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Carcinogen macromolecular adducts and their measurement

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Damage to DNA induced by carcinogenic chemicals reflects exposure and is directly related to tumor formation, whereas modification of protein provides relatively precise dosimetry for stable adducts of proteins with a known half-life. Sophisticated methods for the detection and quantitation of DNA and protein adducts have been developed during the last ~25 years. For DNA adducts the most widely used methods include electrochemical detection, mass spectrometry, fluorescence and phosphorescence spectroscopy, immunoassays and immunohistochemistry and ^{32}P-post-labeling. Detection limits for quantitative assays are typically in the range of 1 adduct in 10^7 or 10^9 nucleotides. However, accelerator mass spectrometry, which is highly sophisticated but less accessible, has a detection limit of ~1 adduct in 10^{12} nucleotides. Methods for the determination of protein adducts include immunoassay and a variety of elegant high-resolution mass spectrometry approaches. The detection limit of ~0.1 fmol for protein adducts, is based primarily on method specificity and the availability of large quantities of sample material. Using these highly sensitive methods a major achievement has been the biomonitoring of chemically exposed human populations. Validation of macromolecular adduct formation in humans has been predicated on studies in animal models. Adduct formation in humans appears to be indicative of molecular dosimetry and suggestive of increased human cancer risk. However, despite the large body of literature documenting DNA and protein adduct molecular dosimetry for many carcinogen exposures, the relationship between adduct formation and human cancer risk has been defined for only a few carcinogens. Thus, elucidation of this association remains a compelling challenge. For the future, integration of DNA and protein adduct measurements together with documentation of correlative and subsequent events, and host susceptibility factors, within the context of valid molecular epidemiologic study designs, will further our understanding of human disease mechanisms.

Abbreviations: 4-ABP, 4-aminobiphenyl; AFB_1, aflatoxin B_1; AMS, accelerator mass spectrometry; B[*a*]P, benzo[*a*]pyrene; GC, gas chromatography; HBV, hepatitis B virus; HPLC, high performance liquid chromatography; MS, mass spectrometry; NNK, 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone; PAH, polycyclic aromatic hydrocarbon; PHIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; RR, relative risk.

Introduction

Genetic damage that produces a heritable loss of growth control comprises a major mechanism of chemical carcinogenesis. Most chemical carcinogens require metabolic activation to reactive electrophiles that can modify cellular macromolecules (nucleic acids and protein). The first observation that a metabolically activated carcinogenic chemical species became bound to a cellular macromolecule was made by E.C.Miller and J.A.Miller who showed that the hepatic carcinogen *p*-dimethylaminoazobenzene bound covalently to rat liver protein (1). During the same decade, DNA, and not protein, was recognized to be the genetic material (2), and cancer researchers began seeking, and finding, evidence that chemical carcinogens damage DNA (3). Now, at the beginning of the 21st century, the weight of evidence supports the notion that exposure to most chemical carcinogens results in damage to the structural integrity of DNA, which occurs primarily as covalent carcinogen binding and is referred to as carcinogen–DNA adduct formation (4,5). Damage to DNA is generally considered to be causative and directly related to tumor formation (4,6–9), while modification of protein indicates a particular exposure and provides relatively precise dosimetry if the adduct is stable and the protein has a known life-time (10–14).

The last two decades have witnessed the development of sophisticated assays for the measurement of DNA and protein adducts. Previously, macromolecular adduct measurements typically required administration of a radiolabeled carcinogen, and measurement of adducts in chronic exposure studies or in human tissues was extremely rare. The development of alternative methods has made possible the investigation of adduct dosimetry within the context of chronic study designs in experimental models, and numerous investigations have documented adduct formation, removal and persistence. A major achievement has been the application of highly sensitive DNA and protein adduct detection assays to the biomonitoring of chemically exposed human populations. Based on data from experimental models, it is considered likely that macromolecular adduct formation in humans may be both a valid indicator of molecular dosimetry and suggestive of increased human cancer risk (6,7,10,12,13,15,16).

