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Review

A Critical Evaluation of DNA Adducts as Biological Markers for Human Exposure to Polycyclic Aromatic Compounds

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The causative role of polycyclic aromatic hydrocarbons (PAH) in human carcinogenesis is undisputed. Measurements of PAH-DNA adduct levels in easily accessible white blood cells therefore represent useful early endpoints in exposure intervention or chemoprevention studies. The successful applicability of DNA adducts as early endpoints depends on several criteria: *i.* adduct levels in easily accessible surrogate tissues should reflect adduct levels in target-tissues, *ii.* toxicokinetics and the temporal relevance should be properly defined. *iii.* sources of inter- and intra-individual variability must be known and controllable, and finally *iv.* adduct analyses must have advantages as compared to other markers of PAH-exposure. In general, higher DNA adduct levels or a higher proportion of subjects with detectable DNA adduct levels were found in exposed individuals as compared with non-exposed subjects, but saturation may occur at high exposures. Furthermore, DNA adduct levels varied according to changes in exposure, for example smoking cessation resulted in lower DNA adduct levels and adduct levels paralleled seasonal variations of air-pollution. Intra-individual variation during continuous exposure was low over a short period of time (weeks), but varied significantly when longer time periods (months) were investigated. Inter-individual variation is currently only partly explained by genetic polymorphisms in genes involved in PAH-metabolism and deserves further investigation. DNA adduct measurements may have three advantages over traditional exposure assessment: *i.* they can smooth the extreme variability in exposure which is typical for environmental toxicants and may integrate exposure over a longer period of time. Therefore, DNA adduct assessment may reduce the monitoring effort. *ii.* biological monitoring of DNA adducts accounts for all exposure routes. *iii.* DNA adducts may account for inter-individual differences in uptake, elimination, distribution, metabolism and repair amongst exposed individuals. In conclusion, there is now a sufficiently large scientific basis to justify the application of DNA adduct measurements as biomarkers in exposure assessment and intervention studies. Their use in risk-assessment, however, requires further investigation.

Keywords: Biomarkers, DNA adducts, Human biomonitoring, Polycyclic aromatic hydrocarbons

Introduction

Polycyclic aromatic hydrocarbons (PAH) have been found to be carcinogenic in laboratory animals and are suspected to be carcinogenic in humans (IARC, 1983). Classic epidemiological techniques can be employed to study the association between reduction of PAH-exposure and cancer prevention. However, these techniques are usually compromised by the obligatory use of large numbers of volunteers/ patients and the lack of quantitative exposure data. Molecular epidemiology, which aims to integrate techniques of analytical chemistry, biochemistry, molecular biology and epidemiology, has the potential to contribute to cancer research in a number of areas, including assessment of the biologically effective exposure to carcinogens and the early identification of individuals at potentially high cancer risk (Schulte and Perera, 1993). Exposure biomarker analysis comprises the assessment of agents or their metabolites either in tissues, secreta, excreta or any combination of these, to evaluate exposure as compared with an appropriate reference.

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*This article is dedicated to Harald zur Hausen on the occasion of his retirement as head of the German Cancer Research Center (DKFZ) with gratitude and appreciation for 20 years of leadership.

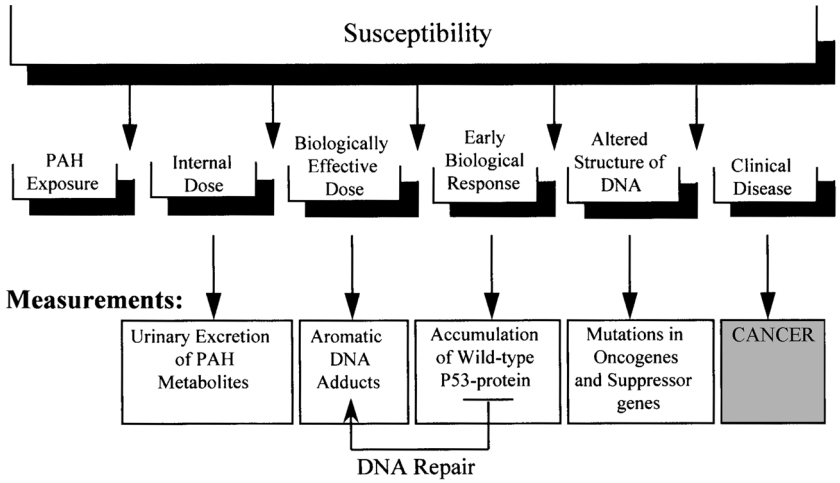

Fig. 1. The framework of molecular epidemiology, which provides a theoretical background for the application of biological markers as early end-points in testing the efficacy of exposure intervention and for cancer prevention studies.

