DNA Adducts as Exposure Biomarkers and Indicators of Cancer Risk

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Quantitation of DNA adducts in human tissues has been achieved with highly sensitive techniques based on adduct radiolabeling, antisera specific for DNA adducts or modified DNA, and/or adduct structural characterization using chemical instrumentation. Combinations of these approaches now promise to elucidate specific adduct structures and provide detection limits in the range of 1 adduct/10^9 nucleotides. Documentation of human exposure and biologically effective dose (i.e., chemical bound to DNA) has been achieved for a wide variety of chemical carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aromatic amines, heterocyclic amines, aflatoxins, nitrosamines, cancer chemotherapeutic agents, styrene, and malondialdehyde. Due to difficulties in exposure documentation, dosimetry has not been precise with most environmental and occupational exposures, even though increases in human blood cell DNA adduct levels may correlate approximately with dose. Perhaps more significant are observations that lowering exposure results in decreasing DNA adduct levels. DNA adduct dosimetry for environmental agents has been achieved with dietary contaminants. For example, blood cell polycyclic aromatic hydrocarbon–DNA adduct levels were shown to correlate with frequency of charbroiled meat consumption in California firefighters. In addition, in China urinary excretion of the aflatoxin B_1-N^7-guanine (AFB_1-N^7-G) adduct was shown to increase linearly with the aflatoxin content of ingested food. Assessment of DNA adduct formation as an indicator of human cancer risk requires a prospective nested case–control study design. This has been achieved in one investigation of hepatocellular carcinoma and urinary aflatoxin adducts using subjects followed by a Shanghai liver cancer registry. Individuals who excreted the AFB_1-N^7-G adduct had a 9.1-fold adjusted increased relative risk of hepatocellular carcinoma compared to individuals with no adducts. Future advances in this field will be dependent on chemical characterization of specific DNA adducts formed in human tissues, more precise molecular dosimetry, efforts to correlate DNA adducts with cancer risk, and elucidation of opportunities to reduce human DNA adduct levels. — *Environ Health Perspect* 105(Suppl 4):907–912 (1997)

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Introduction

Many compounds classified as human carcinogens are known to form DNA adducts *in vivo* (*1*). Impetus to apply DNA adduct formation as a biomarker of human cancer risk comes from extensive studies in animal models in which DNA adduct formation has been shown to be necessary but not sufficient for tumorigenesis. Estimation of biologically effective dose of chemical exposures in humans and projection of DNA adduct-based cancer risk are dependent on sensitive and specific methods for carcinogen–DNA adduct detection (*2,3*). The observed adduct levels presumably reflect variables that comprise extent and frequency of chemical exposure, xenobiotic metabolism (a balance between carcinogen activation and detoxication), rate of covalent binding of ultimate metabolites to DNA, and rate of DNA adduct repair (*4,5*). Currently, the presence of a DNA adduct in human tissue indicates that exposure has occurred, although the amount of that exposure and the individual's cancer risk remain unknown. This presentation will discuss major methodological advances in human DNA adduct quantitation, give examples of exposure monitoring and molecular dosimetry, and describe one study in which human DNA adduct formation has been shown to correlate with incidence of a human cancer. The examples chosen will also provide an opportunity to focus on relevant issues related to monitoring of human DNA adducts induced by exposure to environmental carcinogens.

Methodological Approaches to Human DNA Adduct Monitoring

Single methods currently in use for carcinogen–DNA adduct detection include immunoassays (*6*), immunohistochemistry (*7,8*), ^{32}P-postlabeling (*9,10*), fluorescence and phosphorescence spectroscopy (*11*), gas chromatography–mass spectrometry (GC–MS) (*12*), atomic absorbance spectrometry (*13,14*), and electrochemical conductance (ECC) (*15*). These methods, applied individually, are typically not able to chemically characterize specific adducts. Therefore, an important aspect of more recent approaches to human biomonitoring is the development of preparative strategies for sample purification that can be applied prior to the ultimate adduct quantitation (*16*). Recent advances combining preparative chromatography with immunoassays, ^{32}P-postlabeling, synchronous fluorescence spectrometry (SFS), and GC–MS have allowed identification and quantitation of specific DNA adducts in human tissues, thereby strengthening human exposure documentation.