Currently, a large body of literature validates the use of molecular dosimetry for both DNA adducts and protein adducts for many carcinogen exposures (17). However, the relationship between macromolecular adduct formation and human cancer risk has been defined for only a few carcinogens (18–22). Clearly, elucidation of this association remains a compelling challenge for the future. This review will focus on some of the major advances in research on carcinogen macromolecular adducts that have been achieved in the last two decades.

DNA adducts

The presence of a DNA adduct in a critical gene provides the potential for occurrence of a mutagenic event, resulting in subsequent alterations in gene expression and a loss of growth control. A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis since other events, such as mutagenesis and cell proliferation, must also take place (9,10,12,23,24). DNA adduct levels, measured at any point in time, reflect tissue-specific rates of adduct formation and removal, which depend upon carcinogen activation, DNA repair, adduct instability and tissue turnover.

Reaction of chemical carcinogens with DNA, either directly or after metabolic activation, typically involves covalent binding of an electrophilic carcinogen with a nucleophilic site in DNA (3). Guanine is by far the most prevalent target, although adducts have been reported for all bases (5,25). Fundamental to the advances made in the last two decades has been a vast effort to elucidate the chemical structures of DNA adducts. Adduct species formed in *in vitro* reactions as well as in experimental biological model systems have been characterized using chemically specific techniques (mass, fluorescence and nuclear magnetic resonance spectrometry) (4,5,9, 25–27). Further information, on a molecular level, has been obtained by subjecting purified adducts to nuclear magnetic resonance and other methods to determine stereospecificity and three-dimensional structure (28,29).

Associations have been observed between DNA adduct formation, and mutagenesis (9,30), and tumorigenesis (6,7,15); while reductions in DNA adduct levels have been associated with chemoprevention (8,12). However, molecular mechanisms for these and other biological consequences of carcinogen exposure remain obscure. Subsequent to characterization of a specific DNA adduct, site-specific mutagenesis and/or tumor studies have been employed to determine its biological effectiveness. These studies have demonstrated that some adducts are highly mutagenic and associated with carcinogenesis, while other adducts are not (9,23). Particularly notable have been studies in animal models that have demonstrated an association between mutation 'hot-spots' in proto-oncogenes and tumor suppressor genes and specific adducts. Mutations considered carcinogen specific have been observed in *p53*, *ras* and other reporter genes in humans (31–33); however, caution must be used in the interpretation of such data because of the complex nature of human exposures. Furthermore, the relative roles of chemical reactivity in the formation of a lesion and the carcinogenic potency of a particular lesion in the establishment of clonal growth advantage remain enigmatic. Elucidation of these interactions might best be achieved by concomitant application of a spectrum of biomarker assays.

DNA adduct detection methodologies

Substantial achievements, over the last two decades, in our understanding of carcinogen–DNA interactions, have resulted largely from the development of sensitive and specific methods for DNA adduct measurement (4,17,34–36). The most frequently used methods include immunoassays (37–41) and immunohistochemistry (37,42–44) using adduct-specific antisera, ^{32}P-post-labeling (45–47), fluorescence and phosphorescence spectroscopy (27,36,48), electrochemical detection (49) and mass spectrometry (MS) (36,50–52). The sensitivities of individual methods vary and often depend on the amount of DNA that can be analyzed, but some assays may detect as little as 1 adduct/10^9 nucleotides. The most sensitive quantitative method, accelerator mass spectrometry (AMS) (53,54) requires administration of very low levels of ^{3}H- or ^{14}C-labeled compound and is sensitive in the range of 1 adduct/10^{12} nucleotides.

