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COMMENTARY

DNA adducts, mutations and cancer

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Anyone having lectured on DNA adducts is likely to be familiar with the first question after the presentation: 'What is the relationship of DNA adducts and cancer?'. Although there are a number of reviews on the topic (1–5), my personal response, instead of going into lengthy circumstantial argumentation and hand-waving, has been a quotation from Mortimer Mendelson: 'I wouldn't like to have my DNA messed up'. In this commentary I will indulge in this still circumstantial argumentation. The reason being that never before has it appeared to be so easy, or less difficult, to entertain the question. Hopefully this effort will spare colleagues confronting the same question some time. At the same time I will take the opportunity to describe developments in the identification of DNA adducts. A vivid history of the development of ideas on chemical causes of cancer has been published by Lawley (2).

Known DNA adducts

A meeting organized in Sweden at the end of 1992 attempted to summarize the world literature on DNA adducts (6). The speakers were asked to critically evaluate the level of structural evidence for the adducts discussed. The criteria implied structural characterization. Mere radioactivity on DNA, cross-linking, immunoassay or spots in postlabelling were not considered as sufficient evidence. The adducts of over 200 different DNA binding agents were reviewed. These agents form close to 550 known adducts. The number of adducts is, however, smaller, because many compounds form the same adduct. This figure does not include diastereomers or enantiomers of the same structure nor radical types of damage, which are numerous but somewhat hypothetical. Undoubtedly, multi-author works also miss adducts because of difficulties in precisely defining work tasks.

Over the years numerous monographs have been written on DNA adducts. A review compiled 10 years prior to the meeting in Sweden listed some 230 different adducts of some 90 compounds (7). The increase in number since then is almost entirely due to new compounds studied.

The literature was dominated 10 years ago by simple alkylating agents, aromatic amines and epoxides. Since then entirely new groups of chemicals, such as nitro-polycyclic aromatic hydrocarbons, food-derived heterocyclic amines, aldehydes, allylic compounds, acylating agents and chemotherapeutic drugs have been investigated. Elegant work has been carried out with the complex DNA-binding metabolites of polycyclic aromatic hydrocarbons. Sixty years after the description of the first purified polycyclic aromatic hydrocarbon, benz[*a*]anthracene (8), the adducts of some 10 different species have been established.

Adducts of known carcinogens

Whether DNA-binding agents are always carcinogenic is an unanswered question, because of the limitations of animal carcinogenicity tests. However, although the question has been taken up in several meetings, no *bona fide* examples of DNA-binding non-carcinogens have resulted.

The question can be reversed and DNA-binding of the known carcinogens can be analysed. This is not a test of causality but simply a test of our knowledge of DNA-binding agents. On the International Agency for Research on Cancer's list of known human carcinogens there are 18 single organic chemicals which are not hormones (IARC Monographs 1-58, 1993). Among these, azathioprine and cyclosporin are immunosuppressive agents, lacking DNA-binding data; chloromethyl methyl ether and bis(chloromethyl)ether (classified as one entry), chlornaphazine and treosulfan, industrial and pharmaceutical compounds, also lack DNA-binding data, but should be expected to bind to DNA. The remaining 13 compounds are presented in Table I. For all except benzene, unequivocal DNA-binding products have been demonstrated *in vitro*. Adducts have been identified for eight compounds in experimental animals, but for only two, aflatoxin B1 and 4-aminobiphenyl, in humans.

Adducts in humans

The development of sensitive methods based on ^{32}P-postlabelling (9–11) and immunoassay (12) has laid the groundwork for determination of DNA adducts in humans. Gas chromato-

Table I. Identified DNA adducts of known human carcinogens

Agents	Adducts		
	In vitro	*In vivo*	
		Animals	Humans
Aflatoxins	+	+	+
4-Aminobiphenyl	+	+	+
Benzene	–	–	nd
Benzidine	+	+	nd
1,4-Butanediol dimethanesulfonate (Myleran)	+	+	nd
Chlorambucil	+	nd	nd
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea	+	nd	nd
Cyclophosphamide	+	+	nd
Melphalan	+	nd	nd
Mustard gas (sulphur mustard)	+	+	nd
2-Naphthylamine	+	+	nd
Thiotepa	+	nd	nd
Vinyl chloride	+	+	nd

+ = positive, = negative, nd = no data.

