DNA–protein crosslinks, a biomarker of exposure to formaldehyde—in vitro and in vivo studies

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Introduction

Formaldehyde (FA*) is a widely produced industrial chemical. Sufficient evidence exists to consider FA as an animal carcinogen. In humans the evidence is not conclusive. DNA–protein crosslinks (DPC) may be one of the early lesions in the carcinogenesis process in cells following exposures to carcinogens. It has been shown in in vitro tests that FA can form DPC. We examined the amount of DPC formation in human white blood cells exposed to FA in vitro and in white blood cells taken from 12 workers exposed to FA and eight controls. We found a significant difference (P = 0.03) in the amount of DPC among exposed (mean ± SD 28 ± 5%, minimum 21%, maximum 38%) than among the unexposed controls (mean ± SD 22 ± 6%, minimum 16%, maximum 32%). Of the 12 exposed workers, four (33%) showed crosslink values above the upper range of controls. We also found a linear relationship between years of exposure and the amount of DPC. We conclude that our data indicate a possible mechanism of FA carcinogenicity in humans and that DPC can be used as a method for biological monitoring of exposure to FA.

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FA is rapidly metabolized to formate, which is partially incorporated via normal metabolic pathways into the one carbon pool of the body or further oxidized to carbon dioxide. It also reacts with proteins (36) and with a variety of cellular nucleophiles, including glutathione, forming adducts of varying stability (37–39), like N^6-hydroxy methyldeoxyadenosine and N^6-hydroxymethyldeoxyguanosine, which are the major adducts formed by reaction with FA (37–39). In addition to FA (40–43), many established or suspect carcinogens, such as UV, radiation, BCNN (44), alkylating agents (45) and some metal compounds such as nickel (46), chromium (47,48) and cis- or trans-Pt (II) diamine dichloride (49), are also known to produce DNA–protein crosslinks (DPC). FA selectively

Abbreviations: FA, formaldehyde; DPC, DNA–protein crosslinks; WBCs, white blood cells.

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induces histone DPC which are not disrupted by chelating or reducing agents (50). FA induces the formation of DPC in the nasal respiratory mucosa of rats following chronic inhalation exposure (51,52). It reacts with single-strand DNA, but not with double-strand DNA. This link is reversible, unlike the protein crosslinks, which are stable (53).

DNA crosslinks are persistent over long time intervals following removal of the crosslinking agents (48,54) and, due to poor repair capacity present during DNA replication, cause a loss of genetic material. The apparent low capacity for repair renders this type of DNA damage a potentially important lesion to use as a biomarker of exposure. Measuring DPC can indicate the dose of FA at a critical target site. Many attempts have been made to develop assays to detect DPC, the most popular being the alkaline elution test (55–58). The main limitation of this method is that it detects DPC indirectly and it is a time-consuming procedure allowing only a limited number of samples to be analyzed (59).

Zhitkovich and Costa (60) used a method which is a modification of the K-SDS assay originally developed by Liu et al. (61) and Muller et al. (62) to detect DNA–topoisomerase complexes with the same sensitivity as alkaline elution but more efficiently. With it they detected DPC induced by chromate, cis-Pt (II) diaminodichloride and FA in cultured cells and in white blood cells (WBCs) of rats and mice exposed to chromate by intraperitoneal injections. We report here the results of a study done on human WBCs exposed to FA in vitro and in vivo.

Materials and methods

In vitro studies

We examined the amount of DPC in human WBCs following exposure for 1.5 h to increasing concentrations (0–10 mM) of FA. Samples treated with FA were then frozen at −20 or −70°C for 3 weeks and DPC were measured.

In vivo studies

After the assay was established and we were convinced that it could be used to detect DPC formed following exposure to FA, we implemented this assay in our in vivo studies. We examined the amount of DPC in peripheral WBCs taken from workers occupationally exposed to FA. Our study population consisted of 12 workers and the control group was made up of eight workers who had never been exposed to FA. Characteristics of the study population are shown in Table 1. Every worker completed a questionnaire on demographic data, occupational and medical history and smoking and hygiene habits.

Venous blood was withdrawn into EDTA vacutainer tubes and WBCs were isolated after 3 h. The resulting cell pellets were resuspended in storage medium and placed at −20°C. DPC were measured within 3 weeks. In order to detect DPC due to FA exposure we adopted Zhitovich and Costa's assay (60) and implemented it in human WBCs exposed to FA in vitro and in vivo. Briefly, the assay is based upon the binding of SDS to proteins and its lack of binding to DNA. Fragments of protein-free DNA and protein-bound DNA were easily separated, since free DNA remained in the supernatant while protein-linked DNA precipitated with protein-bound SDS when the cation was changed from Na to K. Thus the amount of DNA in the SDS pellet provides a direct measurement of DPC. DNA was mixed with 1 ml freshly prepared Hoechst dye reagent (200 ng/ml) at pH 7.5 to determine the DNA concentration to calculate the percentage of DNA crosslinks.

Results

In vitro studies

We found that exposure of human WBCs to FA caused the formation of DPC. These lesions can be detected with the assay we used at concentrations of FA as low as 0.001 mM or even lower. The DNA crosslinks are induced in a dose-
DNA-protein crosslinks / total DNA

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<th>MEAN</th>
<th>STD</th>
<th>MIN</th>
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**Fig. 2.** DNA–protein crosslinks due to formaldehyde exposure.