Each method has specific advantages and disadvantages and most have been successfully applied in experimental models where only one compound is administered. However, for human samples where multiple adducts are present, it is difficult to obtain either exact quantitation of individual adducts or chemical characterization of a specific adduct, unless combined with preparative techniques. For example, MS typically employs a preliminary derivatization before analysis (50,51). Alternatively, immunoaffinity chromatography, high performance liquid chromatography (HPLC), gas chromatography (GC) or other types of chromatography have been used to prepurify sample DNA and concentrate the adduct of interest, which is further assayed by chemically specific methods (36). Using preparative techniques, specific DNA adducts have been identified and quantified in human tissues, despite the presence of many different types of adducts (36,55,56). Specificity is also obtained using AMS, which offers the potential to administer and monitor the fate of minuscule doses (~1 μg/kg body weight) of pure radiolabeled carcinogens to human subjects. For example, the administration of radioactive 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PHIP) to patients prior to colon cancer surgery, using doses lower than those occurring in a small portion of well-cooked meat, has allowed the demonstration of PHIP–DNA adducts in human colon (53).

DNA adduct dosimetry in experimental models

The classic methodology for DNA adduct detection has comprised administration of a radiolabeled carcinogen and determination of tracer either in whole DNA or DNA subjected to digestion and chromatography (25). Studies that have employed this approach for DNA adduct dosimetry in single-dose model systems (cells in culture or laboratory animals) are too numerous to catalogue in detail here; however, linearity of DNA adduct formation with dose at non-toxic levels has generally been observed (4,5). Of particular note is the concurrent development of *in vitro* culture methods for epithelial cells, and the capability of correlating DNA adduct dosimetry with metabolism and cell transformation in human target tissues (23).

Most human cancers are associated with chronic low level exposures to xenobiotic agents. In the past, chronic carcinogen exposures have been difficult to model in animals due to the high cost of radiolabeled compound. However, the recent development of highly sensitive alternative methods, including immunoassay and ^{32}P-post-labeling, has made long-term dosing possible. Because a number of tumor studies have employed chronic carcinogen exposures at multiple dose levels, it has been possible to compare tumor incidence with DNA adduct levels in target organs after administration of carcinogen at the same doses for 1–2 months, which is sufficient time for DNA adducts to reach steady state. The comparison between DNA adduct formation and tumorigenesis has been investigated for 2-acetylaminofluorene, 4-aminobiphenyl (4-ABP), aflatoxin B_1 (AFB_1), *N*,*N*–diethylnitrosamine, 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[*a*]pyrene (B[*a*]P) (15,30,57). The results of multiple different tumor/adduct comparisons have demonstrated that (i) DNA adduct formation is typically linear at the lowest doses; (ii) DNA adducts form

in the absence of tumors but tumors do not form in the absence of DNA adducts; and (iii) other tissue-specific events (e.g. cell proliferation) are sometimes necessary for tumors to appear (15,30,57). An examination of 27 chemicals (7) led to the conclusion that the range of DNA adduct concentrations associated with a 50% tumor incidence was 53–5543 adducts/10^8 nucleotides for rat and mouse liver. The same study suggested that a non-detectable 5% increase in liver tumor incidence in rodent models would be associated with an increase in 200–500 DNA adducts/10^8 nucleotides (7).

Human DNA adduct biomonitoring

Since the first reported measures of alkyl–DNA adducts in human DNA (58) the variety of human exposures that can be detected by determination of DNA adducts has expanded rapidly. Exposures occurring as a result of lifestyle choices (tobacco products and UV light), diet (charbroiled and mold-contaminated foods), medical treatment (anti-cancer drugs, coal tar, UV light and psoralens), occupation (coke ovens, foundries, smelters, burning forests and oil wells) and contaminated environment have been documented (44,55,59–62). In addition, formation of endogenous DNA adducts, frequently at levels higher than xenobiotic DNA adducts, has also been observed (52).

While blood is the most frequently used source of human DNA, because it is readily and repeatedly available, many studies have also used tissues collected at the time of biopsy, surgery or autopsy; whereas fresh/frozen materials have been most widely studied, fixed paraffin-embedded materials may provide a valuable resource. Urinary excretion of DNA and RNA adducts, including alkylation products, oxidized bases and bulky adducts, are excellent markers of exposure (63). It is often difficult to use multiple methods to confirm the same result, although this is clearly desirable. The large interindividual variability in DNA adduct formation, observed in individuals experiencing similar exposures, suggests that genetic differences in carcinogen metabolism, DNA repair and cell-cycle control modulate individual response to exposure (64).