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Table II. Specific DNA adducts demonstrated in humans

Adduct	Source	Method	Tissue
7-Methylguanine	Dimethylamine	HPLC	Liver
	Endogenous	Chromatography	Lung, WBC
	Tobacco	Postlabelling	WBC, PBL
	Procarbazine, Decarbazine	Electrochemical detection	WBC
O^6-Methylguanine	Procarbazine	Repair assay	WBC
7-Ethylguanine	Endogenous	HPLC + postlabelling	WBC, lung
Cisplatin–guanine	Cisplatin	HPLC + immunoassay	WBC
O^6-Styrene oxide–guanine	Occupational	Postlabelling	WBC
Benzo[*a*]pyrene–guanine	Environmental	SFS, GC–MS	WBC
	Occupational	Postlabelling–immunoassay	Lung
4-Aminobiphenyl	Tobacco	Postlabelling	Bladder
	Exhaust	GC–MS	Lung
	Tobacco	IA–postlabelling	Placenta
4,4′-Methylenebis-(2-chloroaniline)	Occupational	Postlabelling	Bladder
Aflatoxin B1	Food	IA, HPLC	Urine
8-Oxoguanine	Endogenous	GC–MS, HPLC	Urine
Thymine glycol	Endogenous	GC–MS, HPLC	Urine
3-Methyladenine	Methylating agents	IA, GC–MS	Urine

GC–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; IA, immunoaffinity column purification; PBL, peripheral blood lymphocytes; WBC, white blood cells.

graphic–mass spectrometric techniques have been used to determine protein adducts (3). These techniques are new, ever-improving and expanding (13), and due caution must be exercised concerning the accuracy of such measurements. Inter-laboratory comparisons carried out on the postlabelling technique, immunoassays or both have shown reasonable correlations (14–16), but unknown variables exist and there is no proof that in human DNA the two assays measure identical adducts.

One of the problems in measuring DNA adducts is specificity, i.e. detection of a particular type of adduct using stringent structural criteria. This has been achieved for only a few human DNA adducts (Table II). The data are compiled from references (17–21). In the postlabelling method, such specificity has apparently been achieved in humans for 7-methylguanine (22,23), O^6-methylguanine (24) and benzo[*a*]pyrene–DNA adducts (16,25). It is not yet clear how specific immunoassays are without prepurification of the adducts. Immunoassay of cisplatin adducts after high-performance liquid chromatography is a likely method for detecting specific adducts (26). Antibodies to DNA adducts are useful for prepurifying adducts by affinity column chromatography and will probably facilitate the identification of specific adducts. A competitive DNA repair assay has been used to measure O^6-methylguanine in human DNA (27).

Although quantitation of the adducts in these studies may be somewhat uncertain, internal or external standards are useful, if not mandatory, for accurate results. Interestingly, two studies using standards gave almost identical quantifications of lung and lymphocyte 7-methylguanine levels (28–30).

DNA adducts and mutations

Many known mutagens form covalent DNA adducts (6,7,31) that are released from DNA either spontaneously or by biological repair processes. Ample evidence suggests that mutations arise either during DNA replication at the damaged site or during error-prone DNA repair (32).

The use of shuttle vectors with built-in specific adducts has provided the ultimate piece of evidence for the promutagenic role of adducts in mammalian cells. Mutations induced by a large number of compounds, from small alkylating agents to arylating agents, and irradiation have been scored and characterized using shuttle vectors (33–35). Up to the present, dozens of specific adducts have been tested in such systems. Interesting aspects of these studies are the sequence specificity of adduct formation and of subsequent mutations (mutational hot spots) and the mutational efficiency of different adducts, from barely mutagenic to those that frequently cause mutations. The data obtained to date provide unequivocal evidence that many, if not most, adducts have the potential to cause mutations.

An example of the mutational specificity of five chemical agents, UV light and spontaneous mutations on exon 3 of the hypoxanthine phosphoribosyltransferase (*HPRT*) gene is shown in Figure 1 (generously provided by Alexandra Kat and William Thilly; 36).

One aspect worth pointing out is that the mutations usually occur at bases that are known targets for the agents, e.g. guanine for MNNG, BPDE; guanine and adenine for cisplatin; adenine for hydrogen peroxide (36). Another notable feature is the site-specificity. Even with the limited number of mutants scored, the site-specificity is striking, but unexplained.