The theoretical basis for molecular epidemiology (Fig. 1) provides a broad range of possible biomarkers or measurements that might be of use in studies regarding PAH-exposure. For instance, the internal dose of PAH can be assessed by measuring PAH concentrations or PAH-metabolites in body fluids, of which urine is most commonly used (Strickland *et al*., 1996). However, such measurements do not necessarily represent the biologically effective dose, which is the dose of a carcinogen that actually affects DNA in a tissue in which ultimately tumors may occur (i.e., target organs). Therefore, the most promising biomarker seems to be the measurement of DNA adducts, since it takes into account individual differences in exposure, absorption and distribution of the chemical, its metabolism into DNA reactive forms, detoxification to reactive intermediates, as well as cell turnover and repair of DNA damage. However, for the effective and reliable use of DNA adducts in PAH-exposure monitoring or intervention studies, several aspects of PAH-DNA adduct measurements need to be known in detail and will be discussed in this overview.

Criteria for DNA Adducts as Biomarkers of Exposure to Aromatic Compounds.

Causative role of DNA adducts in the etiology of cancer
The metabolic activation of PAH, including B[a]P, to their reactive derivatives has been discussed many times and the main metabolic pathways leading to metabolites that can bind to DNA are summarized in Fig. 2. Chemically induced carcinogenesis is a complex multistage process, which involves mutations in growth-regulatory proto-oncogenes (e.g. *ras*) and tumor-suppressor genes (e.g. *p53*) (Hall and Grover, 1990). The initial step is thought to be the interaction of electrophilic metabolites with DNA, which may result in a changed nucleotide sequence due to misincorporation of a nucleotide opposite the damaged base. Aberrant gene products or modified gene expression may ultimately disrupt normal regulatory processes of cell growth. There are several lines of evidence regarding the role of PAH-DNA adducts in transformation of normal cells into cancerous cells. Firstly, experiments with cultured cells exposed *in vitro* to PAH. Secondly, studies using animal models, particularly laboratory rodents. Thirdly, studies in healthy human volunteers and cancer patients, who were exposed to PAH.

A. *In vitro* studies
Important evidence for the promutagenic role of DNA adducts in mammalian cells was provided by *in vitro* studies using shuttle vectors with DNA adducts at specific localizations (Hemminki, 1993). However, the best evidence for the role of PAH-DNA adducts in human cancer has emerged relatively recently in a study by Denissenko *et al.* (Dennisenko *et al*., 1996). It was found that the strong and sequence-specific formation of BPDE-DNA adducts in *p53* of bronchial epithelial cells *in vitro* occurred at the same positions as the major mutational hotspots in *p53* found in DNA from human lung cancer tissue. Furthermore, other studies on cultured cells *in vitro* exposed to PAH, showed that aromatic carcinogens have the potential to alter or "activate" H-*ras* proto-oncogenes (Marshall *et al*., 1984; Vousden *et al*., 1986; Yang *et al*., 1987). In these studies, the most predominant point mutations induced by BPDE were G to T transversions and to a lesser extent G to A transitions, similar to those found in *ras* oncogenes and *p53* tumor suppressor genes in human cancers (Vineis and Caporaso, 1995). Recent studies strongly suggest that both preferential adduct formation and slow DNA-repair lead to such mutational hotspots (Denissenko *et al*., 1998).

