ELSEVIER

Toxicology Letters 134 (2002) 39–49

Toxicology Letters

www.elsevier.com/locate/toxlet

Carcinogen biomonitoring in human exposures and laboratory research: validation and application to human occupational exposures

Glenn Talaska *, Andrew Maier, Scott Henn, Angela Booth-Jones, Yutaka Tsuneoka, Roel Vermeulen, Brenda L. Schumann

The Department of Environmental Health, The University of Cincinnati College of Medicine, Cincinnati, OH 45267-0056, USA

Received 21 September 2001; received in revised form 1 February 2002; accepted 4 February 2002

Abstract

A multiple biomarker approach is required to integrate for metabolism, temporal response and exposure-response kinetics, biological relevance, and positive predictive value. Carcinogen DNA adduct analysis can be used in animal and in vitro studies to detect absorption permutations caused by mixture interactions, and to control metabolic variation when specific CYP450 genes (1A1 or 1A2) are knocked out. These enzymes are not critical to the metabolic activation of model Polycyclic Aromatic Compounds (PAC) and aromatic amines, respectively, as suggested by in vitro analysis. Several human studies have been carried out where multiple biomarkers have been measured. In a study of benzidine workers, the similarities in elimination kinetics between urinary metabolites and mutagenicity is likely responsible for a better correlation between these markers than to BZ-DNA adducts in exfoliated cells. In a study of rubber workers, the relationship between specific departments, urinary 1 HP and DNA adducts in exfoliated cells coincided with the historical urinary bladder cancer risk in these departments; the same relationship did not hold for urinary mutagenicity. In a study of automotive mechanics, biomarkers were used to monitor the effectiveness of exposure interventions. These data reinforce the notion that carcinogen biomarkers are useful to monitor exposure, but that a complementary approaches involving effect and perhaps susceptibility biomarkers is necessary to obtain the necessary information.

Keywords: Biological monitoring; Carcinogens; DNA adducts; ^{32}P-postlabelling; Rubber workers; Passive smoking; Auto mechanics

1. Introduction

The measurement of carcinogen-DNA adduct levels is central to the understanding of chemical carcinogenesis both in animal models to determine mechanisms, and human exposure assessment. This is a strong statement and perhaps over-reaching in the sense that the measurement of DNA adducts has not come into routine use in occupational or environmental exposure assessment. Yet, because adducts are the ultimate mutagenic lesion, they play a central role in

* Corresponding author. Tel.: +1-513-558-0519; fax: +1-513-558-4397

E-mail address: glenn.talaska@uc.edu (G. Talaska).

0378-4274/02/$ - see front matter
PII: S0378-4274(02)00161-3

determining the relationships between all the intermediate steps between external dose and ultimate cancer outcome. Continued development of non-invasive biological monitoring tools for carcinogen-DNA adduct analysis should be an important priority for researchers in occupational health and preventive medicine. The focus of this paper is to review the usefulness of adduct analysis using recent examples.

Internal dose markers estimate uptake from all routes (Fig. 1). This is particularly important when ingestion or dermal exposure are significant routes as is the case with many polycyclic aromatic compounds (PAC) and aromatic amines. It has been shown recently that the organ distribution of carcinogens applied topically can be altered by co-exposure to materials like kerosene, which damage the skin and promote distribution of PAC to the lung and other internal organs. Higher levels of the parent carcinogen, in this case benzo(a)pyrene (BaP), were found in the lungs and livers of treated animals (Schumann et al., 2002). However, the biological significance to tissue distribution studies in isolation is limited. For example, if the material distributed to these organs was metabolized to a harmless moiety, findings of altered distribution could be considered interesting, but unimportant. However, since it was seen that kerosene treatment significantly elevated the levels of DNA adducts, the results of the distribution studies are much more relevant and suggest that extra caution is required when humans are exposed to a mixture of this type (Bhatnagar and Talaska, 1999).

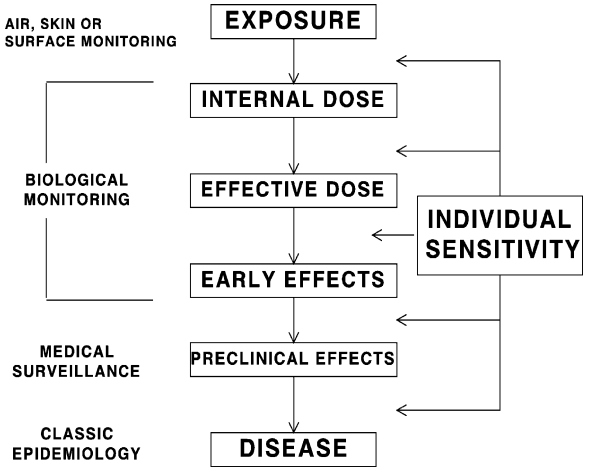


Fig. 1. Schematic for the continuum between exposure and disease outcome.