The two most frequently employed DNA adduct methodologies, immunoassays and ^{32}P-postlabeling, will be discussed briefly. Antisera elicited against DNA adducts and carcinogen-modified DNA samples (*17–19*) have been widely utilized to quantify and localize xenobiotic-induced DNA damage (*20–23*) and to measure DNA adduct formation in human tissues (*24,25*). Competitive radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) are able to detect human DNA damage with sensitivity in the range

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Abbreviations used: AFB_1-N^7-G, aflatoxin B_1-N^7-guanine; B[*a*]P, benzo[*a*]pyrene; CB, charbroiled; ECC, electrochemical conductance; ELISA, enzyme-linked immunosorbent assay; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; PAHs, polycyclic aromatic hydrocarbons; RIA, radioimmunoassay; SFS, synchronous fluorescence spectrometry; TLC, thin layer chromatography.

of 1 adduct in 10^8 unmodified nucleotides. Immunoassays are reliable and inexpensive, and they allow for the analysis of many samples in one day. Disadvantages include the requirement for relatively large amounts of DNA (200 µg) and a lack of absolute specificity because of antibody cross-reactivity. Cross-reactivity with unmodified nucleotides or carcinogen alone occurs very rarely (*26*), but there may be recognition of other adducts of the same carcinogen or adducts of other chemically related compounds (*27*), resulting in detection of multiple, chemically similar DNA adducts. ^{32}P-postlabeling is based on DNA digestion to 3′-phosphates, 5′-radiolabeling of adducts with high specific activity ^{32}P from γ^{32}P-ATP by T4 polynucleotide kinase, and separation of the *bis*-phosphates on thin layer chromatography (TLC). The method, widely used for human DNA adduct detection (*10,22,25*), has the advantage of high sensitivity (often 1 adduct in 10^9 nucleotides) and application to small quantities of DNA (2–10 µg). However, identification of the detected adducts has very rarely been achieved, and the assay has the additional disadvantage that unknown adducts may become 5′-phosphorylated with varying efficiencies, resulting in underestimation of adduct concentrations in a human samples.

For investigation of human DNA samples, the immunoassays and ^{32}P-postlabeling, discussed above, provide indications of exposure but lack chemical specificity. Methodological combinations devised to improve the specificity of DNA adduct detection typically involve either conventional chromatographic separation by high- pressure liquid chromatography (HPLC) or immunoaffinity chromatgraphy as a first step. When a human DNA sample is digested and subjected to HPLC, even though the adduct levels are too low to be detectable by conventional monitoring, the fractions that should contain specific adducts (identified by appropriate standards) can be subjected to SFS, ^{32}P-postlabeling, or GC–MS. Thus, the sensitivity and specificity of ELISAs have been enhanced by combination with prior HPLC. The approach has been applied to human gastric mucosa samples (*28*) and human liver samples (*29*) using antisera specific for alkyl-modified nucleosides. Chromatographic separation by HPLC has also been combined with ^{32}P-postlabeling, and a recent review (*30*) covers the general subject. One line of experimentation has combined two chromatographic steps with ^{32}P-postlabeling for the detection of specific O^6- and N^7-alkyl-deoxyguanosine adducts in human lung and lymphocytes (*31–33*). The development of this method has facilitated the use of internal and co-chromatography standards. In an additional approach (*34*), two chromatographic steps were used prior to the GC–MS determination of 3-methyladenine and 7-methyl-guanine adducts in the urine of smokers. Finally, HPLC has been used as the first step in a procedure combining ^{32}P-postlabeling with immunoprecipitation for the detection of O^6- and O^4-alkyl-deoxyguanosine adducts in human liver and leukocyte DNA samples (*35*).

Human Exposure Monitoring and Molecular Dosimetry

Xenobiotic exposures that have been examined by immunoassay in DNA samples from human subjects include aflatoxins (*36–38*), 4-aminobiphenyl (*39,40*), *N*-nitrosamines (*28,29,41*), and polycyclic aromatic hydrocarbons (PAHs) (*42–44*). In addition, adducts have been determined in DNA of patients receiving medicinal exposures, including cisplatin (*45*), procarbazine (*46*), dacarbazine (*47*), coal tar (*48*), and 8-methoxypsoralen (*49*). Oxidative damage (*50*) and ultraviolet light photoproducts (*51*) have been measured in DNA by immunoassay. ^{32}P-postlabeling has been applied to DNA from multiple human tissues (*43,52–55*), with indications that aromatic adducts increased in individuals with documented high occupational or tobacco exposures (*10,56–64*). This technique has also been used to examine DNA from individuals given coal tar (*65*) and mitomycin C (*66*) for medicinal purposes. For mitomycin C and styrene (*67*) it is the only method available for detection of human DNA adducts. Luminescence spectroscopy has been highly successful in documenting DNA adducts of aflatoxins (*68,69*), benzo[*a*]pyrene (B[*a*]P) (*70,71*) and PAHs (*72,73*), while GC–MS is routinely applied for 4-aminobiphenyl (*74*), *N*-nitrosamines (*12,75–78*), and tobacco-specific nitrosamines (*79*). Atomic absorbance spectrometry is used for cisplatin (*80,81*) and electrochemical detection is used for oxidative DNA damage (*82,83*).