B. Studies in laboratory rodents
Despite this convincing body of evidence supporting the

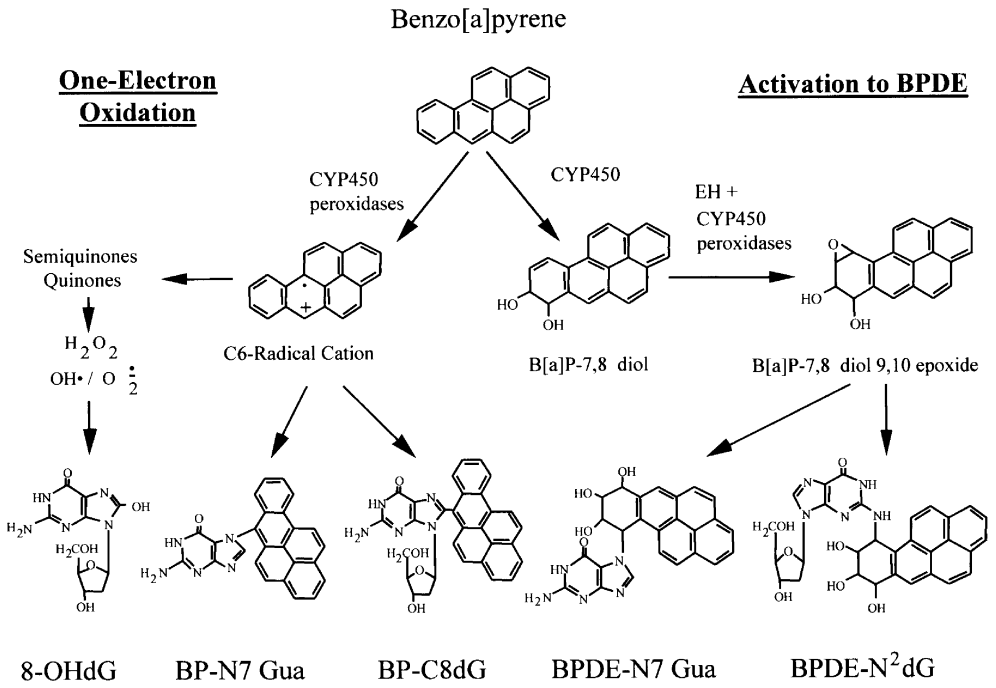

Fig. 2. Simplified scheme for the induction of DNA damage by polycyclic aromatic hydrocarbons via multiple metabolic pathways, as exemplified for B[a]P. Left side: One-electron oxidation, Right side: Two-electron oxidation to bay-region diol-epoxides.

critical role of DNA adducts in PAH-induced cancers, much remains to be uncovered about the quantitative relationship between PAH-DNA adduct formation and cancer risk *in vivo*. One might expect that higher concentrations of PAH-DNA adducts would imply a higher likelihood of critical mutations leading to cancer. However, studies with laboratory rodents have led to the understanding that there is no simple algorithm for translating DNA adduct levels into cancer risk. For example, DNA adducts appeared to be not specific for target organs, but are also formed in tissues in which no tumors are induced (Ross *et al*., 1990; Godschalk *et al*., 1997). Thus, DNA adducts are not solely responsible for tissue specific cancers, but additional factors are involved. Nonetheless, the overall data in target organs of animal models seem to point out that the level of DNA adducts is related to cancer risk. In some cases, but not all, the target organs of sensitive animal species had higher adduct levels than the same organs in resistant species (Wogan and Gorelick, 1985).

Chronic administration of a carcinogen resulted in the attainment of steady-state DNA adduct levels, which were related to the dose over a wide range and were generally correlated with the carcinogenic outcome (Poirier and Beland, 1992). Furthermore, inhibition of adduct formation resulted in decreased carcinogenicity of B[a]P (Huang *et al*., 1992). In studies concerning acute exposure to a single dose of a carcinogen, the relationship between DNA adducts and the carcinogenic outcome is more complex, because DNA adduct levels change as a function of time and may follow different pharmaco-kinetics for different carcinogens. Ross *et al.* (1995) showed that the adduct level integrated over time (i.e., area under curve) correlated with the induction of lung carcinoma by several PAH. This suggests that both formation and removal of PAH-DNA adducts determine their carcinogenic potency.

C. Studies in humans

Recently, a prospective study directly linked DNA adduct levels with lung cancer outcome (Tang *et al*., 2001), but most other studies on DNA adduct formation and subsequent cancer risk were on a cross-sectional basis, which may obscure the temporal relationship between exposure, DNA adduct formation and cancer (the presence of a tumor may affect DNA adduct levels) (Perera *et al*., 1989; Tang *et al*., 1995). There is sufficient circumstantial evidence to assume that DNA adducts are actually related to cancer risk in man. For example, Perera *et al.* (1994) reported a correlation between DNA adduct levels and the *in vivo* mutation frequency in individuals occupationally exposed to PAH. Although the gene (*HPRT*) and tissue (white blood cells, WBC) under investigation, are not relevant in occupationally related carcinogenesis, their results provide evidence for the mutational effects of PAH *in vivo* in humans. In general, aromatic-DNA adduct levels in critical organs and cancer risks are both related to PAH-exposure (Phillips *et al*., 1988; Cuzick *et al*., 1990). Moreover, correlations have been observed between DNA adduct levels in various groups of exposed subjects and their corresponding cancer risk. For instance, higher adduct levels were observed in lung of smoking

females as compared with males (Ryberg *et al*., 1994), which is in line with epidemiological data showing that women are at a greater risk of tobacco-induced lung cancer (Harris *et al.*, 1993, Risch *et al*., 1993). Furthermore, higher PAH-DNA adduct levels in lung parenchyma (Perera *et al*., 1989) and peripheral blood leukocytes (Tang *et al*., 1995) were observed in lung cancer patients as compared with healthy controls. Finally, adduct levels were found to be higher in lean subjects as compared to overweight individuals, which is in agreement with the epidemiological observation that lean smokers have a higher lung cancer risk (Palli *et al*., 2000; Godschalk *et al*., 2002). Thus, taken together all the available *in vitro* and *in vivo* data, it can be concluded that it is possible to use DNA adduct data not only for exposure assessment, but also as a measure of human cancer risk.