**Fig. 3.** Correlation between DNA–protein crosslinks and occupation.

dependent manner (Figure 1). We also found that samples of WBCs can be stored at −20 or −70°C for 3 weeks without loss of DPC (Figure 1).

**In vivo studies**

We found a significant difference ($P = 0.03$) between the levels of DPC in peripheral WBCs among exposed (mean ± SD 28 ± 6%, range 21–38%) and unexposed control workers (mean ± SD 22 ± 6%, range 16–32%). Of the 12 exposed workers four (33%) showed DPC values above the upper range of the controls (Figure 2).

We divided the exposed group into physicians and technicians because, according to the questionnaire and ambient measurement, we knew that the technicians were exposed daily for longer periods and to a higher concentration of FA. We found a significant difference ($P < 0.05$) in the levels of DPC in peripheral WBCs between these two groups (mean ± SD 26 ± 4%, range 26–38% among the technicians, mean ± SD 26 ± 4%, range 21–34%, among the physicians) (Figure 3).

We also found a linear relationship between years of exposure and the amounts of DPC (Figure 4). This finding emphasizes the fact that DPC can be used as a biomarker to measure chronic exposure.

We found a significant difference ($P < 0.03$) in the levels of DPC between the group of exposed and unexposed non-smokers and the group of exposed and unexposed smokers, as well as between exposed and unexposed workers (Figure 5). Based on these findings we can assume that DPC were not influenced by smoking habits in our study population and that the results we found were due to occupational exposure only.

**Discussion**

Studies of several species, including humans, have shown that FA undergoes rapid biotransformation immediately after absorption and therefore no increase in its concentration in tissue or blood can be detected even moments after exposure (63,64).

FA is genotoxic, including gene mutation, deletion, chromosomal aberration, sister chromatid exchanges and cell transformation (65). FA can induce DNA adducts such as hydroxymethyl adducts in DNA (66) and crosslinks with biologic macromolecules. Under its influence structural proteins that normally do not bind to DNA become covalently crosslinked to DNA. Characteristically FA selectively formed histone DNA crosslinks (43,66–68). Using the assay described in Materials and methods we were able to detect DPC formed following exposure to FA in vitro and in vivo. We also found a significant different between the amounts of DPC in peripheral WBCs of exposed workers and unexposed control workers (Figure 2). Based on these results we conclude that the assay we used is sensitive enough to discriminate between exposed and controls.

Our findings of a linear correlation between years of exposure to FA and the amount of DPC and those of Sugiyama et al. (48) and Tsapakos et al. (54) that DPC can persist for a long period after removal of the crosslinking agent, most probably due to poor repair capacity, render this lesion an important biomarker of chronic exposure. Because DPC are irreversible they remain present as an obstacle during DNA replication and may possibly lead to a loss of genetic material that may, among other things, inactivate tumor suppressor genes (p53). These assumptions were illustrated in a model system of
genetic consequence in the Chinese hamster X chromosome, where deletion of the long arm of the chromosome was found after exposure to nickel, a human carcinogen which is also known as a crosslinking agent (69,70).

FA induces squamous cell carcinoma in the nasal passage of rats following chronic inhalation exposure (71,24). Casanova et al. (72) found formation of DNA—protein crosslinks in the nasal mucosa of rats following exposure to FA, so these lesions can be regarded as a measure of FA dose at the site of tumor formation. Recio et al. (73) examined cDNA of the tumor suppressor gene p53 from 11 primary FA-induced tumors from the nasal passage of F344 rats for mutation using DNA sequence analysis. Point mutations in the p53 cDNA sequence were found in 5/11 of the tumors analyzed. The p53 point mutations observed by Recio et al. are common alterations in certain rat (71) and human cancers, including squamous cell carcinoma of the respiratory tract (74,75). The study of Recio et al. presents a possible role of human suppressor gene alteration in FA-induced nasal squamous cell carcinoma.

The relationship between a genetic lesion and the development of a malignant tumor is difficult to establish. However, the fact that loss of DNA is an important genetic consequence in many different types of tumors and the realization that tumor suppressor genes such as p53 maintain normality of cells (76) emphasize that DPC may be one of the primary and early lesions of the carcinogenesis process.

The method we used is inexpensive, sensitive, rapid and easily applicable to multiple samples, required in epidemiological studies. Samples can be stored at -20 or -70°C for at least 1 month without loss of DPC. Measuring DPC in peripheral WBCs can be regarded as a surrogate for the presence of the same lesion in other tissues, such as the upper respiratory tract, which receives considerably higher exposure than peripheral blood cells and can indicate the dose of FA at a certain target site.

We conclude that our finding of DPC in WBCs of workers exposed chronically to FA indicates a possible lesion that may represent an early event in the mechanism of FA carcinogenesis in human cells. We also conclude that DPC can be used as a biomarker of occupational exposure to FA, making it possible to follow up high risk populations more closely for secondary prevention.

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References

36. French, D. and Edsall, J.T. (1945) The reactions of formaldehyde with...


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Formaldehyde exposure biomarker