DNA adduct dosimetry cannot be ascertained for most environmental exposures because precise documentation of the dose received is impossible; however, certain trends are noted when multiple studies are examined. For example, in several reported investigations in which ambient B[*a*]P concentrations were compared to blood cell PAH–DNA adducts, increased ambient pollution was associated with higher levels of blood cell PAH–DNA adducts (*44,61,84,85*). In addition, measures taken to reduce the ambient PAH levels result in lowered DNA adduct levels (Table 1). For example, in two studies of Finnish foundry workers, performed several years apart, decreasing the B[*a*]P levels from 12 to 200 ng/m^3 down to <5 to 60 ng/m^3 significantly reduced the PAH–DNA adduct levels (*44,48*). In addition, the same workers showed lower PAH–DNA adduct levels after time spent on vacation (*60*). In another study, U.S. Army soldiers went from a very clean environment in Kuwait in August 1991 to significantly higher pollution levels in Germany in October 1991, and DNA adducts increased significantly (*86*). An example of reducing pollution in the environment and lowering DNA adduct levels occurred in the Silesian region of Poland in the summer of 1992, where it was demonstrated that the air was about 5-fold cleaner than in the winter and the adducts in lymphocytes were about 5-fold lower at that time (*87*). In analyzing these data a number of confounding factors must be recognized. The use of ambient B[*a*]P measurements provides an indicator of the pollution levels, but the actual hydrocarbon components vary and are not always measured. In addition, cohorts are grouped according to the highest exposure documented, but the range of

Table 1. DNA adduct levels (adducts/10^8 nucleotides) in human blood cells decrease with a reduction in airborne B[*a*]P concentration.

	Airborne B[*a*]P concentration, ng/m^3					
Cohort	<1	<5	5–12	12–60	50–50,000	References
Finnish foundry	2.2[a]	–	–	8.0	21.0, 50.0	Perera et al. (*44*)
Finnish foundry	–	5.2	6.1	9.6	–	Paleologo et al. (*48*)
Polish coke ovens	–	–	–	8.2	24.5	Hemminki et al. (*84*)
Polish Silesia region	–	1.3, Summer[b]	–	6.4, Winter[b]	–	Grzybowska et al. (*87*)
U.S. Army soldiers	1.6, Kuwait	–	4.0, Germany	–	–	Poirier et al. (*86*)

[a]Controls: ambient monitoring was typically not conducted for individuals serving as controls. [b]DNA adduct values are for lymphocytes only.

exposures for one job at one worksite can vary considerably. In some studies DNA adduct levels correlate with extent of pollution, but the discrepancies suggest that B[*a*]P may not be the compound responsible for producing the majority of PAH–DNA adducts observed by immunoassay and ^{32}P-postlabeling in human blood cell DNA samples.

Because much of the available human DNA adduct dosimetry for occupational and environmental exposures depends upon ambient biomonitoring, precise dose–response relationships have not been possible. However, in some studies with dietary carcinogen exposure, dosimetry has been demonstrated. In one study of California firefighters (*88*), a blood sample was taken before the summer firefighting season began and another after 8 weeks of firefighting for approximately 12 hr/day. PAH–DNA adducts were shown not to correlate with the extent of firefighting. However, these individuals often ate charbroiled food cooked over an open flame. Comparison of dietary habits with DNA adduct values showed that individuals (n = 19) consuming charbroiled food one to two times in the previous week had a mean PAH–DNA adduct value of 1.6 adducts in 10^8 nucleotides (Figure 1). However, 23 individuals who reported consumption three to five times in the previous week and 5 individuals who reported consumption > 5 times in the previous week had mean adduct values 3.0 and 6.7 adducts/10^8 nucleotides, respectively (Figure 1). For