The use of surrogate tissues due to inaccessibility of target organs Practical and ethical considerations limit the types of tissues available for analysis of DNA adducts in man. Most target organs for PAH induced carcinogenesis can not be reached for routine sampling. Therefore, DNA adducts have predominantly been studied in easily available peripheral white blood cells (WBC). Gupta *et al*. (1988) demonstrated the capacity of human peripheral blood lymphocytes to *in vitro* metabolize a number of carcinogens, including B[α]P, to their DNA binding species and high inter-individual variations (up to 62 fold) in binding capacity of reactive PAH derivatives were observed. This variation in B[a]P related DNA adduct formation *in vitro* was found to be genetically controlled (Nowak *et al*., 1988) and may be indicative for individual differences in lung cancer susceptibility (Hawke *et al*., 1986; Nowak *et al*., 1992). However, WBC are not necessarily target cells for PAH or the most sensitive cells for DNA adduct formation and the question remains whether these cells provide a reliable estimate of PAH exposure *in vivo*. Studies on occupationally exposed workers showed that, in general, adducts can be detected in a significant proportion of the exposed workers and to a lesser extent in control subjects (reviewed in Schut and Schiverick, 1992; Peluso *et al*., 2001). Studies on smokers and nonsmokers showed conflicting results on the effect of smoking on PAH-DNA adduct levels in WBC. Several studies did not reveal any significant differences in PAH-DNA adduct levels between the two groups (Phillips *et al*., 1986; Phillips *et al*., 1990; Van Maanen *et al*., 1994a), suggesting that adducts in WBC may result from other sources than tobacco smoking. In a study among roofers, PAH-DNA adduct levels in WBC correlated with the amount of PAH in skin wipes, but not with the amount of PAH in personal air samples, indicating that dermal contact might be a major route for DNA adduct formation in WBC (Herbert *et al*., 1990). Furthermore, several studies showed that the consumption of PAH-containing foods significantly enhanced DNA adduct levels in peripheral blood lymphocytes (Rothman *et al*., 1993a, 1993b; Van Maanen *et al*., 1994b). The possibility for multiple routes of exposure may further complicate the relationship between adduct levels in target tissues and surrogate tissues. Therefore, for a better understanding of data obtained by DNA adduct analysis in humans, it is important to know the contribution of the different exposure routes to DNA adduct formation in different WBC-subpopulations. Exposure route dependent adduct formation by B[a]P was studied in rats acutely exposed to B[a]P via intra-tracheal instillation, gavage and dermal application (Godschalk *et al*., 2000). Higher adduct levels were observed in WBC after intratracheal and oral exposure of rats as compared with dermal application of B[a]P. In the same rats, similar results were found for lung tissue, which is a major target organ for B[α]P induced carcinogenesis, and a relationship between DNA adduct levels in WBC and lung tissue, independent from the route of exposure, was found.

In human studies, a relationship between DNA adducts in WBC and lung was initially not observed (Phillips *et al*., 1990; Van Schooten *et al*., 1992), but in more recent studies using mononucleated blood cells (lymphocytes plus monocytes, MNC) instead of total WBC, this relationship was actually found (Wiencke *et al*., 1995). Also in eczema patients topically treated with coal-tar ointments, it was suggested that analysis of total WBC instead of MNC would decrease the strenght of the relationship between adduct levels in target tissue (skin) and surrogate tissue (Godschalk *et al*., 1998a). Overall, these results indicate that DNA adduct formation in MNC might reflect DNA adduct levels in relevant target organs.

WBC can be subdivided in monocytes (5-10% of total WBC), lymphocytes (20-40%) and granulocytes (40-75%). The life-span of lymphocytes varies from a few days to several years and these cells are therefore potentially useful in determining long-term exposure. On the other hand, the half-life of monocytes and granulocytes is much shorter (hours-days) and therefore DNA adducts in these cells may represent recent exposure only. The variable life-span of WBC-subsets makes the number of adducts persisting in total WBC after a given amount of time extremely uncertain; any immune-disturbance, like a common cold, may profoundly affect numbers and life-span of WBC subtypes. Furthermore, it has been observed that the various WBC-subpopulations differ in metabolic and repair capacities (Okano *et al*., 1979; Knudsen *et al*., 1992). Thus, separation of the different WBC-subpopulations might be necessary to obtain a more reliable estimate of DNA adduct levels. Indeed, most studies using isolated lymphocytes, monocytes or MNC showed clear differences between smoking and nonsmoking individuals (Wiencke *et al*., 1995; Godschalk *et al*., 1998b).