The mutations induced by UV light are particularly interesting. The predominant, but not the only, UV-induced adducts are cyclobutane or 6,4-photoproducts at dipyrimidine sites. In exon 3 of *HPRT* thymine bases are hot spots, but mutations also occur at guanines. However, in several other studies, including the *HPRT* gene, it has been shown that UV light induces typical tandem double mutations at dipyrimidine sites (36). This is important for later discussion.

The other well characterized genes, adenine phosphoribosyl transferase and dihydrofolate reductase, also demonstrate mutations at nucleotides that are known targets of the mutagen used (4,37). Mutations induced chemically in the *ras* oncogene family also usually follow the known base-specificity of the mutagen used (4,37).

DNA repair of adducts depends on a number of factors, including the type of adduct. In the dihydrofolate reductase (*DHFR*) and *HPRT* genes of many types of cultured cells it has been demonstrated that for certain adducts removal is faster in

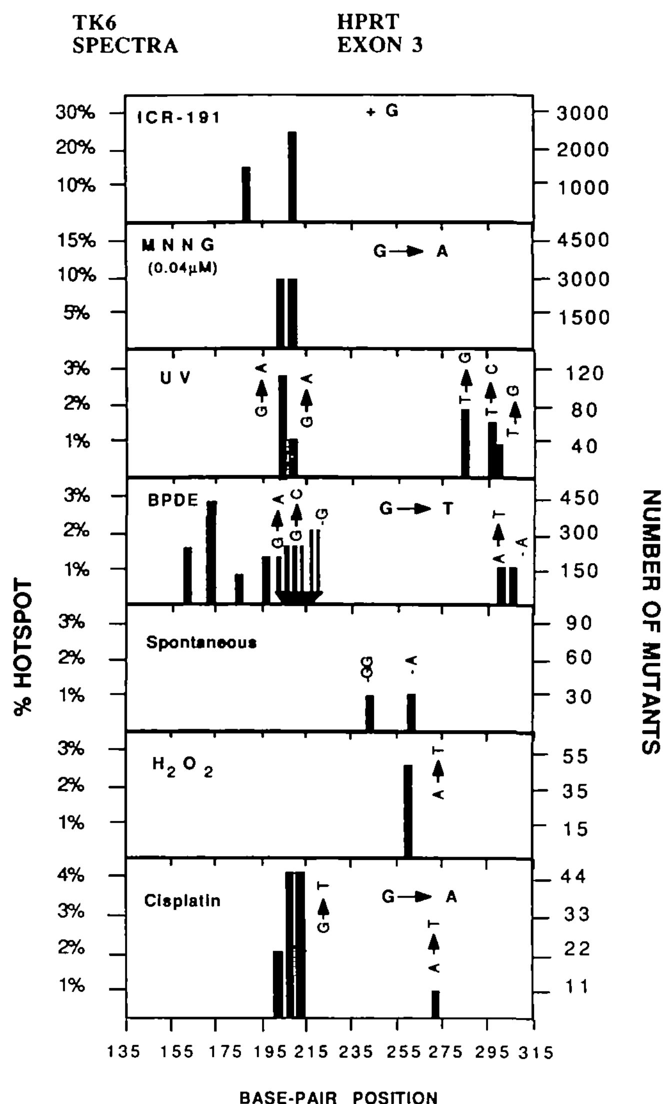

Fig. 1. Mutational spectra induced by seven different mutagens in *HPRT* exon 3. Spectra consist of shared alterations determined by the population approach in at least two populations. Each mutagen is listed in the upper left corner of the spectrum. The horizontal axis shows the *HPRT* exon 3 coding region from bp 135 to 318. The left vertical axis gives the percentage of 6-thioguanine-resistant point mutants accumulated within the hot spots induced by each mutagen, while the right vertical axis lists the mean number of mutants. The predominant kind of alteration, if any, is given in the upper right hand corner of the spectrum and other kinds of alterations are listed above the bars representing the individual hot spots (36).

the gene than in the non-coding region close to the gene, or faster in the transcribed as compared to the non-transcribed strand (38). Both preferential repair of the active region and of the transcribed strand have been described for UV-, nitrogen mustard- and cisplatin-induced adducts. By contrast, neither type of preference was seen with dimethyl sulphate or 4-nitroquinoline 1-oxide.