Human studies that include both internal and effective dose measurements are similarly of greater utility and relevance. For example, a trimodal distribution of *N′*-acetylbenzidine DNA adduct levels was found in a group of workers exposed to benzidine (Bhatnagar and Talaska, 1999). The adduct levels in one group of several workers was almost indistinguishable from the control group, suggesting that these workers had much lower chronic exposure than others who were grouped as benzidine 'exposed'. The possibility that these data were artifactual because of the new method (exfoliated urothelial cell DNA adduct analysis) applied to an occupational group for the first time was decreased when it was also shown that the levels of excreted benzidine metabolites were highly correlated in the group. Thus, the persons whose daily exposure was low, as indicated by the low levels of excreted metabolites, also had low chronic exposure, as indicated by the levels of DNA adducts in their exfoliated urothelial cells.

It was also reported that the skin appears to be the major route of exposure for benzidine-based dye workers. Persons working with these dyes, which are known to be poorly absorbed, but rapidly converted to benzidine once they are absorbed, had much lower levels of the specific acetylated benzidine DNA adduct in exfoliated urothelial cells than did workers exposed to the parent benzidine which is well absorbed through the skin (Rothman et al., 1996).

These data indicate the utility of DNA adduct measurements as effective dose markers in integrating data from all routes of exposure in both humans and animals. Similar data could have been obtained using the analysis of metabolites alone. However, the adduct measurements were extremely useful to confirm a genotoxic effect and support the biological plausibility of the results.

An area where DNA adduct analysis is essential to accurate estimation of effects is when individual susceptibility may play a role in the outcome.

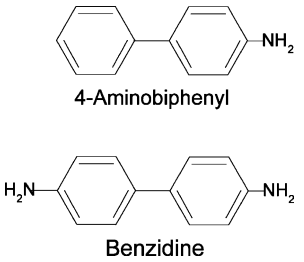


Fig. 2. Structures of 4-aminobiphenyl and benzidine, carcinogenic aromatic amines affected differently by NAT2 status.

Referring to Fig. 1, effective dose biomarkers integrate for differences in toxicokinetics (absorption and metabolism). We have seen that *N*–acetyltransferase 2 (NAT2) modifies the effect of 4-aminobiphenyl (4-ABP) exposure in tobacco smoker, but not in workers exposed to benzidine (Vineis et al., 1994; Rothman et al., 1996). These data showed dramatically that there can be significant differences in the effect of a metabolic susceptibility marker due only to minor changes in the nature of the metabolized substance. The structures of 4-ABP and BZ are given in Fig. 2.

1.1. Animal and in vitro studies

1.1.1. CYP 1A2 activation of 4-ABP in vivo

The impact of specific CYP isozymes in the toxicity and DNA binding of 4-ABP was recently investigated. As scientists, we explore the truth in all its complexity, yet as humans we seek to distill complex relationships down to a kernel of truth to make matters easier to understand. For example, Butler and her co-workers published two landmark papers several years ago which showed the relative reaction rates for *N*-hydroxylation of 4-ABP and methylene bis-2-chloroaniline (MOCA) for several of the CYP gene products (Butler et al., 1989a,b). Their report that CYP1A2 was the most active for 4ABP has evolved into the truism that CYP1A2 is THE enzyme responsible for the activation of aromatic amines. This is in spite of the fact that the Butler reports showed that other CYPs could account for more than half of the total *N*-hydroxylation of 4-ABP and that CYP1A2 was only a minor player in the activation of MOCA. One way to get at the truth of the matter is to conduct whole animal studies in mice where the specific CYP genes were knocked out. Dan Nebert has knocked out the CYP1A2 and CYP1A1 genes in separate lines of mice, derived from C57 black 6. These lines of animals have been used to determine the impact of knocking out the specific genes on the DNA adduct levels in target organs. Animals were treated with sub-carcinogenic doses of 4-ABP topically and the livers and urinary bladder adduct levels were determined using ^{32}P-postlabelling. The results were extremely interesting: the specific 4-ABP DNA adduct levels were no lower in the knockout than in the wildtype animals. These data were consistent in liver and in urinary bladders of males and females. It was hypothesized that at these low doses other CYP enzymes are able to compensate for the absence of the CYP1A2 and *N*-hydroxylate 4–ABP. By integrating for metabolic differences, the measurement of DNA adducts as effective dose has helped to determine that the overall CYP metabolism appears to be more important than the activity of any one isozyme.

