MINIREVIEW

Biological Relevance and Consequences of Chemical- or Metal-Induced DNA Cross-Linking (43964)

DENNIS J. PAUSTENBACH,* BRENT L. FINLEY,* AND SAM KACEW†,[1]

McLaren/Hart Environmental Engineering Corporation, Chem Risk Division, Alameda, California 94501, and Department of Pharmacology,† University of Ottawa, Ottawa, Ontario, Canada*

Abstract. **A vast number of chemicals are known to induce mutagenesis and/or carcinogenesis in mammals. Although disruption of cellular nuclear material resulting ultimately in mutagenesis/carcinogenesis can be accomplished by various mechanisms, the search for biomarkers of chemical-induced toxicity continues. This review focuses on the ability of certain metals or chemicals to bind to DNA in a cross-link fashion in whole animal as well as under *in vitro* conditions. The methodologies currently used to determine DNA cross-linking are described. The biological relevance of the presence of chemical- or metal-induced DNA cross-linking as a measure of carcinogenesis in humans is still under debate, as there is no clear correlation between the disease and the DNA cross-link reaction.** [P.S.E.B.M. 1996, Vol 211]

Inadvertent exposure to chemicals in the biosphere, be it through air, food, or water, continues to be a concern for the health of humans and mammals, particularly if chemical toxins exceed permissible threshold limits and induce adverse effects. Further, self-administration of pharmaceutical agents for therapeutic reasons such as in the treatment of cancer, exposure to drugs of abuse, or ingestion of naturally occurring foodstuffs may also produce mammalian toxicity. In controlled laboratory conditions the consequences of chemical exposure to mammals or invertebrates can be established and related to factors including the dose or concentration of compound, duration, sex, nutritional status, etc. In humans, the measurement of contaminant in urine or blood indicates merely the presence of chemical but not necessarily that a toxic reaction has occurred. With the sophistication of scientific technology, methods have been developed to assess the potential damage arising from exposure to contaminants in nonlaboratory settings. Clearly, the use of cellular material such as proteins, fat, carbohydrates, etc., and in particular DNA has been the focal point for establishing human exposure to chemicals for over 40 years (1).

Currently, a number of techniques exist for the analysis of the interaction between DNA and a chemical. One of the known methods for pinpointing the sites at which DNA is modified by chemicals is the DNA-sequencing technique in which DNA cross-links can be detected. The interaction between an electrophilic chemical and a nucleophilic guanine residue of DNA results in primarily covalent bond formation. However, chemical binding of DNA to protein can be noncovalent in nature in certain conditions (2, 3). In conditions where the same chemical has an additional electrophilic site which then attaches in a covalent

[1] To whom requests for reprints should be addressed at Department of Pharmacology, University of Ottawa, 451 Smyth Road, Ottawa, Ontario K1H 8M5, Canada.

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fashion to either a second guanine or another nucleophilic site, be it an amino group, histone, or sulfhydryl radical of protein, this can result in the linking of two nucleic acid chains to one chemical (DNA-DNA cross-linking) or the linking of one DNA chain to a protein (DNA-protein cross-linking). The nuclear proteins involved in the DNA-protein cross-link reaction may vary from H1 histone to nonhistone proteins such as topoisomerase II, the major component of nuclear matrix (4, 5).

For purposes of definition, the DNA-DNA cross-link thus involves primarily covalent bonding of a chemical to either two different strands (interstrand) or covalent bonds of a chemical to two nucleophilic sites in one DNA strand (intrastrand). Covalent bonding of a chemical with nucleophilic sites in a DNA strand and to an additional nuclear protein site is referred to as a DNA-protein cross-link. The importance of the DNA-DNA or DNA-protein cross-link reaction lies in the scientific area of mutagenesis and carcinogenesis. The formation of covalent cross-links involving DNA was suggested to be responsible for the cytotoxic and antitumour effects of bifunctional alkylating agents as early as 1948 (6). It was shown that binding of nitrogen mustard compounds with DNA resulted in damage such that the inability to repair this process yielded a nonfunctional DNA which could not replicate and thus induced cellular lethality (7, 8). Subsequently, numerous pharmaceutical agents, especially those in the treatment of cancer, and natural plant products were found to induce a DNA-protein cross-link reaction (9–11). Further, induction of carcinogenesis was associated with the presence of these chemicals. These data thus suggested a correlation among cytotoxicity, mutagenicity, and drug-induced DNA-protein cross-linking. At present, the precise role of DNA-protein cross-links in chemical-associated carcinogenesis remains to be established. The aim of this review is to identify some industrial chemicals and heavy metals known to elicit a DNA-DNA or DNA-protein cross-link reaction, and establish the relevance of this phenomenon as a biomarker of exposure and potential role in carcinogenicity.

