ORIGINAL ARTICLE

DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde

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Background: Formaldehyde (FA) is classified as a probable human carcinogen.
Aims: To examine DNA protein crosslinks (DPC) and p53, which are generally known to be involved in carcinogenesis, in peripheral blood lymphocytes of workers exposed to FA.
Methods: DPC and p53 ("wild type" and mutant) were examined in peripheral blood lymphocytes of 186 workers exposed to FA (mean years of exposure = 16) and 213 unexposed workers. Every worker completed a questionnaire on demographic data, occupational and medical history, smoking, and hygiene.
Results: The adjusted mean level of DPC in the exposed and the unexposed workers differed significantly. Adjustment was made for age, sex, years of education, smoking, and origin. Exposure to FA increased the risk of having a higher level of pantropic p53 above 150 pg/ml (OR 1.6, 95% CI 0.8 to 3.1). A significant positive correlation was found between the increase of pantropic p53 protein and mutant p53 protein, as well as between pantropic p53 >150 pg/ml and mutant p53 protein. In the exposed group a significantly higher proportion of p53 >150 pg/ml was found among workers with DPC >0.187 (55.7%) (0.187 = median level of DPC) than among workers with DPC ≤0.187 (33.3%). The risk of having pantropic p53 protein >150 pg/ml was determined mainly by levels of DPC. Workers with DPC above the median level had a significantly higher risk of having pantropic p53 >150 pg/ml (adjusted OR 2.5, 95% CI 1.2 to 5.4).
Conclusions: Results suggest that DPC and mutation in p53 may represent steps in FA carcinogenesis and a possible causal relation between DPC and mutation in p53. These biomarkers can be applied in the assessment of the development of cancer due to FA exposure.

Formaldehyde (FA) is a flammable, colourless, and readily polymerised gas at ambient temperature. It is present in the environment as a result of natural processes,[1 2] and from man made sources, including motor vehicle exhaust, wood burning stoves,[3] and cigarette smoke.[4] It is found in small quantities in every human cell, where it is derived from the metabolism of serine, glycine, sarcogine, choline, and methionine.[5]

Occupational exposure to FA occurs in the production of urea, acetal, melamine, and phenol FA resins. These resins have numerous commercial applications and are used in the production of adhesives and binders for wood, plastics, textile, and leather.[6] FA itself has an important function in medicine as a bactericide and tissue preservative.[6] Because of its widespread use, a relatively large number of workers are exposed to FA.[7]

According to the IARC (International Agency for Research on Cancer), there is sufficient evidence in experimental animals, and limited evidence in humans for carcinogenicity of FA. Based on these facts FA is classified as probably carcinogenic to humans (group 2A).[8] Because of the lack of consistency between cohort and case control studies, epidemiological studies can do no more than suggest a causal role of occupational exposure to FA and squamous cell carcinoma of the nasal cavities and the peranasal cavities.

Numerous studies have shown that FA is genotoxic and mutagenic to mammalian cells and that it induces a broad spectrum of genetic effects.[8–10] The primary and direct genotoxic effect of FA seems to be the formation of DNA–protein crosslinks (DPC) in target tissues. DPC have been described in various in vitro experiments, using *E coli*,[11] *Saccharomyces cervisiae*,[12 13] mouse cells,[14] and human lymphocytes.[15 16] It has been shown in *E coli* that at least 50% of these DPC bind the two strands of DNA together, even after exposure to low doses of FA.[17]

In vivo experiments in animals showed that chronic inhalation of FA leads to formation of DPC in the nasal respiratory mucosa of rats and monkeys.[18 19]

Despite the wide evidence from both in vitro and animal studies of the formation of DPC following exposure to FA, no studies have been conducted in humans.

Our in vivo pilot study was the first to measure DPC in humans.[15 16] In that study, we measured the amount of DPC in peripheral lymphocytes taken from 12 workers from a pathology department who were occupationally exposed to FA, and from eight unexposed workers. We found that the amount of DPC was significantly higher ($p = 0.03$) among exposed workers compared to unexposed workers.[15 16]

One of the most common somatic genetic alterations in human cancers involves the loss of inhibitory function of the p53 tumour suppressor gene products.[20] In many cases, a common mechanism is the loss of one normal p53 allele and/or point mutation of the other p53 allele.[20] The resultant effect is a paradoxic increase in intracellular levels of the p53 protein. Since many mutant forms of p53 have a much longer half life than wild type p53, the mutant form of p53 can bind to the wild type, stabilising its normally rapid degradation and elongating its half life.[20]

The intracellular accumulation of mutant p53 protein in cells in culture results in a corresponding increase in p53 protein in the extracellular supernatant.[21] Similarly, individuals

Abbreviations: DPC, DNA–protein crosslinks; FA, formaldehyde; OR, odds ratio; SCC, squamous cell carcinoma; SE, standard error