Human DNA adduct determinations in molecular cancer epidemiology

Potentially the greatest strength of human carcinogen–DNA adduct biomonitoring has been derived from analysis of DNA adduct measurements within the context of epidemiologically sound study designs. Sophisticated molecular epidemiologic studies have the potential to elucidate the relationship between DNA adduct formation and human cancer risk. To date, few such studies have been published. The most instructive examples are for AFB_1–DNA adducts in the case of dietary AFB_1 exposure and liver cancer (18,19), and polycyclic aromatic hydrocarbon (PAH)–DNA adducts in the case of tobacco smoking and lung cancer (65–67).

The success of the AFB_1 study rests on the ability to demonstrate dosimetry for exposure and adduct formation (17), the availability of a highly specific method for the adduct measurements, and the choice of a population having a high incidence of liver cancer. The study (18,19) was designed to resolve questions pertaining to the proposed viral (hepatitis B virus; HBV) and chemical (AFB_1) etiologies of liver cancer. Blood and urine samples were collected from a cohort of 18 244 Chinese men (45–64 years old) between 1986 and 1989. During a 7 year period, 50 liver cancers developed. A prospective nested case-control study was constructed by assaying blood for HBV and urine for AFB_1–N^7-guanine in samples from the 50 liver cancer cases and 267 age- and residence-matched controls. In individuals having measurable urinary AFB_1–N^7-guanine adducts and no serum anti-HBV antibodies, the relative risk (RR) of liver cancer was 9.1. In persons with a history of HBV infection (serum anti-HBV antibodies) and no evidence of AFB_1 exposure, the RR for liver cancer was 7. Besides establishing AFB_1 exposure and HBV infection as independent liver cancer risk factors, this study (19) showed that individuals having both exposures experienced a multiplicative liver cancer risk (RR = 59.4).

Despite the overwhelming evidence that tobacco smoke is a human lung carcinogen, development of molecular epidemiological studies that reveal the role of PAH–DNA adduct formation in lung cancer risk have been fraught with difficulties. These studies have been hampered by the lack of assays able to measure adducts from specific PAHs, lack of well-established molecular dosimetry, and the virtual impossibility of obtaining target tissue for DNA adduct measurements. Discrepancies in PAH–DNA adduct measurements between peripheral blood leukocytes and target tissues in the same individual have been demonstrated for lung and skin (68–70). A New York City hospital-based case-control study (65–67) was undertaken to investigate the relationship between biomarkers of PAH exposure and lung cancer, using peripheral leukocyte DNA samples and an immunoassay that employs an antibody specific for multiple PAH–DNA adducts. The odds ratio for elevated PAH–DNA adducts in lung cancer cases was 7.7 (66) and a clear dose–response was demonstrated between PAH–DNA adduct levels and reported PAH dose (current, former or never smokers) irrespective of case/control status. These data were consistent with the subsequent finding that, in females with lung cancer, there was a higher proportion of individuals with the *GSTM1* null genotype compared with controls (65). Because tobacco and tobacco smoke contain carcinogenic chemicals of many classes, including PAHs and nitrosamines, the relative role of particular carcinogens in lung carcinogenesis might only be resolved when case-control studies are able to compare levels of many different types of carcinogen–DNA adducts.

Future of human DNA adduct biomonitoring

Major advances in methods to determine carcinogen–DNA adducts over the last 2 decades have made possible the identification and chemical characterization of adduct species synthesized as standards, the determination of DNA adducts formed during chronic dosing with low levels of carcinogenic chemical and molecular dosimetry in human populations. Challenges that still confront this area include procurement of a suitable surrogate tissue, resolution of adducts occurring as a result of exposures to complex mixtures, and validation of data obtained using different adduct detection methods. Correlations between DNA adduct levels in the target tissue of interest and in available surrogate tissues (blood, exfoliated cells) have not been found (44,70). Moreover, complex mixtures of adducted materials are problematic since different assays have characteristic strengths and weaknesses and no single assay can determine adducts of multiple chemical classes simultaneously (24,42). Several studies have compared DNA adducts in human tissues using multiple different methods, and while correlations have been reasonable, differences in adduct levels are common and reflect the assay being used (44,56).