Methodology

The ability to detect and extract DNA-protein cross-links has been modified over the years to enhance sensitivity. In general, it should be noted that this technology is based on *in vitro* methods where the chemical is incubated with cells, but there are some instances where there is *in vivo* mammalian exposure followed by extraction of cellular material and then *in vitro* determination. The basic method for detection of DNA-DNA and/or DNA-protein cross-links is by the alkaline elution technique (12). In order to distinguish whether a compound produces DNA damage by strand breaks or cross-linking, the principle of elution rate developed by Kohn (13) was incorporated. Essentially, cells are incubated with a chemical. After incubation and removal of the chemical, the remaining solution is subjected to 300 or 600 rads of x-rays (irradiation) and then deposited on a filter of pore size ranging from 0.8 to 2 μm. The function of irradiation is to introduce DNA strand breaks, while any cross-links adhere to the filter and elute more slowly. The success of alkaline elution is dependent on the pore size of the filter. The assay is based on the principle that an alkaline medium will pass through the filter and elute denatured DNA but any DNA that is cross-linked will remain behind on the filter. The subsequent step involves the lysis of cellular material on the filter with a biological detergent such as sodium dodecyl sulfate (SDS) to release DNA (14). It is possible to dissociate DNA-DNA interstrand cross-links and DNA-protein cross-links from other DNA damage by using filters of specific pore diameter, and a difference in the composition of the lysis medium distinguishes types of cross-links. For the measurement of DNA-DNA cross-links, proteinase K is present in the lysis medium, while proteinase K is removed from the lysis medium for the assay of DNA-protein cross-links. The alkaline filter elution method is designed to determine the presence of DNA-DNA interstrand and DNA-protein cross-links. The occurrence of DNA interstrand cross-links reduce the elution rate by linking two or more strands, thereby preventing passage through the filter. In contrast, DNA-protein cross-links decrease elution rate as protein tend to adsorb onto the filters under alkaline conditions. DNA interstrand cross-links can be distinguished from DNA-protein cross-links by enzymatically catabolizing the protein adsorbed to the filter. The introduction of potassium chloride to SDS to precipitate detergent-resistant protein was found to further enhance the sensitivity for measurement of DNA-protein cross-links especially when the amount of DNA is low (15–17). The successful detection of DNA-protein cross-links is dependent in part on the size of the DNA fragment where the ability to detect cross-links is inversely reduced as the DNA fragment size is increased (18). In order to circumvent the variability in the data and increase sensitivity, DNA must be a uniform, homogenous size (15, 19). Successful fragmentation of DNA to a homogenous, uniform length is produced by a shearing action which is induced by the mechanical passage of DNA either pipetting the sample six times through a 1-ml pipette tip or through 18- to 20-gauge needles (15, 20). By using the shearing of DNA prior to filter binding the endogenous background cross-link formation was 0.5%–2.5% (15, 17) compared with 8%–15% using nonsheared DNA. Though it is not possible to eliminate the incidence of spontaneous cross-link formation, increased sensitiv-

ity of detection by means of mechanical fragmentation of DNA to a uniform size enables one to distinguish easily control form chemical-exposed populations (19).

Cross-Link Formation and Genetic Monitoring

Genetic monitoring is concerned with markers of exposure such as measurement of a chemical, pharmacokinetics, cumulative dose, etc., as well as effect including genotoxicity, functional change, etc. A number of industrial chemicals with varying uses were reported to produce primarily DNA-protein cross-links under *in vitro* conditions. However, the findings that chemical-induced DNA-protein cross-linking occurs *in vivo* and that these agents are commonly present in the environment based on the multitude of current uses (Table I) suggested that biomarkers for human exposure are essential.

