DNA Adducts and Mutations in Occupational and Environmental Biomonitoring

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The methods applied for DNA adduct determination in humans have become more reliable. Yet there is a need to characterize the adducts studied better and, when possible, to identify them with the help of the available standard compounds. Use of standard compounds also allows quantification of adduct levels. There is a lack of knowledge on the adduct levels and their half-lives in target and surrogate tissues. Most adduct studies have been carried out on occupational populations exposed to complex mixtures. White blood cells have been the most common source of DNA. Other exposures and tissues should be a subject of study. Notably, dietary exposures have been largely neglected. Biomonitoring of mutations is a relatively new field and a few exposures have so far been investigated. The results have been promising, but logistics of the studies have to be improved to make large field studies possible. Future biomonitoring studies should make an effort to combine many end points, with emphasis on adducts, mutations, and constitutional metabolic factors. — Environ Health Perspect 105(Suppl 4):823-827 (1997)

Key words: 32P-postlabeling, DNA damage, pollution, metabolism, risk assessment

Introduction

Application of DNA adducts and mutations in human biomonitoring is recent and the related technologies are continuously improving. Since the 1980s when DNA adduct studies became possible in humans (1,2) the question about their significance has been asked. There is no direct answer to the question but much of circumstantial evidence points to their mechanistic role in cancer (3). Yet direct evidence is lacking and it would be simplistic to assume that one measurement in time would tell much about the risk. However, if a particular exposure has lasted for an extended time, or if it has been excessive, such as anticancer chemotherapy or an accident involving exposure to radioactive material, some increase in risk may be predicted. Some, but by no means all, of the reasons DNA adducts are important are the following:

- Adducts cause mutations: e.g., construction of a specific mutation in a phage or virus leads to a specific mutation at a site of adduct; DNA repair defects.
- Mutations in viral and cellular oncogenes, tumor suppressor genes; transgenic animals can cause cancer.
- Adducts, cross-links in particular, are cytotoxic and thus mitogenic; cell proliferation may contribute to cancer.
- Adducts show individual exposure, metabolic capacity, and DNA repair capacity.
- Adducts indicate present DNA damage (cf. epidemiology).
- Adduct-forming chemicals—e.g., many anticancer agents—are potentially dangerous.
- Adducts can show active ingredients in complex mixtures.

Although first emphasized in the “initiation” phase of carcinogenesis, the recent evidence of genetic lesions in multiple steps of cancer development, suggests that DNA damage plays a role in many stages of oncogenesis. Among the available methods, only 32P-postlabeling is discussed here because of its wide application in the occupational and environmental studies.

The role of mutations has not been questioned to the same extent as that of DNA adducts because mutations play an important role in the development of cancer (4). However, the appearance of mutations in surrogate tissues of healthy individuals may not be directly informative of the events in target tissues. Additionally, all the mutational systems available for human biomonitoring have their own features and limitations (5). Only one mutational system, based on hypoxanthine-guanine phosphoribosyl transferase (HPRT), is discussed here.

Development of the 32P-Postlabeling Method

The 32P-postlabeling method, introduced about 15 years ago, made it possible for the first time to analyze DNA-adducts existing in DNA in vivo. The method has been used extensively to compare adduct patterns in various tissues and in various exposures. Most studies have focused on unidentified aromatic adducts because the original technique selects for these types of adducts (6). More recently, standard compounds have been used in the identification and quantification of adducts (2). Although this is widely accepted now, it was long thought by a large section of the postlabeling community that labeling of all adducts was complete. By now it has been demonstrated with tens of different synthetic postlabeling standards that, depending on the adducts and conditions of labeling, the recoveries vary between 0 and 100%. Even diastereomers can label differently. In an illustrative experiment, DNA adducts of a number of 3H-labeled polycyclic aromatic hydrocarbons (PAH) were prepared in a microsomal system and used for optimization and measurement of recoveries in the postlabeling assay. The optimal labeling conditions for all tested compounds were very similar. The recoveries varied from 3 to 60% among different PAHs, indicating that the levels of these adducts could be considerably...
underestimated in analyses of human samples from PAH-exposed populations. Because we have found similar results with an entirely different group of compounds, it generally can be concluded that different adducts require different conditions for optimal labeling (7). Thus the absence of proper standards, or analysis of unknown adducts, impedes quantitative interpretation of the postlabeling results. The most important parameters for optimal postlabeling are that the adduct must be known; the standard must be synthesized and its stability tested; the standard’s labeling efficiency must be tested; and the total recovery of the tested DNA adduct must be determined. Below some illustrative examples of the application of the postlabeling method are given. Extensive surveys of the literature are available (1,6).

Surrogate versus Target Tissues

In biomonitoring of almost any end point, surrogate rather than target tissues have to be used. The information on the applicability of surrogate tissues in humans is scanty. In some animal experiments the question of the correlation of adducts in surrogate and target tissue has been addressed (18) and references therein. Rats were exposed by inhalation to individual alkenes from ethene to octene; DNA adducts in liver and lymphocytes and hemoglobin adducts were measured (Figure 1). For all these adducts the levels decreased from ethene to octene. However, the decrease was 5-fold in liver, 30-fold in lymphocytes, and 2000-fold in hemoglobin. This was interpreted as indicating a complex interplay of tissue uptake, metabolism, and diffusion out of organs.

