neither when including only genotype as predictor nor when including both particle exposure and genotype [33] Such results have also been found by other studies on non-occupationally exposed subjects, which suggest that with low to moderate concentrations of PAHs no simple association between biomarkers of susceptibility and genotoxicity can be detected.

An involvement of GST polymorphism in relation to benzene toxicity has been suggested, as toxic benzene metabolites generated by CYP2E metabolism can be detoxified by GSTs. Significant higher excretions of the benzene metabolite ttMA has been found in subjects with GSTM1 null genotype [126] and GSTT1 null genotype [127] as compared with the wild types. However, another study on outdoor working policemen failed to find any involvement of either GSTM1 or GSTT1 polymorphisms in relation to excretion of the benzene metabolites ttMA and PMA [128]. A study on benzene exposure in 40 healthy subjects in Copenhagen included polymorphisms of GST's as well as urinary excretion of benzene metabolites (ttMA and PMA), as well as DNA damage [38]. The GSTM1 or GSTT1 wild type was accompanied by increased excretion of ttMA in men in an additive manner, whereas no significant difference could be distinguished among the women or for PMA. In addition, a recent study found that among subjects occupational exposed to benzene, those with GSTM1 wild type or both GSTM1 and GSTT1 wild types, showed signs of increased

bone marrow toxicity as compared with those with the null genotypes [129]. This was explained by an enhancement of activity in redox cycling by glutathionyl conjugation of benzoquinone that might thus be a toxification reaction. However, these conjugates appear from spontaneous reaction with glutathione [130] and a role for GST's in this respect has not been clarified.

NQO1 is another important phase II enzyme. It catalyses a detoxification of reactive quinones that can produce ROS through redox cycling. NQO1 is thus believed to be an important part of the antioxidant defense. Genetic polymorphism has been detected in NQO1 causing a proline to serine substitution, which inactivates the enzyme. Increased frequency of the variant NQO1 allele has been found in patients with urological malignancies [131], whereas the wild-type allele has been associated with increased risk of lung cancer in another study [132]. NQO1 polymorphism in relation to benzene exposure has drawn some attention. One study found that humans homozygous of the variant allele were more susceptible to occupational benzene exposure, measured as risk of developing signs of benzene poisoning [133]. Benzene is primarily metabolized by CYP2E1 to a variety of metabolites, some of them reactive quinones [134]. In rodent studies, benzene exposure has been shown to induce 8-oxodG in lymphocytes and bone marrow and to cause DNA SB [135,136]. Elevated levels of FPG sensitive sites have also been detected in the bone marrow of benzene-treated mice [137]. In the Copenhagen study described above, a significant correlation was found between excretion of PMA and 8-oxodG levels in lymphocytes (Rs = 0.39). The correlation seemed to be related to NQO1 genotype, as the highest correlation (Rs = 0.58) was found in the group with only one variant allele, although there was no significant difference in the associations of the two different genotype groups [38]. These findings of positive associations between benzene exposure and oxidative DNA damage in relation to GST and NQO1 genotypes implies that even low levels of benzene exposure could have genotoxic effects in susceptible individuals.

6. Conclusion

The overall epidemiological evidence is consistent with the hypothesis that particulate air pollution is an important risk factor in cardiopulmonary disease and mortality even at low levels of exposure. The biological linkages are not fully understood, although the research to date point to an involvement of oxidative stress and inflammation. The use of biomarkers together with careful individual exposure monitoring has been shown to be important tools in gaining insight into these mechanisms.

Several biomarkers have been measured and tested for association with exposure to $PM_{2.5}$ and benzene. Three biomarkers of oxidative stress were found to be significantly associated with the personal $PM_{2.5}$ exposure, namely 8-oxodG, PLAAS and MDA. These findings support the hypothesis that exposure to $PM_{2.5}$ causes an increase in oxidative stress and add to ongoing research by finding that this seems also to be true in the non-occupational population. Especially, 8-oxodG seems a promising biomarker in future research of health effects caused by PM.

The biomarkers 1-HP and bulky DNA adducts were not associated with ambient or personal $PM_{2.5}$ exposure. As they have only been found associated with exposure in occupationally exposed sub-populations they are probably not sufficiently sensitive to be used as biomarkers of particle exposure in the non-occupational exposed population.

No effects of the susceptibility biomarkers, GST's and NQO1 genotype, were found in relation to non-occupational particle exposure. These results have been confirmed by other studies on non-occupationally exposed subjects, and conclude that with low concentrations PM no simple association between biomarkers of susceptibility and biomarkers of biological effective dose can be detected. Nevertheless, GSTM1, GSTT1 and NQO1 were important for the response to exposure to benzene.

Much research is still needed to understand the mechanisms between exposure in the non-occupational exposed population and health effects. However, interesting results of relationships between exposure and oxidative damage have been obtained supporting the hypothesis that increased oxidative stress is involved.

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