The ability of chemicals with diversified chemical structures and actions to induce a similar DNA-protein cross-link reaction indicates the potential importance of this reaction as a marker for low-risk environmental exposure, effect, and susceptibility (21). However, the usefulness of this technique as a relevant index for genotoxic effects must be tempered based on the circumstances under which this reaction was initiated under *in vivo* conditions as shown in Table II. Data on acetaldehyde *in vivo* is rather limited, and relevance to humans is derived primarily from *in vitro* findings. In the case of formaldehyde, the DNA-protein cross-link reaction can be detected in nasal mucosa at concentrations that are not cytolethal or carcinogenic (35). Clearly, the formation of DNA-protein cross-links in the nasal cavity is effective as a measure of exposure to formaldehyde (23, 24), but this assay is limiting as this reaction is site specific (36) and the ability to obtain human nasal turbinates is impractical (24). Further, epidemiologic studies of human exposure to formaldehyde are inconsistent. McLaughlin (37) found that in professionals who use formaldehyde, such as embalmers and pathologists, there was an excess of deaths from cancer of the brain and leukemia but not nasopharyngeal carcinoma. In industry workers, exposure to formaldehyde did not appear to induce excess cancer risk and the presence of nasopharyngeal cancer after close examination was likely due to misclassification following multiple subgroup analysis. It was concluded that epidemiologic data on formaldehyde and human cancer failed to provide credible causal evidence (37).

Peripheral lymphocytes are the cell population of choice for studies in genetic monitoring as these cells are (i) readily obtained; (ii) in contact with most body

Table I. Uses of Industrial Chemicals Known to Induce DNA-Protein Cross-Linking

Chemical	Uses
Diepoxybutane	Preparation of erythritol Sterilizing agent for food and medical equipment
Acetaldehyde	Manufacture of disinfectants, drugs, dyes, explosives, perfumes, resins, pesticides
Glutaraldehyde	Tissue fixative Disinfectant Intermediate in chemical and plastics industry
Formaldehyde	Fungicide Disinfectant Component of embalming fluid Manufacture of silk, latex, urea, furniture, paper, drugs
Vinyl acetate	Polymerization processes for emulsions, resins, adhesives, paints, textiles
Ethylene oxide	Fumigant for foodstuffs Fungicide Sterilization in hospitals Intermediate for synthesis of plastics, glycols, acrylonitrile
Acetylaminofluorene	Pesticide
Chloroacetaldehyde	Fungicide Tree bark remover

Table II. Metal- or Chemical-Induced DNA-Protein Cross-linking Following *In Vivo* Exposure

Compound	Site	Status	Reference
Acetaldehyde	Rat nasal turbinates	Present	22
Formaldehyde	Rat nasal respiratory tissue	Present	23–25
	Rhesus monkey nasal mucosa	Present	26
	Mouse liver	Present	20
	Rat liver	Absent	20
	Mouse lung	Absent	20
Ethylene oxide	Human peripheral lymphocytes	Present	27
Chromium	Human peripheral lymphocytes	Present	19
	Rat hepatoma cells	Present	28
	Chick embryo hepatocytes	Present	29,30
	Human peripheral lymphocytes	Absent	31
Nickel	Rat liver	Present	32,33
Arsenic agents	Mouse lung Rat hepatoma	Present	5,34

tissues, which is an important factor in transport of reactive metabolites from liver to target site; and (iii) indicative of an integrated measure of biologic response to a chemical (21). Further, lymphocytes possess an inherent metabolic capacity which enables measurement of parent or reactive metabolite following chemical exposure. Although one can incubate human lymphocytes with formaldehyde and induce DNA-protein cross-links (18), inhalation of formaldehyde *in vivo* would not be expected to produce DNA-protein cross-links in circulating blood cells as the site of action is primarily the nasal cavity (26). This has prompted the U.S. Environmental Protection Agency (38) to utilize the number of formaldehyde-induced DNA-protein cross-links as a measure of internal dose for low-risk estimation but not as a phenomenon by which to determine the mechanism of carcinogenesis. The use of peripheral human lymphocytes proved successful as a tissue site to monitor genotoxic damage in ethylene oxide-exposed hospital workers (Table II). Extraction of lymphocytes from individuals using ethylene oxide as a disinfectant in hospitals followed by alkaline filter elution techniques revealed the presence of a significant increase in DNA-protein cross-links, suggesting that this assay may serve as a sensitive parameter for genotoxicity monitoring (27). Similarly, Costa *et al.* (19) reported an increase in DNA-protein cross-links in peripheral human lymphocytes of chromium-exposed workers. However, Gao *et al.* (31) failed to observe any damage in lymphocytic DNA in chromium-exposed workers. At present, a positive correlation between the level of chromium exposure, increased risk of lung cancer and the presence of a DNA-protein cross-link is speculative (39). Epidemiologic data has not identified that chromium at a specific concentration was related to human lung cancer (37). Chromium, as formaldehyde, produces DNA-protein cross-links at concentrations that are not cytotoxic. The use of the DNA-protein cross-link in the case of chromium may be an index of a metal-induced effect, the biological relevance of which is not known, and is highly dependent on the amount of chromium. It should be noted that chemical-induced DNA-DNA cross-link reaction and human peripheral lymphocytes play an important role as a screen for blood disorders. Incubation of human peripheral blood lymphocytes with diepoxybutane was found to induce DNA-DNA cross-linking (40). The biological importance of this observation is that this mechanism of diepoxybutane action is currently used to identify patients with Fanconi anemia (41, 42). Peripheral blood lymphocytes are incubated with or without diepoxybutane and scored for the number of spontaneous chromosome breakages (43). In patients with Fanconi anemia, diepoxybutane was found to increase chromosome fragility, but this was not the case for lymphocytes of patients with other hematologic disorders (42).