Other kinds of surrogate tissues have been used in the assessment of PAH exposure. Izotti *et al*., (1991) and de Flora *et al*. (1993) suggested that cells obtained by broncho-alveolar lavage (BAL) can be used as source of exposed DNA, specifically for inhaled carcinogens. The predominant cell-type in BAL is alveolar macrophages (AM), which can be considered as a non-peripheral WBC-subfraction, since these

cells are thought to originate from peripheral blood monocytes that have left the circulation by passing through the walls of the alveolar capillaries. Furthermore, we should keep in mind that the applicability of surrogate tissues in field studies largely depends on their accessibility, and therefore the WBC-subpopulations seem to have the advantage above BAL-cells, but recent developments made it possible to use 'sputum induction' to obtain alveolar macrophages *via* an easy and relatively non-invasive procedure (Pavord *et al*., 1997; Besarati Nia *et al*., 2000). Of all the mentioned surrogate tissues, DNA adduct levels appeared to be highest in BAL-cells (or induced sputum as alternative), followed by respectively MNC and granulocytes. These results suggest that DNA adduct measurements in BAL-cells or MNC would provide the most sensitive analysis for assessing exposure to inhaled aromatic compounds.

The problem of selecting the appropriate surrogate tissue in chemopreventive studies might be even larger, due to tissue-specific reactions to chemopreventive agents. For example, a recent study on the chemo-preventive properties of *N*-acetylcysteine (NAC) in human smoking volunteers (Van Schooten *et al*., 2002) showed a clear reduction of DNA adduct levels in BAL-cells, but not in the peripheral blood cells. Moreover. the effect of NAC on adduct formation in BAL-cells was only observed in light smokers, but not in heavy smokers, indicating an exposure-dose dependent action of the chemopreventive agent. Dose response relationships will be further discussed below.

Defined pharmaco-kinetics and temporal relevance
Knowledge on dose-response relationships and adduct persistence is inexpendable for exposure monitoring studies, which use DNA adduct levels as end-points (Schulte and Perera, 1993). For example, researchers should know how, and how fast adduct levels might change upon changes in exposure. Thus, understanding the kinetics of DNA adducts in surrogate and target tissues will guide the interpretation of adduct data in humans and is important in designing future studies for choosing the frequency and timing of sampling (Schulte and Perera, 1993). The dose-response relationship between PAH-exposure and subsequent DNA adduct formation has been studied to evaluate the strength of DNA adduct measurements to distinguish between two or more different exposure levels. In laboratory rodents exposed to low doses of PAH, adduct formation seemed to follow first-order kinetics, i.e. the degree of binding in target organs is proportional to the administered dose. However, human data on adduct formation do not demonstrate a strong proportional relationship between exposure and adduct levels (Lutz, 1990; Lewtas *et al*., 1997; Van Schooten *et al*., 1997). In lung tissue of smokers, a correlation was found between DNA adduct levels and the amount of cigarettes smoked per day (Phillips *et al*., 1988), but the course of the dose-response relationship in WBC (subpopulations) flattened at high levels of exposure, i.e., more than 20 cigarettes per day (Dallinga *et al*., 1998). In general higher DNA adduct levels or a higher proportion of subjects with detectable DNA adduct levels were found in exposed individuals as compared with non-exposed subjects, but saturation may occur at high exposures.

PAH-DNA adduct measurements should have the ability to integrate exposure to PAH over a relatively long (a few weeks up to a few months) period of time and also detect previous exposures after a long period without exposure. In lung tissue, DNA adduct levels were found to decrease after smoking cessation (Schoket *et al*., 1993 and 1998; Dunn *et al*., 1991 and Garner *et al*., 1990). Removal of aromatic-DNA adducts from lung tissue apparently occurred in two phases; an initial fast reduction of DNA adduct levels, which is followed by a period in which adduct elimination is much slower. Studies in mice, suggest that adduct loss in lung is largely due to cell turnover (Van Schooten *et al*., 1990). To investigate whether DNA adduct measurements have the ability to integrate exposure over a relatively long period of time (a few weeks up to a few months), adduct removal was studied in smokers who stopped smoking, and in patients after their treatment with coal-tar ointments (Paleologo *et al*., 1992; Mooney *et al*., 1995; Godschalk *et al*., 2002) and DNA adduct levels in MNC were significantly reduced. The calculated half-life of aromatic-DNA adducts in MNC was approximately 10-12 weeks, based on logarithmically transformed DNA adduct levels. Additionally, DNA adduct formation and removal was studied in separated WBC-subpopulations of individuals topically treated with coal-tar ointments for a short period of time (Godschalk *et al*., 1998a); one week after the end of treatment, DNA adduct levels in monocytes and granulocytes were back to base-line levels, whereas in the lymphocyte fraction adduct levels were more persistent. These data indicate that if exposure changes, DNA adduct levels in WBC sub-populations change accordingly and thus reflect exposure levels. Furthermore, to study prior exposures to aromatic compounds after a period of non-exposure, the lymphocyte fraction should be used as surrogate tissue. However, if a particular research question focuses on recent exposure only, and bias by previous exposures is not wanted, the monocyte fraction could also be of importance.