Preferential repair of adducts in the transcribed strand poses the question of fixation of mutations. In genes such as *HPRT* and *DHFR* in many types of cultured cells it has been demonstrated that many mutagens cause mutation primarily in the non-transcribed strand (33). Strand-bias is also seen in tumours. In mice irradiated by UV light all the dipyrimidine mutations were on the non-transcribed strand (39)

Extensive work carried out on *p53* tumour suppressor gene mutations has shed light on the origin of 'spontaneous mutations'. Among the point mutations, 'hot spots' in colon cancer are codons 175, 248 and 273, each a CG dinucleotide (more than half of colon cancers have a mutation at C). As CG is the recognition site for cytosine methylase, it is assumed that the mutagenic lesion is deamination of 5-methylcytosine to thymine. Such a lesion would appear spontaneous, but why it should be particularly prevalent in colon cancer and practically non-existent in many other cancer sites remains unexplained (40).

Other mechanisms of spontaneous mutation may involve insertions and deletions. An analysis of deletions in the *p53* gene revealed a copy of the deleted sequence in close vicinity to the deleted one. The insertions were usually duplications of an existing sequence (41). The mechanism of both types of mutations may be slipped mispairing in DNA synthesis.

Additionally, it is known that a number of endogenous DNA-binding or -damaging chemicals exist. *S*-Adenosylmethionine is capable of methylating DNA. Hydroxyradicals can damage DNA. Depurination and deamination are reactions proceeding at a constant background rate and are likely to contribute to spontaneous mutations.

DNA adducts and cancer

DNA adduct levels have been studied in animal models in target and non-target tissues in relation to known tumour responses. This work has led to some understanding of the role of DNA adducts. Firstly, adducts, if detectable at all, are usually found in target and non-target tissues, indicating that adducts are not the only reason for tissue specificity (1,3). It is not unexpected that lipophilic, directly reactive carcinogens, such as ethylene oxide, alkylate DNA in many tissues to a roughly equal extent (42,43). Secondly, in target tissues of a particular class of carcinogens, DNA-binding may be correlated with carcinogenic potency (1,44). Unfortunately, *N*-nitroso compounds, on which a large amount of literature is available, have not been analysed quantitatively for such correlations (45). Thirdly, DNA binding to tissue DNA appears to be linear over a wide dose range for many compounds, irrespective of whether tumours are formed in the tissue (3,46). Fourthly, carcinogens that react with specific sites in DNA appear to have different efficacies in causing tumours: *O*-alkylation appears to be effective for small alkylating agents (3,13), while many potent, large carcinogens react with the *N7*-position of guanine (47). Fifthly, the stability of adducts in DNA appears to be related to the probability that tumours will occur (48–50). Sixthly, in some, but not all, cases the target organs of sensitive animal species have higher levels of DNA adducts than the same organs in resistant species (51).

These observations show that there is no simple algorithm for translating DNA adduct levels into cancer risk in a particular tissue. While some of the paradoxes may only be apparent and explicable technically, the biological complexity of cancer may be such that simple algorithms never hold. The adducts of only five compounds have been measured in chronic carcinogenicity studies, as part of a bioassay or in long term simulation experiments (52). These studies include liver and bladder adducts of 2-acetylaminofluorene and 4-aminobiphenyl, liver adducts of aflatoxin B1 and diethylnitrosamine and lung adducts of a tobacco-specific nitrosamine, 4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone. In all cases the adduct levels were linear at low doses. For most of the compounds, adduct levels correlated with tumour yields. Poirier and Beland conclude: 'Taken together, these data suggest that when extrapolating from higher doses to low doses within an animal model, the extent of DNA adduct formation will most often correlate with the extent of tumorigenesis.

However, in animal model experiments where the number of animals is limited, tumour incidences may not be measurable when DNA adduct levels are low or in the absence of toxicity and/or cell proliferation' (53).

In the case of 4-aminobiphenyl, adducts have been measured in urinary bladder DNA both in experimental animals and in humans. Poirier and Beland (53) calculated that the 50% tumour incidence equalled 156 fmol adducts/μg DNA in mice as compared to 0.815 fmol/μg in humans. The levels refer to the main 4-aminobiphenyl adduct at the C8 position of guanine. A correction for life-span still showed that higher levels of adducts were required in mouse than man to induce tumours. Although such calculations rely on a number of assumptions (52), they show that new technologies allow such comparisons. The conclusion from 4-aminobiphenyl was that, per unit dose, humans appeared to be more sensitive than mice for the induction of bladder tumours.