Fig. 3 demonstrates another useful application of carcinogen-DNA adduct analysis. Absorption and metabolic phenotype can modify the effect of the specific exposure to produce the toxic effect. In the same way, the genotype for genes like p53 can modify the outcome, the measurement of carcinogen-DNA adducts can assist in the interpretation of the data. Addressing the question: can the relationship between DNA adduct levels

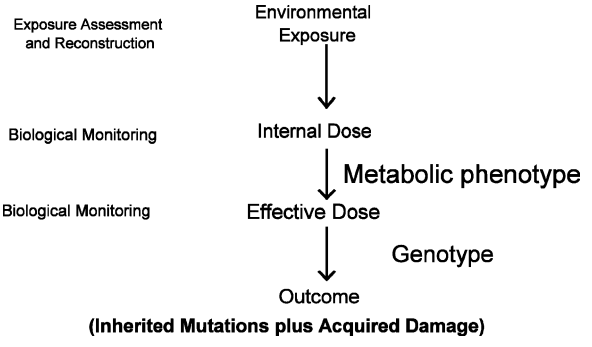


Fig. 3. Utility of effective dose markers to: 1. Integrate for differences in metabolic phenotype and; 2. provide an accurate dose assessment for genotypic differences in response to damage.

and carcinogenicity be altered when the animal is already mutated in the p53 gene or a gene which will bring blood supply to the tumor, or promote oxidative damage? Dr Shuker (this volume) has alluded to this use of DNA adduct measurements and similar studies are in progress at the University of Cincinnati with Roy Albert, Susan Heffelfinger and Dave Warshawsky.

1.2. DNA adducts and analysis of complex mixtures

1.2.1. Mechanisms of PAC and arsenic interaction in vitro

Arsenic is a metal that has been recognized as a carcinogen for more than 30 years (ACGIH, 2001), however, its mechanism of action has, thus far, been resistant to clear understanding. Arsenic is known to react with thiols and affect many enzymes and proteins in the cell. One hypothesis is that arsenic inhibits enzymes associated with DNA repair. A second, epigenetic, mechanism has been proposed where arsenic interferes with normal regulation of gene expression by altering DNA methylation. A third mechanism of arsenic interaction proposes that the metal competitively interacts with the reactive thiols of the glutathione molecule and allows reactive metabolites of carcinogenic materials to bypass this important detoxification pathway.

There is epidemiologic evidence supporting an interaction between arsenic and PAC. Several workers (e.g. Pershagen et al., 1981; Higgins et al., 1981) have suggested smokers were more prone to develop bronchogenic carcinoma in cohorts of arsenic exposed copper smelter workers. The mechanisms proffered above support an interaction between these materials. To determine the root of arsenic-PAC interactions, mouse hepatoma Hepa 1 cells were exposed to low concentrations of arsenic and then BaP, and it was shown that arsenic pre-treatment increased the BaP DNA adduct levels in the cultured cells by almost 9-fold (Maier et al., 2002). One of the major impressions was that, although arsenic effects at high doses were significant, the interaction was strongest at very low doses of BaP.

Factors that influenced the arsenic-induced increase in BaP DNA adducts were then studied. First, two variant HEPA 1 cell lines were obtained: c57 which does not express CYP enzymes and C × 4 cells in which CYP 1A1 had specifically been added back. cultures were treated with arsenic and either BaP and BaP-7,8-diol, the first metabolite along the DNA binding pathway which led to BaP-7,8-diol, 9,10-epoxide. The doses used were 10 μM for arsenite, 0.5 μM for BaP and 0.5 μM for BaP-7,8-diol. It was seen that CYP activation was required for arsenic to have an impact on BaP adduct levels. In addition, although the adduct levels following exposure to the 7,8-diol of BaP were higher than when the parent compound was used, the results could be explained by a focusing of the initial metabolism away from the mono-hydroxylated metabolites of BaP (3-OH-BaP, for example) to the activating diol-epoxide pathway. There was no evidence that arsenic was selectively activating BaP-diol to a greater degree than BaP itself. This result, plus studies of enzymatic activities revealed that arsenic was acting at a step downstream of CYP-mediated metabolism.

Next, the hypothesis that modulation of glutathione levels by arsenic might explain the arsenic-induced increase in BaP DNA adduct we observed was tested. Cell cultures were pretreated either with the glutathione synthesis inhibitor buthionine sulfoximine (BSO), or supplemented with glutathione using glutathione ethylester. Following these pre-treatments the cells were co-treated with arsenic and BaP. BSO pretreatment (GSH depletion) significantly increased the degree to which arsenic enhanced BaP-DNA adduct formation, while adduct formation was efficiently blocked when cells were pretreated with glutathione ethylester. These data indicated that at least one step where arsenic has a significant interaction with BaP is related to the competition between glutathione and nucleophilic sites in DNA for the binding of the reactive epoxide metabolites of BaP.

Since arsenic has been shown to inhibit DNA repair, the impact of arsenic treatment on removal of BaP-DNA adducts was studied by following the adduct removal time course. After accounting for the initial difference in adduct levels caused by arsenic when the cells were treated with arsenic, the slope of adduct removal curves was essentially identical with both treatments indicating that arsenic did not have an impact on the excision step of DNA repair. To determine whether these changes in adduct level resulted in downstream toxicological responses, the effect of arsenic on mutation rate in the cell cultures was examined. It was found that arsenic increased the rate of BaP mutations, but not out of proportion to the levels of BaP-DNA adducts, suggesting that arsenic did not have an effect on the conversion of BaP-DNA adducts to mutations. Overall, in this cell culture system it appears that there is a significant interaction and potentiation of effect between arsenic and BaP, providing mechanistic support for the interactions between PAC and arsenic exposures reported in epidemiology studies. These data show the utility of using ^{32}P-postabelling in animal and in in vitro studies to determined interactions between complex mixtures.