Understanding inter- and intra-individual variability
Inter- and intra-individual variations should be characterized before PAH-DNA adducts can be applied as exposure marker in large scale studies (Schulte and Perera, 1993). It is known that DNA adduct formation is subject to a greater variability than external exposure assessment, because the body actively participates in the absorption, distribution, and elimination of PAH. In other words: two individuals with a similar level of external exposure, might have different aromatic-DNA adduct levels. Variations may be due to differences in metabolic phenotypes, possibly related to genetic polymorphisms in a variety of enzymes involved in the activation or detoxification of PAH or repair of PAH-DNA adducts.

Electrophilic metabolites of PAH may be detoxified by

glutathione-S-transferases (GST's) before they might react with cellular macromolecules (Ketterer *et al*., 1992). Especially *GSTM1* and *GSTP1* seem to have a high affinity towards epoxides, and may thus play an important role in the protection against reactive PAH-diol-epoxides. BPDE has a slow spontaneous rate of reaction with GSH. However, if this rate is sufficiently enhanced by GST-catalysis and the resulting reaction may compete effectively with DNA adduct formation (Ketterer *et al*., 1992). Interestingly, the expression of GST's is highly variable between different tissues and individuals. For example, about 50% of the caucasian population lack the *GSTM1* gene and has therefore no GSTM1-1 activity and it has been reported in molecular epidemiological studies that these individuals might have an increased risk for developing lung cancer (D'Errico *et al*., 1996). In some studies, the absence of the GSTM1-1 isoenzyme in lung of smoking individuals was associated with relatively high PAH-DNA adduct levels, which implies an important role of *GSTM1* in scavenging reactive PAH-metabolites (Ketterer *et al*., 1992; Shields *et al*., 1993; Kato *et al*., 1995; Rojas *et al*., 1998), however these findings were not consistent.

Other important PAH-detoxifying enzymes are UDP-glucuronosyl transferase (UGT) and sulfotransferase (ST), which catalyze the conjugation of PAH-derivatives that posses HO-functions with respectively glucoronic acid and sulfate. The dose-dependent conjugation of mainly quinones and phenol metabolites results in sufficiently hydrophilic compounds that can be excreted in urine or feces. Genetic polymorphisms of glucuronidation and sulfation are known to exist (Burchell *et al*., 1997), although the characterization and assessment of the importance of these variations need further study. Activities of enzymes involved in the activation of PAH are highly variable between individuals as well, and the basis for this variability is often a genetic polymorphism in genes that encode for these enzymes. For example, the gene that codes for the inducible cytochrome P450 (CYP) isoenzyme CYP1A1, which is characterized by affinity for planar substrates like PAH and its ability to oxygenate molecules in conformationally hindered positions (bay-regions), was found to be polymorphic in the 3' noncoding region (Kawajiri *et al*., 1990). A *MspI* restriction site polymorphism seemed to be associated with an increased risk for lung cancer as initially reported by Kawajiri *et al*. (1990). In another study, they postulated that the *MspI* polymorphism was genetically linked with an Isoleusine-Valine polymorphism in exon 7, which is related to a stronger capacity of the enzyme to bind the substrate (Hayashi *et al*., 1991). In a Japanese population, individuals with the susceptible *CYP1A1 MspI* genotype combined with deficient *GSTM1* had a remarkably high risk for developing carcinoma in lung (OR = 16) (Fig. 3). The risk was even higher in individuals who had the exon 7 susceptible genotype of *CYP1A1 (Val/Val)* combined with the *GSTM1* null genotype (OR = 41)(Nakachi *et al*., 1993). So far, the number of reports supporting Kawajiri's first observation for