Highly specific antisera elicited against DNA adducts and modified DNA samples will continue to provide useful approaches for quantitative and immunohistochemical biomonitoring. High affinity antisera are typically used for adduct quantitation, while low affinity antisera are used in preparative procedures. Polyclonal antisera, which include high affinity species, are economical to produce, while monoclonal antisera are more costly because the high-affinity clones are difficult to find. Quantitative immunoassays have the disadvantage that they require large amounts of DNA ($\geq$100 μg), but this difficulty can be overcome by immunohistochemical adduct localization. Recent advances in antigen retrieval, signal amplification, and the capacity to quantify nuclear staining have vastly improved the capacity of immunohistochemical staining to provide quantitative comparisons between samples. This approach is likely to become widely applied in the future due to the ready availability of small amounts of exfoliated human cells and tissue biopsies.

The ^{32}P-post-labeling assay, which radioactively labels adducts digested from sample DNA, has been widely applied because of its high sensitivity and the requirement for only microgram amounts of DNA. This assay has been especially useful for detection of adducts in single exposure experimental systems and as a means of elucidating the metabolic activation of previously uninvestigated potential carcinogens (46,47). For human biomonitoring ^{32}P-post-labeling can give an impression of total adduct burden, but it is rarely possible to quantify specific adducts accurately in human samples. Advances may lie in the use of better chemical standards, more advanced preparative techniques, standard curves and use of corroborative assays.

Carcinogen–DNA adduct detection by fluorescence has been applied to compounds that lead to either highly fluorescent products or adducts that can subsequently be derived to highly fluorescent chemical species. This approach has been useful for adducts of PAHs and AFB_1 but remains limited. Increased sensitivity may ultimately be possible by concentration of larger amounts of the fluorescent material for analysis, or the development of more intense fluorophores (27,36).

Physicochemical methods, including MS, offer the advantage of high chemical specificity. Major improvements in sensitivity have allowed the measurement of increasingly smaller amounts of adducted species in biological matrices. Although ever improving hard-ware together with separatory techniques may lower detection limits for human biomonitoring, this approach will continue to require expensive equipment and large quantities of DNA.

Molecular-epidemiologic studies that employ DNA adduct measurements are likely to be widely applied in the future and have the potential to generate hypotheses regarding underlying basic biologic mechanisms that subsequently can be tested in the laboratory. Large interindividual variations, specifically in carcinogen–DNA adduct formation, suggest that host (genetic) determinants of carcinogen metabolism, DNA repair and cell-cycle control may contribute to consequences and outcome of DNA damage. The relationship between formation of DNA adducts and polymorphisms in some carcinogen metabolizing genes has provided evidence to this effect, although the large number of genes involved indicates that large scale studies will be needed to understand the complex nature of such gene–environment interactions. Elucidation of underlying mechanisms will be necessary to support interpretation of DNA adduct data currently being collected in epidemiologic studies (71–73).

Protein adducts

Protein adduct formation is considered to be a valuable surrogate for DNA adduct formation since, particularly in animal models, many chemical carcinogens bind to both DNA and protein in blood with similar dose–response kinetics (10,12,74). Hemoglobin and serum albumin are the proteins of choice because they are readily accessible, more abundant than DNA, and have known rates of turnover. In addition, efforts have been made to validate histone and collagen adducts as indicators of more long-term exposures (74).