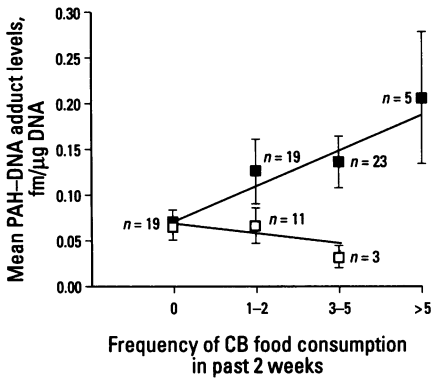

Figure 1. Frequency of charbroiled (CB) food consumption and level of PAH–DNA adducts determined by immunoassay in blood cell DNA from California firefighters (*88*). Dose response (■) is shown for frequency of CB food consumption during the immediately previous week (Pearson r hr = 0.24, p hr = 0.06). There is no dose response when consumption was 7 to 14 days previously (□), suggesting that adduct removal may be essentially complete by 7 days.

dosimetry of aflatoxin exposure, 42 individuals in the Guanxi region of China (*89*) were studied. A portion of the actual food consumed was assayed for aflatoxin content and urinary output was assayed for excretion of aflatoxin B_1-N^7-guanine by both males and females (*37,90*). The dosimetry data showed an excellent correlation between dietary aflatoxin intake and urinary adduct excretion (*91*). Dose–response relationships for DNA adducts have also been demonstrated for cancer chemotherapeutic agents (*47,92–94*) but will not be discussed here because the exposures are medicinal, rather than environmental.

DNA Adduct-based Risk Assessment

A major goal of studies in this field is the use of DNA adduct values to predict human cancer risk. If formation of a specific DNA adduct in exposed individuals parallels risk for cancer induction and comparison is made with appropriate controls, it may be possible to identify at-risk groups of individuals. This has been accomplished in one large study linking dietary aflatoxin exposure and hepatitis seropositivity with liver cancer in China, using a prospective and nested case–control study design (*95,96*). Samples of urine and blood were banked from more than 18,000 men in Shanghai, China. Within several years 50 cases of liver cancer were reported and matched for age, sex, and neighborhood with 267 controls. At that time the blood was assayed for evidence of hepatitis seropositivity and the urine was assayed for aflatoxin DNA adducts. Individuals with evidence of DNA adduct formation had a 9.1-fold increased relative risk of developing hepatocellular carcinoma, and individuals who showed evidence of both hepatitis and urinary aflatoxin adducts had a 60-fold increased relative risk as compared to controls. This important investigation was the first to show an association between DNA adduct formation and cancer risk.

Conclusions and Future Directions

To date, DNA adduct measurements are routinely performed on a wide variety of human tissues from individuals experiencing a broad spectrum of environmental and other exposures. The most frequently used approaches to DNA adduct quantitation do not identify or chemically characterize specific adducts. However, methodological advances that involve preparative chromatography steps are becoming widely applied and permit determination of specific adducts in a human tissue sample. The ability to characterize adducts in a human tissue will facilitate molecular dosimetry, although some highly specific end points, such as GC–MS, may not be adaptable to routine screening efforts.

Future directions for this field will focus on the implementation of epidemiologically sound study designs to assess the association between DNA adduct formation and human cancer risk. Whereas this association is strongly supported by animal studies, it remains to be seen whether adducts are also a necessary component of tumorigenesis in humans. In the one study of liver cancer and urinary aflatoxin–DNA adducts in China, an association appears to be present. However, background levels of DNA adduction are essentially universal and it is not clear to what extent low levels of genotoxic damage contribute to human cancer risk. To address this issue the prospective nested case–control study design is essential. However, pitfalls in such endeavors include the costly demands of prospective studies and the necessity to choose a cancer that has a short latency to generate sufficient study subjects within a reasonable time frame.

In the field of human biomonitoring the potential correlation of DNA adducts with markers of susceptibility, exposure, and effect (*97*) may substantially alter conventional approaches to risk assessment. Many studies are now being designed to correlate metabolic polymorphisms, urinary metabolites, mutagenesis, chromosomal aberrations, protein adducts, and other markers with DNA adduct levels. The usefulness of these correlations is still being determined, but it is possible that future approaches to cancer risk assessment will eventually reflect the results of a battery of biomarker tests, including DNA adduct analyses.

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