In humans, smoking has been the main model of exposure used. Smoking is a known risk factor of laryngeal cancer. Aromatic adducts of laryngeal tissue obtained from surgery patients were analyzed and a relationship to smoking was found. Both tumor and normal laryngeal tissues showed a correlation of about 0.9 to the total white blood cells (9).

Tobacco smoke contains methylating and hydroxyethylating principles, originating, for example, from tobacco-specific nitrosamines and ethene, respectively. The levels of 7-methylguanine were highest in the bronchial DNA of smokers, exceeding the level in nonsmokers by almost four times (10). In a small number of smokers, both target (bronchial) and surrogate (lymphocyte) DNA were available, showing a correlation of 0.8. We now know that the 7-methylguanine adduct spot also contains 7-(2-hydroxyethyl)guanine adducts, which, however, were poorly labeled and contributed little to the radioactivity of the spot (11). Larynx tissue samples obtained from surgery patients were also assayed for 7-methylguanine DNA adducts. There was a relationship to smoking, and larynx adduct levels exceeded those of white blood cells by twice (12). There was a modest correlation only between 7-alkylguanines and aromatic adducts (above). The smokers had higher aromatic adduct levels in lymphocytes than in granulocytes, which indicated that in chronic exposure the main focus should be on lymphocytes (13). Smokers also had elevated levels of 7-methylguanine, particularly in their lymphocytes as compared to the granulocyte DNA (14). However, taking into consideration the very different half-lives of lymphocytes and granulocytes, the isolation of cells only clears away the "noise" caused by granulocytes and improves the precision, as only about 25% of the DNA in total white blood cells is from lymphocytes. This should be an important principle applied in biomonitoring studies.

Aromatic Adducts

In spite of problems in interpretation of postlabeling results of complex mixtures, most published literature concerns exposures in which PAHs are of primary concern. Many of the groups studied have been at risk of cancer according to epidemiological reports that reflect exposures a few decades ago. The main questions posed have been: a) do the exposed groups show higher, work-related adduct levels than the controls; b) is there a correlation between exposure measures (air concentration or urinary 1-hydroxypyrene) and adducts; and c) how large are the individual variations and metabolic genotypes on the level of adducts?

Occupational Populations

The study populations have included foundry and coke workers, aluminum and electrode workers, and chimney sweeps. Additionally, some other occupational groups have been studied as reference groups in environmental studies, discussed below. Examples are given on some collaborative studies in which our laboratory has participated. The foundry study has involved blood and urine sampling of the same individuals each December for four years. The last sampling was done in December 1993. It is a multi-end point study, including some 15 parameters. Only results from the first 2 years on certain outcomes have been published but an intense compilation of the total material is underway. The first published papers showed elevated total white blood cell DNA adduct levels as measured by immunoassay (15) and postlabeling (16,17), relating to exposure. Among the other occupational groups, coke workers had higher levels of aromatic adducts than the local controls (18,19). Somewhat elevated but not statistically significant differences were seen in electrode and aluminum workers even though air concentrations of PAHs and urinary 1-hydroxypyrene levels indicated excessive exposure (20,21). Adduct levels were also slightly increased in total white blood cell DNA of chimney sweeps. However, the difference to a control group became significant only after adjustment for the CYP1A1 and glutathione S-transferase (GSTM1) genotype (22). Yet each of the genotypes alone had a rather small effect on DNA adduct levels. In all the studies cited, the interindividual variation in the levels of adducts has been large, over 10-fold. The variation is usually larger in the exposed than in the control populations, suggesting that exposures as well as constitutional factors contribute to such a variation.

Environmentally Exposed Populations

Two series of environmental studies have been conducted, one in Poland and the other mainly in Sweden. The Polish study was initiated several years ago in response to the alarming reports of environmental
pollution in Silesia, a heavily industrialized area. The first study on the Silesian population showed an elevated level of adducts, by postlabeling and immunoassay, in the total white blood cells of the residents (23). This was followed by reports of seasonal differences in adduct levels, which matched the air concentrations of PAHs (18,24). The effects were mainly seen in DNA of the long-lived lymphocytes, while granulocytes showed no clear effect. Sampling in both summer and winter allowed a rough estimation of the half-lives of aromatic adducts in lymphocytes of 1 to 2 months (24). Also, cyto genetic damage was seen in the Silesian population (25).

The nature of the adducts detected by postlabeling has been studied in more detail by comparing nuclease P1, butanol extraction, and immunoaffinity purification of the adducts. Adduct recovery was approximately equal by the P1 and butanol techniques, suggesting that the adducts are of PAH-type. For immunoaffinity chromatography an antibody raised against benzo[a]pyrene deoxyribose–DNA was used. Only about 25% of the adducts were bound by the antibody, indicating that most of the adducts in DNA are not closely related to benzo[a]pyrene. However, in winter, a time of high air pollution, the relative binding by the immunoaffinity column was higher than in the summer (19). In HPLC analysis using flow-through radioactivity detectors, typical seasonal adduct peaks were noted and they were particularly prominent in lymphocyte DNA collected in the winter. They eluted in the area of PAH–DNA adducts, giving additional support to the conclusion that the adducts are PAH-like (Figure 2) (26).