^{32}P-postabelling is a very sensitive method of detecting DNA adducts in the vast sea of un-adducted nucleotides within a cell. In addition, postlabelling has these excellent detection limits for a wide variety of aromatic and polyaromatic compounds. This means that similar methodology can be applied to many exposures and that the identity of the specific chemical does not need to be known in advance before beginning analysis. This makes it a useful tool for determining the effects of complex exposures. ^{32}P-Postlabelling cannot, by itself, provide information regarding the identity of specific adducts. However, it has been coupled with methods such as HPLC and MS to identify specific adducts of 4-ABP and acetylbenzidine to deoxyguanosine residues in human urinary bladder and/or exfoliated cells. In addition it has been used in conjunction with standards and metabolites to discern the metabolic pathways in the activation of other compounds (Dowty et al., 2000; Talaska et al., 1995).

2. Human studies: validation of biomarkers

Human studies are essential validations for biomarkers for which biological plausibility and dose–responses have been established in animal studies.

2.1. Human studies with known exposure risks

Successful validation of a human biomarker must occur on several levels (Schulte and Talaska, 1995). Once biological plausibility is demonstrated and animal studies are concluded, estimates of how the biomarker performs relative to exposures, where the risks are known, should be made. Prospective studies of exposure-effect are the best type of evidence that a marker is associated with disease. A less robust alternative method to associate a cancer biomarker with a disease risk is to apply biomarkers to study a population whose risk has been characterized earlier in epidemiological studies. The clear weakness of this approach is that measures of exposure and disease incidence are not made on the same population. This laboratory has conducted two human studies involving exposures identified as having excess cancer risk in the epidemiological literature: tobacco smoke and benzidine. Tobacco smokers are at 2–7-fold increased risk of urinary bladder cancer (Mommsen and Aagaard, 1983). When the levels of 4-aminobiphenyl DNA adducts were measured in the urothelium of smokers, the levels of the specific 4-ABP DNA adduct were elevated about 4.5-fold in the smokers (Talaska et al., 1991). Slow acetylators, both smokers and non-smokers, had increased rates of Hb and DNA adducts compared with fast acetylators in the same smoking categories (Vineis et al., 1994). These results are consistent with a link between 4-ABP-DNA adducts and smoking induced bladder cancer.

Persons working directly with benzidine appear to be about 24 times more likely to develop urinary bladder cancer, on average, than the unexposed population (Bi et al., 1992). The risk is dose related, with persons being in low, medium and high exposure categories found to have 5, 40 or 160 times the normal rate of this disease,

respectively. Earlier reports from the US indicated that about 70% of the exposed workers developed urinary bladder cancer in their lifetime (Zavon et al., 1973). The levels of benzidine DNA adducts in the exfoliated urothelial cells were studied in groups of workers with exposure to benzidine or benzidine-based dyes (or controls). It was reported that benzidine workers had levels of the specific benzidine DNA adduct which were increased in almost the same proportions as the cancer risk in the workers in the Bi et al. (1992) study. These data affirmed that non-invasive determinations of DNA adducts from cell in the target organ can be used to assess exposure; levels are also well-correlated with ultimate disease outcome. As an interesting aside, benzidine exposure was formally banned in the country where these exposures took place at the time the results were published.

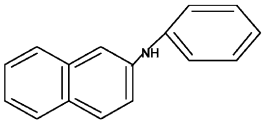
2.2. Human studies with low exposures and uncertain risks

Once it has been shown that biomarkers respond in concert with the exposure risk, they can then be applied to situations where exposures are low and the risk of the exposure is uncertain. This is an exciting phase of biomarker application as they are used in new territory and will either show or not show their strengths. As an introduction to studies of this nature it becomes extremely important that all samples be analyzed blind, i.e. without knowledge of exposure status.

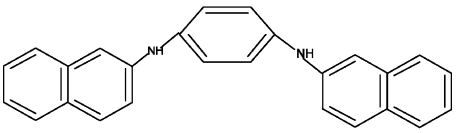
Three studies will be discussed: DNA adducts in exfoliated urothelial cells and urinary 1-hydroxy-pyrene (1HP) levels in rubber workers; the same markers in the wives of smokers and non-smokers to gauge whether an impact of passive smoking can be determined; and, the impact of a simple intervention on skin contamination, as assessed by 1HP levels and leukocyte DNA adduct levels in a group of young auto mechanic trainees.