Since protein adducts are not removed by active repair processes, chemically stable adducts of hemoglobin or serum albumin provide a more precise measure of dose integrated over the lifespan of the proteins in question (13,75). Interaction of a carcinogen with a protein, either directly or after metabolic activation, typically occurs by substitution at a nucleophilic amino acid (3). For alkylating agents the most common substituted amino acid is cysteine, but modifications have been reported at aspartate, histidine, valine, tryptophan, glutamate and lysine (13,74). Larger carcinogens react with residues that cannot be predicted *a priori* (76). Pioneering human chemical dosimetry studies, using ethylene exposure as a model (11,16), sought to project cancer risk based on protein adduct measurements in an approach analogous to that used in radiation exposure studies.

Protein adduct methodologies

The earliest animal model studies that examined hemoglobin or serum albumin adducts involved exposures to aromatic amines (77), ethylene (75), methylmethane sulfonate and B[*a*]P (13), and primarily exposed rodents to a radiolabeled carcinogen. Extensive studies with AFB_1 have involved immunoassays (12) and protein adducts of 2-amino-3-methylimidazo[4,5-*f*]quinoline have been determined by HPLC with fluorescence detection (74). A number of elegant MS methods have been applied to elucidate protein adducts of methylmethane sulfonate, dimethylnitrosamine, propylene oxide (78), NNK (50), B[*a*]P (79) and styrene (80). These methods are particularly powerful because of the ability to determine the specific chemical structure of the purified protein adduct. In addition, sensitivity typically can be as low as ~0.1 fmol of protein adduct, due to the specificity of the method and the availability of large quantities of sample material (50). In some cases three-dimensional structure has also been determined by X-ray crystallography (74).

For detection of hemoglobin or albumin adducts in humans, samples must be enriched for adducts or adducts must be removed from the protein, before analysis (11,13,74,81). This is accomplished by either chemical or enzymatic release of the adduct or carcinogen from the protein or digestion of the protein into peptides and amino acids. Solvent extraction or immunoaffinity purification may then be used for partial purification. The most commonly used adduct detection method is MS used in conjunction with GC, but immunoassays or HPLC, with UV or fluorescence detection, and AMS, have also been applied to human samples. The development of highly sensitive and specific methods has made possible the elegant monitoring efforts with experimental models and humans that have taken place in the last 20 years.

Protein adduct dosimetry in experimental models

Chronic carcinogen dosing with subsequent examination of protein adducts in either hemoglobin or serum albumin has a characteristic dose–response curve for adducts that are chemically stable. Protein adduct levels in serum and in hemoglobin typically reach steady state by 2–4 months of continuous exposure. The level of adducts at steady state is directly proportional to the daily carcinogen dose and is typically linear over a large dose range (13). Once the dosing is terminated, the decline in adduct levels is linear (first order kinetics) and simultaneous with the protein turnover. The lifespan of hemoglobin is ~60 days in rodents and 120 days in humans, since the protein is removed with erythrocytes. The half-life of serum albumin in humans is 23 days. Because these parameters are constant for stable adducts, and because repair is not involved, protein adducts constitute a much more precise dosimetry tool, when compared with DNA adducts.

Human protein adduct biomonitoring

The potential use of proteins for human dosimetry in environmental and occupational chemical exposures was first recognized by Ehrenberg and Tornqvist (14) who demonstrated the kinetic relationship between protein adduct persistence and protein lifespan (16). This important principle, established for hemoglobin modified by ethylene oxide or alkylating agents, has provided the basis for all studies that have investigated associations between carcinogen–protein adduct levels and carcinogen exposures. Protein adduct biomonitoring has been employed for many human exposures including ethylene oxide, styrene oxide, propylene oxide, acrylamide, tobacco-related, workplace and medicinal (psoriasis) PAHs, tobacco-specific nitrosamines and dietary exposure to AFB_1 (13,60,73). The bulk of these studies fall into two main groups, those that have measured aromatic amine or nitrosamine–hemoglobin adducts in relation to tobacco smoking (50,74) and others that have measured albumin or hemoglobin adducts of PAHs and aflatoxins (12,74,82).