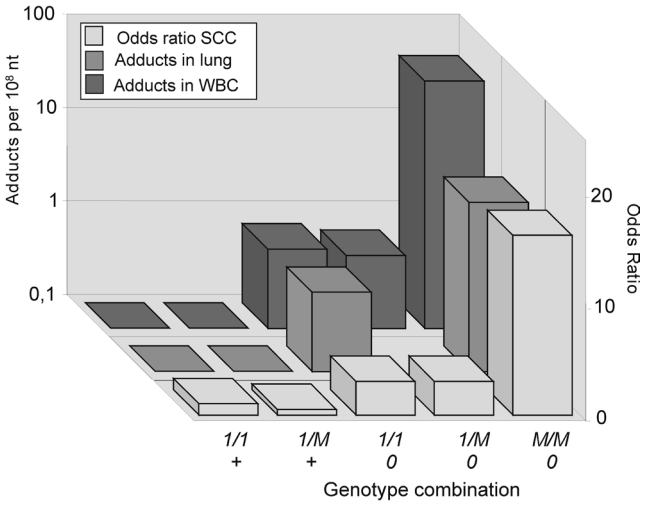

Fig. 3. BPDE-DNA adducts in white blood cells (WBC) from PAH-exposed coke oven workers and lung parenchyma of smoking lung cancer patients (Rojas *et al*., 1998), grouped by *CYP1A1 and GSTM1* genotype combinations, paralleled the Odds Ratio (OR) calculated for squamous cell carcinoma in Japanese populations (Nakachi *et al*., 1993). M, *CYP1A1* mutant allele; 0, *GSTM1* deleted; +, *GSTM1* active.

populations outside Japan is limited (D'Errico *et al*., 1996; Xu *et al*., 1996; Sugimura *et al*., 1994). The influence of the *CYP1A1* polymorphism on DNA adduct levels in lung is still not clear; although many genotyping studies were unable to find a relationship between both parameters. Recently, Rojas *et al*. (1998) found a clear effect of the combination of *CYP1A1* and *GSTM1* genotypes on the formation of specifically BPDE-DNA adducts in human lung and lymphocytes, which remarkably paralleled cancer risk for these genotype combinations (Fig. 3). A study involving phenotyping also showed a clear correlation between aromatic-DNA adducts and CYP1A1 activity among smokers (Alexandrov *et al*., 1992). It is not the purpose of this review to thoroughly discus the scientific literature on the relationship between genetic polymorphisms in phase I or II enzymes and DNA adduct formation. Risk-modifying effects of metabolic genotypes might be more easily identified if specific markers of structurally defined adducts were used, such as the (+)-*anti*-BPDE-DNA adduct (Alexandrov *et al., in press*), because adduct-analysis by the routinely applied NP1- or butanol-enriched ^{32}P-postlabeling methodology may also detect adducts which were activated *via* different pathways (for example aromatic amines, and bulky adducts from lipid peroxidation products) (Godschalk *et al*., 2001). Overall, it is thought that the balance between activation and inactivation of PAH may play a significant role in the susceptibility towards the formation of DNA adducts and subsequent risk for developing chemically induced cancers. Thus, it is expected that assessment of the complete set of putative 'high risk genotypes' will allow the identification of susceptible

individuals or subgroups. Currently, however, genetic polymorphisms are promising, but still cannot completely identify individuals at higher cancer risk. Therefore, other factors that could affect adduct formation *in vivo* should be taken into account, like gender (Ryberg *et al*., 1994), body composition (Godschalk *et al*., 2002), diet (Palli *et al*., 2000) and DNA repair efficiency (Tang *et al*., 2002).

Since the variation in adduct formation within or between individuals can be high, the question remains whether aromatic-DNA adduct levels in WBC can be applied to discriminate between individuals in various exposure or risk groups. Such discrimination is practically possible only if the inter-individual variation is not overshadowed by intra-individual variations or uncertainties in laboratory measurements, since these two factors are putatively unrelated to the exposure level or cancer risk (Dickey *et al*., 1997). Thus, the observed range in DNA adducts in WBC is related to three factors: inter-person variability, intra-individual variation and measurement uncertainty. Unfortunately, only few studies focused on intra-individual variations in DNA adduct formation in WBC (Van Schooten *et al*., 1997; Dickey *et al*., 1997; Besarati Nia *et al*., 2000). Intra-individual variation in DNA adduct levels was studied in MNC and BAL-cells from smoking volunteers. In MNC of smokers, the intra-individual variation was found to be low over a period of up to 2 months (Van Schooten *et al*., 1997; Besarati Nia *et al*., 2000), but resampling after a period of 6 months resulted in significantly higher DNA adduct levels than in the first sampling of MNC (Van Schooten *et al*., 1997). In this study, the timing of sampling corresponded with a summer and winter period, respectively and it has been described that environmental exposure is higher in winter as compared with summer (Grzybowska *et al*., 1993). However, other possible explanations cannot be excluded, for example, differences in dietary habits between the two sampling periods. In BAL-cells, intra-individual variation in DNA adduct levels over a period of 6 months was found to be low; both samplings were well correlated.

Control over measurement uncertainty and reproducibility is highly important for future applications of DNA adducts in exposure intervention or chemopreventive studies. The ultrasensitive ^{32}P-postlabeling assay, most frequently used for the detection of aromatic-DNA adducts in human samples, is a complex assay and experimental conditions can vary widely between different investigators or between different batches of enzymes. Interlaboratory trials have led to a much clearer understanding of critical features and procedures in ^{32}P-postlabeling (Phillips and Castegnaro, 1999), which may significantly reduce measurement uncertainties. However, other factors that may hamper accurate DNA adduct analyses, like DNA isolation procedures and RNA contamination (Godschalk et al., 1998c), still deserve further attention.