2.2.1. DNA adducts in exfoliated urothelial cells and urinary 1hydroxypyrene (1HP) levels in rubber workers

Rubber workers are among those known historically to be at increased risk for urinary bladder cancer. The excess risk has been ascribed to β-naphthylamine (β-NA) contamination (~ 0.25–4%) in a material known as NONOX S. This material has been removed from use, yet excesses of urinary bladder cancer have still been reported in rubber workers. There are at least two possible, non-mutually exclusive reasons which may account for the excess. One is that the rubber industry still uses aromatic amines and aromatic amine based materials as antioxidants. Aromatic amines currently used include the recognized carcinogen, methylene-*bis*-2-chloroaniline (MOCA) and methylene dianiline, which may have carcinogenic potential as well (ACGIH, 2001). Several other current antioxidants retain the β-NA nucleus in their structure (Fig. 4), although no β-NA is found free in the commercial products. The other possibility is that workers may have exposure to urinary bladder carcinogens other than aromatic amines. One possibility is exposure to PAC. Two potential sources of PAC are the extender oils that make up as much as 20% of the total weight of the tire. Extender oils are comprise of coal tar pitch, bitumen and/or aromatic oils and four to six ring hydrocarbons which comprise as much as 30% of the total of these materials (IARC, 1981). The carbon blacks added to tires also are a potential PAC source. Technology has favored the use of furnace blacks over channel blacks for this purpose and the latter has been shown to contain PAC (IARC, 1981).



N-phenyl-2-naphthylamine



N,N'-di-naphthyl-p-phenylenediamine

Fig. 4. Structure of two example compounds used as antioxidants in the rubber industry that contain a β-naphthylamine nucleus (IARC, 1981).

For the purposes of the current study 24 h urine samples were collected from 56 non-smoking workers who were stratified into three a priori exposure groups based on the (Ames Salmonella) mutagenicity of total dust collected from air samples and wipes of work surfaces. The groups were high air and high surface mutagenicity, high air and low surface mutagenicity, and low air and low surface mutagenicity. In addition information was collected on job category and urinary mutagenicity. ^{32}P-postabelling was performed on the exfoliated urothelial cells obtained from the workers' urine, and 1HP determinations were made from the same samples.

Neither urinary 1HP levels or the levels of DNA adducts in the exfoliated urothelial cells were correlated with the a priori exposure groups. However, 1HP levels were associated with specific job function, specifically the handling of cured rubber products. The geometric mean of 1HP level among the 20 workers handling cured rubber was 0.62 μg/l (range: 0.4–1.67 μg/l) and 0.48 μg/l (0.35–0.8 μg/l). The individual 1HP values for the workers were also significantly correlated with urinary mutagenicity ($P = 0.005$). The DNA adduct levels were not correlated significantly with urinary mutagenicity for the individuals. However, as with the 1HP values, increased adduct levels were associated with specific job functions, namely, initial scaling and mixing, and curing. The adduct levels in these tasks were significantly higher than the other tasks. Interestingly, while the highest levels were in workers involved with scaling and mixing which were tasks not associated with elevated 1HP levels, there was significant correlation between the individual levels of 1HP and those of adduct number 3 in the exfoliated urothelial cell samples.

It should perhaps not be too surprising that there was no significant correlation between the DNA adduct levels and the level of urinary mutagens. Stronger associations have been reported between markers that integrate exposure over the short-term (urinary mutagenesis and metabolites) than between short-term markers and DNA adduct levels which integrate exposure over a longer time.

Since antioxidants, extender oils and carbon blacks are handled during scaling and mixing, it is possible that the genotoxin causing the DNA adducts seen could be either an aromatic amine or a PAC. It was not possible in this preliminary study to identify the specific adducts. The method used for exfoliated urothelial cell adduct analysis does not discriminate between aromatic amines and PAC as is the general case with the *n*-butanol and nuclease P1 variants of the ^{32}P-postabelling method. Further studies of this group will be needed to identify the specific exposures associated with the adducts. Animal studies with the initial materials may prove to be useful in this regard. This study demonstrates the value of biomarkers in identifying specific job categories that result in biologically significant levels of exposure.

2.2.2. Exfoliated urothelial cell DNA adducts and passive smoking exposure

Tobacco smoke is the most important environmental carcinogen based on its association with greatly increased rates of lung, urinary bladder and several other cancers. The best data are available for lung cancer and it has been estimated that tobacco smoking is responsible for $> 75\%$ of the 700 000 deaths for this cause each year in the US. Estimates of the increased risk of urinary bladder cancer in smokers range from 2 to 7-fold. The risk of cancer in bystanders, so-called passive smoke exposure, has been more difficult to assess. Mixed results have been reported in epidemiological studies of the matter. Passive smoke does contain more of the major urinary bladder carcinogen, 4-ABP, on a per weight basis than does mainstream smoke, owing to differences in burning temperatures and airflow. Among the major problems has been identifying groups of sufficient size and relatively uniform exposure. The range of self reported passive smoke exposures is likely very large. Kristin Anderson, Myron Gross and Stephen Hecht at the University of Minnesota have been working with a group consisting of the wives of smokers and non-smokers. These researchers provided urine samples from these persons for analysis of both exfoliated urothelial cell DNA adducts and 1HP. The study group con-

sisted of 21 wives of non-smokers and 22 wives of smokers.