Hemoglobin adducts formed through the metabolic activation of 4-ABP have proven to be excellent indicators of tobacco smoking since virtually all samples give values above the detection limit. Tobacco smokers are readily distinguished from non-smokers, and a dose–response has been observed between people who use cigarettes with a high 4-ABP concentration (black tobacco) compared with those who use cigarettes containing lower levels of 4-ABP (blond tobacco) (74). Approximately 20 tobacco-related aromatic amine–hemoglobin adducts have also been described (83). In particular, the 3-aminobiphenyl-hemoglobin is an excellent marker for environmental tobacco smoke exposure (passive smoking) because of the differential levels of the parent compound in mainstream and side-stream tobacco smoke (13). Hydroxyethylvaline formation in hemoglobin is also a good dosimeter of tobacco smoking (11); however, it is less specific than 4-ABP–hemoglobin since ethylene oxide has environmental origins other than tobacco smoke.

More work is needed to establish protein adducts in blood as good surrogates for protein or DNA adducts in target tissues. Although there are several studies in animals (10,12,13), there are no studies in humans correlating albumin adducts in blood with DNA adducts in a target tissue. Two reports of PAH–DNA and albumin adducts in blood found no correlation (20,82). Most studies have employed protein adducts as biomarkers of exposure, and few studies have attempted to correlate protein adducts with cancer risk. However, nested case-control studies have demonstrated significant elevations of AFB_1–albumin adducts in individuals who later developed liver cancer, compared with those who remained healthy (21,22,84).

Future of human protein adduct biomonitoring

Because of their abundance, protein adducts represent an attractive option as internal dosimeters for human biomonitoring studies. Methods for the measurement of carcinogen adducts in histone protein and collagen are limited by their tissue availability, but the ease of availability of serum albumin and hemoglobin in blood make them useful surrogates.

Protein adducts are cumulative and related to the known lifespan of the protein of interest. These properties, established in animal models, have been exploited to help appreciate time–exposure relationships in humans. However, new studies that examine how protein adduct burden might impact on protein lifespan will be necessary if time–exposure relationships are to be determined with accuracy.

The impact of protein adduct formation with a critical target, versus a surrogate, has been examined at the cell and tissue level (e.g. lung versus blood), though much work remains to be done in this area (12). Similarly, questions concerning specific genetic targets and the formation of adducts in specific proteins may be of critical concern. For example, if a protein responsible for maintaining a cell-cycle checkpoint was specifically compromised through carcinogen modification, a resulting lack of growth control might contribute to uncontrolled proliferation. Such effects would perhaps be related to the specific amino acids modified on particular proteins.

Molecular epidemiologic studies, similar to those described and proposed for DNA adducts, will increasingly involve the measurement of protein adducts. Such studies will help to resolve questions concerning the relationship between protein adduct levels and disease risk. Future use of protein adducts for surveillance will also benefit from correlations with multiple different biomarkers.

Problems to be overcome in the measurement of protein adducts are similar to those with DNA adducts, and include sensitivity, specificity, methodological discrepancies, correlation with human cancer and biological consequences of the formation of a particular adduct in specific regions of critical proteins. In addition, hemoglobin and serum albumin proteins are surrogates of target tissues, as protein is a surrogate of DNA, and are not considered to be directly involved in the carcinogenic process. Nevertheless, direct associations have been observed between protein adduct formation and human cancer risk.

Conclusions

Consideration of the relative merits of DNA adduct versus protein adduct measurements must take into account the practical purpose of the biomarker in the context of the broader research question. It is therefore necessary to consider the properties of the adducts themselves and their potential role in human carcinogenesis. Study designs that carefully consider and account for the role of a particular adduct in human carcinogenesis are based on the best interpretation of the current literature, including data obtained in experimental models.

Both DNA adducts and protein adducts provide a tangible link between exposure and early biological effects. Macromolecular adduct formation indicates that exposure has

occurred, and associations between DNA and protein adducts and human cancers have been observed for a limited number of chemicals and human tumor types. In the future, integration of data for these intermediate biomarkers together with other environmental and host susceptibility factors in molecular epidemiologic studies of human cancer will assist in the elucidation of human cancer risk.

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