Comparison with other markers of PAH-exposure Several kinds of biomarkers, or combinations of these, have been used to assess exposure to PAH. Comparing one potential biomarker to another can provide useful information on marker characteristics, but does not constitute validation (Schulte and Perera, 1993). In occupational settings (e.g. road-pavers, coke-oven workers and employees in the aluminum industry) the urinary excretion of hydroxylated PAH is considered to be the 'gold standard' for exposure assessment (Strickland *et al*., 1996; Jongeneelen *et al*., 1988). However, hydroxylated PAH-metabolites in urine can only be used to asses the internal dose after recent exposure to PAH. PAH metabolites that have been detected in human urine include 1-hydroxypyrene, 1-hydroxypyrene-O-glucuronide, 3-hydroxy-benzo[a]pyrene and 7,8,9,10-tetrahydroxy-benzo[a]pyrene. The most widely used analysis of these is 1-hydroxypyrene-O-glucuronide, which is often measured as 1-hydroxypyrene after deconjugation of the glucuronide by β-glucuronidase (Jongeneelen *et al*., 1988). Studies regarding these urinary metabolites have been performed in occupational settings, but also in smokers, individuals that consumed charbroiled meat and eczema/psoriasis patients topically treated with coal-tar ointments (Strickland *et al*., 1996; Godschalk *et al*., 1998a). Although the measurement of these metabolites is useful in assessing recent exposure, it does not represent the biologically effective dose. A limited number of studies have examined the association between urinary PAH-metabolite concentrations and DNA adduct levels (Santella *et al*., 1993; Kang *et al*., 1995; Van Schooten *et al*., 1995; Godschalk *et al*., 1998a). However, the results were not consistent, which was probably due to differences in periods and timing of the collection of urine and sampling of blood. In rats acutely exposed to B[a]P, a relationship between 3-OH-B[α]P excretion and DNA adduct levels in target organs (e.g. the site of exposure) was observed (Godschalk *et al*., 2000). This was also observed in coal-tar treated patients; a relationship was found between urinary 3-OH-B[a]P levels and specifically BPDE-DNA adducts (Godschalk *et al*., 1998a). Overall, 3-OH-B[a]P excretion was significantly increased after exposure to PAH, and quickly reduced to base-line levels after exposure stopped.

Perspectives

In weighing the advantages and disadvantages of biological and environmental monitoring to define exposure to aromatic compounds, it is clear that DNA adduct measurements have at least four theoretical advantages over traditional exposure assessment. Firstly, DNA adducts are thought to play a significant role in the causation of certain types of cancer, and therefore represent a measure for exposure as well as risk. Secondly, they can smooth the extreme variability in exposure which is typical for environmental toxicants and therefore reduce the monitoring effort. Thirdly, biological monitoring of DNA adducts in WBC accounts for all exposure routes, i.e. inhalation, ingestion and dermal absorption. Finally, DNA

adducts may account for inter-individual differences in uptake, elimination, distribution, metabolism and repair amongst exposed individuals. On the other hand, sampling and analytical demands as well as the imprecision of assays may lead to significant measurement errors, which will tend to attenuate the underlying relationships. Therefore, future progress in the application of DNA adducts depends on the development and systematic use of high-resolution techniques such as capillary electrophoresis combined with fluorescence detection or mass spectrometry.

Recently, DNA adducts in lymphocytes were found to correlate with cancer risk, indicating their potential use in risk assessment, but additional studies are necessary to strengthen these results. Identification of individuals with high adduct formation, may result in better selection of appropriate study populations in future molecular epidemiological studies. Rapid advances in high throughput gene analysis by DNA chip technology will speed up the identification of (new) mutations in predisposing cancer genes. Progress is therefore to be expected from studies in which predictive intermediate risk markers such as DNA adducts and susceptibility (genetic as well as acquired susceptibility) are combined, providing reasonably short and cost-effective intervention studies. The ability to identify highly susceptible individuals or subgroups will have substantial implications for prevention; once identified, susceptible people might (1) be more easily persuaded to avoid exposures like tobacco use, (2) be targeted for intensive smoking cessation programs, (3) be enrolled in chemoprevention trials and (4) be considered suitable for cancer screening programs not appropriate for application to the general population. Moreover, DNA adducts can be used as promising intermediate molecular markers of human carcinogenesis, to test the efficacy of chemopreventive agents. Overall, it can be concluded that there is now sufficient scientific evidence to justify the application of DNA adduct measurements as biomarkers in exposure assessment and intervention studies.

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