It was found that the 1HP levels were increased in the wives of the smokers (mean = 0.27 μg/l, S.E. = 0.06, vs. 0.192 μg/l, S.E. = 0.02), but the difference was not significant at the 0.05 level ($P = 0.17$). With these exposure levels, measurement of urinary 1HP is essentially an estimate of the exposure which occurred the preceding 24 h. Therefore, these data suggest that there is significant variability in daily exposure of wives of smokers that contributes heavily to the inability to see significant differences in spot samples. On the otherhand, there was a statistically significant difference in the levels of DNA adducts in the exfoliated urothelial cells of the wives of smokers (2.8 adducts per 10^8 nucleotides, S.E. = 0.8) versus the wives of non-smokers ($1.1/10^8$ nucleotides, S.E. = 0.4, $P < 0.05$). Since the DNA adduct levels integrate exposure effects over a much longer time period (the life span of the urothelial cells is ~ 100 days), these data indicate that the variation in daily exposure (as indicated by 1HP levels) is smoothed out by the measurement of the DNA adduct levels. The correlation between the 1HP measurements and the DNA adduct levels was not significant ($r = 0.3$). This further suggests that at these low levels there is too much daily exposure variation to accurately predict biological effect from any one measurement. The data indicate that there would be real value in a biological monitoring program that included both metabolite and DNA adduct analysis, as we have suggested earlier. In addition, repeated measurements of the excretion of urinary metabolites is needed to better assess daily variation in exposure.

2.2.3. Using DNA adduct analysis to evaluate the effective of a simple intervention in automobile mechanic trainees

Probably the most important application of biological monitoring is as a tool to understand exposures and intervene to reduce effects. This is critical for carcinogen biomonitoring because of the chronic and severe nature of the disease. According to the multihit model of carcinogenesis, the probability of disease should increase with the total load of DNA damage (Hemminki, 1993). This is shown in the left panels of Fig. 5. A person (panel A.) whose exposure begins at about age 20 will rapidly come to a steady state DNA damage level based on their usual exposure and the life span of the target tissue and its repair capacity. This steady state level translates into a cancer rate (panel B.) that lags behind the DNA damage curve until the required multiple hits are made, and then rises steeply. If the area under the DNA damage curve can be reduced by an intervention (panel C.), the resulting cancer incidence curve should be reduced in size and shifted to the right, that is, to later in life (panel D). DNA adducts measurements are good candidate for monitoring the steady state level of DNA damage. While DNA adducts, the ultimate mutagens, are along carcinogenesis causal pathway, the probability that any single adduct will cause a consequential mutation is low (Fig. 6). It is also important to institute interventions as early as possible in the exposure history as this minimizes the possibility that an adduct will have already caused a biologically significant mutation.

The pioneering work of Grimmer (Grimmer et al., 1982) and Phillips (Carmichael et al., 1990) demonstrated that used gasoline engine oils (UGEO) contain animal carcinogens and that they induce carcinogen-DNA adducts in the tissues of topically exposed mice. The skin is the

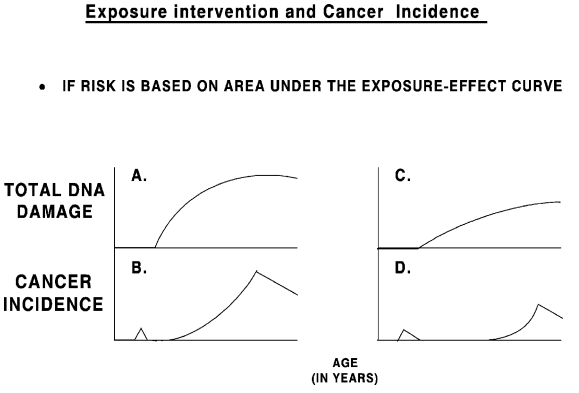


Fig. 5. Scheme for the effect that a reduction in exposure may have on area under the DNA damage curve and cancer incidence rate. A and B depict the DNA damage and cancer incidence curves by age in the absence of intervention. C and D show the same after the intervention.