In the treatment of psoriasis, a group of compounds psoralens are applied topically to the skin which is subsequently irradiated with ultraviolet light (44). The principle is that psoralens bind to DNA to form monoadducts upon radiation and that subsequent irradiation of psoralen monoadducts results in DNA cross-link formation (45, 46). The concentration of psoralens utilized *in vivo* in humans to treat psoriasis is related to the ability to form cross-links and may be responsible for induction of skin cancer (45).

In Vitro Findings

In general, the interaction between an industrial chemical or heavy metal with DNA results in a DNA-protein cross-link formation as opposed to DNA-DNA inter- or intrastrand cross-links which occur with pharmaceutical agents. Incubation of animal or human cells lines with various compounds were reported to induce DNA-protein cross-linking (Table III). Treatment of cells with chromium III which is present in seawater as a complex did not induce a DNA-protein cross-link reaction as it does not penetrate mammalian cells (29, 30). However, exposure to chromium VI, which is released in stainless steel welding, chrome plating, tanning, and leather working, readily penetrates mammalian cells and is subsequently converted by reduction to chromium III within the cell (29, 30). Within the cell, chromium III induces a DNA-protein cross-link reaction. In contrast, nickel, arsenic compounds, or copper induce DNA-protein cross-linking by directly entering cells and do not require conversion to a different form (Table III). The volatile aldehydes, acetaldehyde and formaldehyde, are known to directly induce DNA-protein cross-linking in various cell systems (2, 22). It is of interest that the ability of vinyl acetate, methylene dimethanesulphonate, and dichloromethane to produce DNA-protein cross-links in mammalian cells was dependent on the release of acetaldehyde and formaldehyde, for the latter two compounds, respectively from the parent compounds (15, 20, 25, 28, 60). Similarly it was suggested that vinyl chloride was metabolized to chloroacetaldehyde, which was responsible for the observed genotoxicity (62).

Summary and Conclusions

The number of chemicals known to produce carcinogenesis or mutagenesis is vast; however, chemical-induced cross-link formation has been demonstrated for only a small fraction of compounds. It is possible that this reaction was not determined for many genotoxic agents, as the assay may have lacked sensitivity or the amount of chemical required to in-

Table III. List of Examples of Metals or Chemicals Inducing DNA-Protein Cross-Linking *In Vitro*

Compound	Cell line	Reference
Copper	Chinese hamster ovary cells	4
Arsenic	Human alveolar epithelial L-132 cells	47
	Human fetal lung fibroblasts	48
Nickel	Chinese hamster ovary cells	49
Chromium	Osteosarcoma cells	16
	Chinese hamster ovary cells	50,51
Diepoxybutane	Human bone marrow cells	52
Ethylene oxide	Human lymphocytes	53
Vinyl acetate	Human lymphocytes	54
	Chinese hamster ovary cells	55
Glutaraldehyde	TK6 lymphocytes	56
Acetaldehyde	Human leukocytes	57
Formaldehyde	Mouse leukemia cells	58
	Human bronchial cells	59
Methylene dimethanesulphonate	Mouse Yoshida lymphosarcoma cells	60,61

duce this effect was not reached in sufficient concentration at the target site. As the cross-link reaction is dependent on the interaction of a chemical electrophile with DNA, it is possible that the cellular conditions were such that the chemical failed to generate the electrophile. It is also possible that the chemical electrophile was released but the binding to DNA resulted in chain scission or depurination such that even if cross-linking occurred it may not have been detected. It should be noted that the detection of DNA-protein cross-linking is enhanced as the size of the DNA segment is decreased and that in *in vivo* conditions one cannot regulate the size of the DNA segment that is exposed. It is evident that DNA-protein cross-linking occurs in control conditions and that this rate is increased by chemicals (19). The number of chemicals inducing a cross-linking reaction is probably greater than the list enunciated in this paper as generation of a reactive chemical electrophile is common and does not necessarily result in carcinogenesis.

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