A study of bus drivers from central Stockholm and from the city’s outskirts compared these subjects to a nonoccupational control group of fine mechanics. All participants were nonsmokers. Aromatic DNA adducts in lymphocytes, PAH adducts in albumin and thione, and propene adducts in hemoglobin were not elevated in the urban bus drivers (27). A similar type of study was conducted in Milan, Italy. The study subjects were newspaper vendors from busy streets and from the outskirts of Milan. The levels of DNA adducts did not differ in these populations.

Some occupational groups, exposed to car and diesel exhaust, were positive controls in the above study. They included garage workers who overhauled diesel buses and inhaled diesel exhaust gases, car mechanics exposed to spilled engine oils but not to exhaust, and truck terminal workers, unloading and reloading diesel trucks. All these groups had increased levels of lymphocyte DNA adducts, the highest levels being correlated with air concentrations of diesel exhausts. The estimated air benzo[a]pyrene levels were below 10 ng/m³ (28). GSTM1 and N-acetyltransferase (NAT2) genotypes were determined in the study subjects. In individuals with a combined genotype of slow acetylation but lacking the GSTM1 gene, the adduct levels were significantly increased (29). Neither genotype alone had an effect on the level of adducts.

Figure 2. Aromatic adduct levels of a typical nonsmoker from Silesia as analyzed by HPLC. DNA from lymphocytes collected in the winter was assayed and the peaks numbered in the order of elution. The Roman numerals refer to elution of some standard DNA adducts of PAHs (26).

Styrene

Genotoxicity of styrene has been of interest worldwide because it is one of the few suspected mutagenic compounds that may cause daily exposures in gram quantities (30). Styrene is an example of how the chemical characterization, fascinating in itself, leads to production of standard compounds for postlabeling (31) and to a quantification of O6-guanine adducts in white blood cell DNA of laboration workers (32). Further studies have been carried out to sample the same laboration workers periodically in order to measure lymphocyte and granulocyte DNA. Again it was shown that adducts are essentially only found in lymphocytes. The repair of O6-adducts appeared to be slow, since no essential decrease in adduct levels was noted after workers took a 2-week vacation (33).

The adduct studies suggested that the factory controls, in fact, are not completely unexposed. Strand breaks, measured by the Comet assay, were also increased in the laboration workers. There was a correlation between strand breaks and O6-guanine adducts, but neither correlated with HPRT mutant frequency (34). In vitro data on the effect of styrene oxide (the main metabolite of styrene) on cultured human lymphocytes confirmed the relatively long half-lives of O6-guanine DNA adducts and the induction of strand breaks (35).

HPRT Mutations

Mutations in the HPRT gene in human lymphocytes have been studied extensively, but only a few occupational studies and, as far as we know, no environmental studies on chemical exposures have been carried out (5). The nonoccupational studies include those on smoking-, radiation-, chemotherapy-, and disease-induced mutation rates. The limited number of occupational studies partially reflects logistic problems because cell separation from blood has to be carried out within hours of blood collection, and living cells have to be delivered to the analyzing laboratory. Moreover, large interindividual differences and dependence of the mutation rates on age and smoking may discourage attempts to distinguish small differences between the exposed and control populations.

Among the occupational groups studied, workers producing the anticancer agent cyclophosphamide have elevated levels of lymphocyte HPRT mutations (36). Exposures to ethylene oxide (37) and styrene/dichloromethane have also caused increases in mutation frequency (38). In our studies on laboration workers (above) the HPRT mutation frequency was elevated in workers exposed to styrene but the increase reached statistical significance only when compared to an external rather than an in-house, factory control group (34). Induction of HPRT mutations in cultured human lymphocytes exposed to styrene oxide was considered weak (35).

We have also measured mutant frequencies in occupational populations exposed to PAHs. In the foundry study, HPRT correlated with exposure and adduct levels, while glyphorhin A NO mutations had a moderate, but statistically not significant trend with exposure (16,17). The HPRT mutant frequency was not increased in garage workers, but at an individual level there was a highly significant correlation between adducts and mutant frequency.
Figure 3. Aromatic DNA adduct levels of workers exposed to diesel exhaust [28] as related to HPRT mutation frequency in peripheral lymphocytes [29]. The correlation for the total study population, as shown in the figure, or for the exposed workers was approximately 0.35. MF, mutant frequency.

DNA adduct studies in humans are becoming more quantitative and therefore offer chances for quantitative risk estimation. Since epidemiological studies always show the risks of exposure decades back, DNA adduct studies can be used for current risk estimation. DNA adduct studies are likely to give clues to individual risks and may therefore be useful in protecting sensitive populations. Assays of point mutations, such as those in the HPRT locus have been used to a limited extent in biomonitoring of chemical exposure. The studies published so far are promising. Mutations as compared to DNA adducts are mechanistically closer to the cancer end point and would provide a valuable addition to risk assessment of chemical exposures.

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