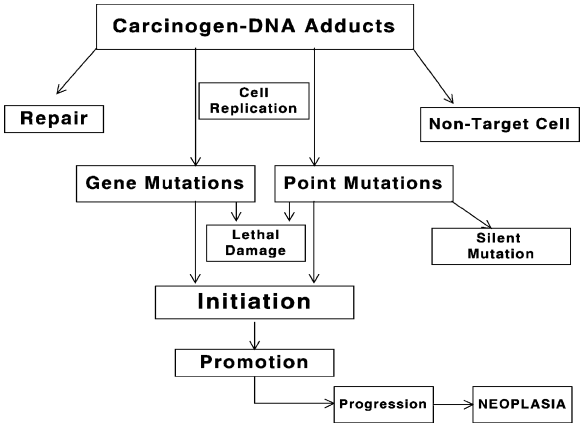


Fig. 6. Scheme for some of the possible outcomes of DNA adduction.

major route of exposure to UGEO making it possible to develop interventions in a mouse model that reduce the DNA adduct levels and may have application to the human exposure experience. It has been shown that when washing with a commercial cleaner occurs shortly after application of the oils, DNA adduct levels are reduced in both skin and lung (Talaska et al., 1996). Certain commercial barrier creams did reduce adduct levels, particularly when washing could not be done quickly after oil application (Drexelius et al., 1999). However, when kerosene was used as a cleaner, the levels of UGEO DNA adducts in the lungs were actually increased to levels higher then the positive (unwashed) controls (Lee et al., 2000). These animal data were used to develop a model for training and intervention. A study of auto mechanic trainees-young men who were in technical schools was begun. Study subjects were selected and gave informed consent, obtaining the pre-intervention skin wipes. Urine and blood were obtained at the same time. Then, the participants were given the detailed results of our animal studies and the potential impact of proper cleaning and hand care was discussed. Participants were given a measured amount of a commercial hand cleaner (provided gratis by GOJO Industries, Akron, OH). Samples were collected initially, at intervals, and finally at the end of the study. Only the data on the pre-exposure and last post-intervention samples have been completed at this time and are discussed here. These samples were collected at least 8 months apart to allow for steady state DNA adduct levels to respond to the new exposure. Only the blood leukocyte adduct levels are reported in this study, as it was not possible to successfully isolate enough DNA from the exfoliated urothelial cells to give a sufficient level of assay sensitivity.

This simple intervention was moderately effective in reducing the amount of PAC collected on skin wipes, the levels of urinary 1HP and the levels of leukocyte DNA adducts. For example, the post-intervention leukocyte DNA adduct levels were reduced in 19 of the 25 participants. On average the levels decrease from 9.4 adducts per 10^9 nucleotides (S.E. = 4.2) to 2.9 adduct per 10^9 nucleotides (S.E. = 1.6) in the post-intervention samples. While it is noted that this result was significant at the $P < 0.1$ level, the fact that the adduct levels, skin wipe levels and 1 HP level were decreased in the majority of the participants is an important finding. In addition, if these reductions are confirmed by further studies, they represent an attempt to reduce the DNA damage burden early in the careers of these participants and hopefully have given them the positive feedback necessary to continue to control their exposures and reduce risk of disease.

3. Conclusions

DNA adduct analysis is a most useful tool for determining mechanisms of activity and a biological monitoring method of carcinogen effective dose in humans. Measurement of DNA adduct levels allows insight into the impact of metabolic variations, the interactions between components of complex mixtures and co-exposure to potentiating compounds which enhance the effect of carcinogens. In human studies, DNA adduct levels in the target organ are consistent with the excess risk noted for populations with specific exposures. It is possible to compliment ^{32}P-postlabelling with other method and identify specific adducts in the target organ for two important exposures: tobacco smoking and benzidine-based dyes. Significant differences in DNA adduct levels

were detected in persons exposed to passive tobacco smoke in the face of only modest differences in exposures. Simple interventions have been shown to reduce DNA adduct levels in the target organ of an exposed population. So, while analysis of carcinogen DNA adducts remains primarily a research tool, these research studies have begun to validate its wider use in biological monitoring of exposed humans.

Acknowledgements

The authors gratefully acknowledge the contributions of other senior investigators in the studies discussed here including Hans Kromhut, Dan Nebert and Alvaro Puga, Kristin Anderson, Stephen Hecht and Myron Gross. This work supported in part by NIOSH RO1-OHO-4124, T42-CCT-510420 and NIEHS P30 ESO6096.

References

ACGIH (American Conference of Governmental Industrial Hygienists), 2001. Threshold Limit Values and Biological Exposure Indices, ACGIH, Cincinnati, OH.

Bi, W., Hayes, R.B., Feng, P., Qi, Y., You, X., Zhen, J., Zheng, M., Qu, B., Fu, Z., Chen, M., Chein, H.T., Blot, W.J., 1992. Mortality and cancer incidence of bladder cancer of benzidine-exposed workers in China. Am. J. Ind. Med. 21, 481–489.

Bhatnagar, V.K., Talaska, G., 1999. Carcinogen exposure and effect biomarkers. Toxicol. Lett. 108, 107–116.

Butler, M.A., Ivasaki, M., Guengerich, F.P., Kadlubar, F.F., 1989a. Human cytochrome P-450^{pa}P-4501A2, the phenacetin-*O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. Proc. Natl. Acad. Sci. USA 86, 7696–7700.

Butler, M.A., Guengerich, F.P., Kadlubar, F.F., 1989b. Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylenebis-2-chloroaniline by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. Cancer Res. 49, 25–31.