Mutations and cancer

The mutational theory of cancer is old, but the best evidence in support of it has emerged relatively recently. The chromosomal instability syndromes, which are linked to increased risks of cancer, provided some early evidence; similarly, the demonstration of specific chromosomal changes in certain malignancies implied the occurrence of mutational events. More recently, molecular mapping of chromosomal changes, such as the translocation between chromosomes 22 and 9 in chronic myeloid leukaemia (Philadelphia chromosome) showed that important growth controlling genes, including some oncogenes, are located in the translocated DNA (53). Molecular characterization of retinoblastoma demonstrated the presence of another class of growth controlling genes, anti-oncogenes (54–56). Functional incapacitation of the two alleles of the retinoblastoma gene confers malignancy. Whether exogenous or endogenous chemicals or aberrations of normal cellular functions, or all of these, underlie these events remains to be established. The common types of activation of oncogenes include point mutations, translocations and amplifications, whereas point mutations and deletions appear to be mechanisms for inactivating tumour suppressor genes (56–58). Recently a type of genetic instability was described in familial colorectal cancer (59–61). This disease, mapped to chromosome 2, is characterized by the insertion of di- and trinucleotide repeats in several chromosomal locations.

Point mutations have been shown to be important in activation of the *ras* and *neu* oncogenes; *ras* is found in many types of human cancers and *neu* particularly in breast cancer (57). Mutations of *ras* and *neu* have been induced in rodents by *N*-nitroso compounds, vinyl chloride, aflatoxin B1, tetranitromethane, polycyclic aromatic hydrocarbons, benzidine derivatives, urethane and ionizing radiation (4). Mutations of *ras* are commonly detected in human cancers, particularly of the pancreas, lung and colon (58). There are, however, a few clues to the origin of these mutations.

The strongest evidence linking adducts, mutations and cancer comes from the *p53* gene, where specific mutations appear to be present in liver cancers of populations assumed to ingest large quantities of aflatoxin B1 (4,40).

The case with UV adducts and skin cancer appears even stronger. As pointed out earlier, UV light is known to induce rare tandem dipyrimidine mutations in eukaryotic systems. These are not unique to UV light, but are usually infrequent with agents such as aflatoxin B1 (62) and oxygen radicals (63). In UV-B-irradiated mouse skin 35 epidermal tumours were analysed for point mutations in the *p53* gene. Of the seven mutations, all occurred at tandem dipyrimidines, and two were tandem dipyrimidine, CC to TT mutations (39).

According to one paper, of 24 human squamous cell carcinomas analysed 14 had *p53* mutations, most involving dipyrimidine sites, three tumours involving a CC→TT dipyrimidine change (64). In a recent study essentially similar results were obtained with basal cell carcinomas (65). In cloned *p53* DNA, after UV irradiation, dipyrimidine photoproducts were detected in mutational hot spots and some other sites, indicating that factors in addition to the photoproducts influence the frequency of mutations (65). In two other studies on squamous cell carcinomas lower frequencies of *p53* mutations were noted, but dipyrimidine sites were overwhelming in basal cell carcinomas also (66). Moreover, *p53* tandem dipyrimidine mutations have also been detected in some cells of normal sun-exposed skin areas (67).

Conclusions

Cancer research has experienced a revolution during the past decade. The emphasis has been on the characterization of new genes and their functions. After completion of the human genome map the emphasis will be on function and regulation. The complex, intricate functional networks make the charts of metabolic pathways of the 1970s look like single pages of a map book.

Arguments about whether chemicals or genes, environment or genetic constitution cause cancer seem obsolete. We have learned that chemicals cause cancer through genetic changes. Furthermore there are genetic changes involved in carcinogenesis that appear spontaneous, or at least endogenous. The mystery of how DNA adducts relate to genetic changes is under intensive study. Why only certain mutations manifest in tumours depend on biological selection and the type of gene affected are areas where we are still apprentices.

What is the relationship between DNA adducts and cancer? It is a chronic messing up of our DNA.

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