Carmichael, P.L., Jacob, J., Grimmer, G., Phillips, D.H., 1990. Analysis of polycyclic aromatic hydrocarbon content of petrol and diesel lubricating oils and determination of DNA adducts in topically treated mice by ^{32}P-postlabelling. Carcinogenesis 11, 2025–2032.

Dowty, H.V., Xue, W., LaDow, K., Talaska, G., Warshawsky, D., 2000. One-electron oxidation is not a major route of metabolic activation and DNA binding for the carcinogen 7H-dibenzo[c,g]carbazole in vitro and in mouse liver and lung. Carcinogenesis 21, 991–998.

Drexelius, R.J., Carwardine, K., Jaeger, M., Talaska, G., 1999. Barrier cream application reduces the formation of DNA adducts in lung tissue of mice dermally exposed to used gasoline engine oil. Appl. Occup. Environ. Hyg. 14, 838–844.

Hemminki, K., 1993. DNA adducts, mutation and cancer. Carcinogenesis 14, 2007–2012.

Grimmer, G., Dettbarn, G., Brune, H., Duetsch-Wenzel, R., Misfeld, J., 1982. Quantification of the carcinogen effect of polycyclic aromatic hydrocarbons in used engine oil by topical application on the skin of mice. Int. Arch. Occup. Environ. Health 50, 95–100.

Higgins, L., Welch, K., Oh, M., Bond, G., Hurwitz, P., 1981. Influence of arsenic exposure and smoking on lung cancer among smelter workes: a pilot study. Am. J. Ind. Med. 2, 31–41.

IARC, 1981. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: The Rubber Industry, vol. 29, International Agency for Research on Cancer, Lyon, France.

Lee, J.-H., Roh, J.H., Burks, D., Warshawsky, D., Talaska, G., 2000. Skin cleaning with kerosene facilitates passage of carcinogens to the lungs of animals treated with used gasoline engine oil. Appl. Occup. Environ. Hyg. 15, 362–369.

Maier, A., Schumann, B.L., Chang, X., Talaska, G., Puga, A., 2002. Arsenic co-exposure potentiates benzo(a)pyrene genotoxicity. Mutat. Res. 517, 101–111.

Mommsen, S., Aagaard, J., 1983. Tobacco as a risk factor in carcinogenesis. Carcinogenesis 4, 335–338.

Pershagen, G., Wall, S., Taube, A., Linnman, L., 1981. On the interaction between occupational arsenic exposure and smoking and its relationship to lung cancer. Scand. J. Work Environ. Health 7, 302–309.

Rothman, N., Bhatnagar, V.K., Hayes, R.B., Kashyap, R., Parikh, D.J., Kashyap, S.K., Schulte, P.A., Butler, M.A., Jaeger, M., Talaska, G., 1996. The impact of interindividual variability in *N*-acetyltransferase activity on benzidine urinary metabolites and urothelial DNA adducts in exposed workers. Proc. Natl. Acad. Sci. USA 93, 5084–5089.

Schulte, P.A., Talaska, G., 1995. Validity criteria for the use of biological markers of exposure to chemical agents in environmental epidemiology. Toxicology 101, 73–88.

Schumann, B.L., LaDow, K., Luse, N., Warshawsky, D., Pickens, W., Hoath, S., Kasting, G., Talaska, G., 2002. Preliminary findings that kerosene alters the distribution of topically applied benzo(a)pyrene in mice. Polycyclic Arom. Comp. in press.

Talaska, G., Al-Juburi, A.Z.S.S., Kadlubar, F.F., 1991. Smoking-related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. Proc. Natl. Acad. Sci. USA 88, 5350–5354.

Talaska, G., Roh, J., Schamer, M., Reilman, R., Xue, W., Warshawsky, D., 1995. 32P-Postlabeling analysis of dibenz[a,j]acridine DNA adducts in mice: identification of pre-genotoxic metabolites. Chem. Biol. Interact. 95, 161–174.

Talaska, G., Cudnick, J., Jaeger, M., Rothman, N., Bhatnagar, V.J., Kayshup, S.J., 1996. Development of non-invasive biomarkers for carcinogen-DNA adduct analysis in occupationally exposed humans: exposure monitoring of chemical dye workers and monitoring the effectiveness of interventions to dermal exposure of used gasoline engine oils. Toxicology 111, 207–212.

Vineis, P., Bartsch, H., Caporaso, N., Harrington, A.M., Kadlubar, F., Landi, M.T., Malaveille, C., Shields, P.G., Skipper, P., Talaska, G., Tannenbaum, S.R., 1994. Genetically-based *N*-acetyltransferase metabolic polymorphism and low level environmental exposure to carcinogens. Nature (London) 369, 154–156.

Zavon, M., Hoegg, U., Bingham, E., 1973. Benzidine exposure as a cause of bladder tumors. Arch. Environ. Health 27, 1–7.