Table 1 Baseline characteristics of the study groups

	Total n=399		Control n=213		Exposed n=186	
	No.	%	No.	%	No.	%
Sex**						
Male	186	46.6	127	59.6	59	31.7
Female	213	53.4	86	40.4	127	68.3
Education**						
1–12	105	26.3	77	36.2	28	15.1
13–15	93	23.3	52	24.4	41	22.0
16+	172	43.1	60	28.2	112	60.2
Missing	29	7.3	24	11.2	5	2.7
Origin**						
Asia/Africa	100	25.1	68	31.9	32	17.2
Europe/America	266	66.7	121	56.8	145	78.0
Israel	32	8.0	23	10.8	9	4.8
Missing	1	0.2	1	0.5	0	
Smoking (NS)						
No	232	58.1	114	53.5	118	63.4
Yes	163	40.9	95	44.6	68	36.6
Missing	4	1.0	4	1.9	0	0
Age**	43.8 (±10.5)		42.1 (±10.7)		45.8 (±9.9)	

**p<0.01.
NS, non-significant.

with tumours that have increased levels of mutant p53 protein can also have high levels of p53 in extracellular fluids, such as serum.[22 23]

The current study is an extension and reconfirmation of the results of our pilot study, as well as the implementation of DPC in a molecular epidemiological study. In addition to DPC we added another biomarker, p53 protein which, we suggest, can help to elucidate the carcinogenesis mechanism of FA in humans. The innovation of our study is the measurement of these markers in humans exposed to FA.

METHODS

Study population

The study population consisted of 399 workers.

The *exposed group* included 186 workers (mean age 45.8 (±9.9) years; 59 (31.7%) men, 127 (68.3%) women) from 14 hospital pathology departments. Concerning smoking habits, 68 (36.6%) of the group were active smokers, and 118 (63.4%) non-smokers. The mean exposure period to FA was 15.9 (range 1–51) years.

Jobs included in the exposed group were: physicians, laboratory assistants and technicians, and hospital orderlies. Occupational histories of both groups showed that in the control group there was no exposure to FA, either past or present, while in the exposed group the exposure to FA occurred only in their present jobs. None of the exposures in the pathology department (such as ionising radiation, chromate, nickel, cisplatin) are known to form DPC in humans.

The *unexposed group* consisted of 213 workers (mean age 42.1 (±10.7) years; 127 (59.6%) men, 86 (40.4%) women) from the administrative section of the same hospitals. Concerning smoking habits, 114 (53.5%) were non-smokers and 95 (44.6%) were smokers.

The age distribution, sex, origin, and education years differed significantly between the exposed and the unexposed group. There were more workers of European/American origin in the exposed group compared to the unexposed group ($p < 0.01$); they were also older ($p < 0.01$) and more educated ($p < 0.01$), and there were more females ($p < 0.01$) (table 1).

Before comparing the results obtained between the exposed and the unexposed groups, adjustment was made for age, sex, origin, and education. These are described in the statistical analysis section.

Every participant in the study population completed a questionnaire eliciting demographic data, history of occupations and exposures, medical history, and smoking habits, the latter because formaldehyde is present in tobacco smoke. Based on the responses to the questionnaire we can assume that nobody in the unexposed group was ever occupationally exposed to FA, and that nobody in the exposed group was occupationally exposed to substances known to form DPC, other than FA.

DNA–protein crosslinks

We examined the amount of DPC in the mononuclear cell fraction in peripheral blood. Venous blood was withdrawn into EDTA vacutainer tubes, and mononuclear cells were isolated using Ficoll-paque gradient shortly after taking the blood samples. As the mononuclear cell fraction was composed mostly of lymphocytes, we will use this term in the paper. The resulting cell pellets were resuspended in storage medium at –20°C. DPC were measured within three weeks. The assay we used is described in our pilot study.[15] Briefly, the assay is based on 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, as 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 DNA in the samples. Fluorescence measurements were made using a digital fluorometer with NB 360 excitation and SC 450 emission filters. We calculated the amount of DPC as the ratio detected in exposed white blood cells to total DNA in the same amount of cells. Each sample was tested in duplicate.

Using the above described K-SDS assay, we were able to detect DPC in human lymphocytes following in vitro exposure to concentrations of FA as low as 0.001 mM or even lower. These concentrations are compatible with real life situations. We also found that this method is inexpensive, rapid, and easily applicable to the multiple samples required in epidemiological studies. Furthermore, samples can be stored at –20 or –70°C for at least one month without loss of DPC.[15]

p53 assay

p53 proteins, wild type and mutant (pantropic p53), in serum were determined using the p53 quantitative ELISA kit (Cat no. QSA07), which is a "sandwich type" immunoassay, according to Oncogene Science protocols. Mutant p53 protein in serum was also determined using p53 mutant selective quantitative

Table 2 Amount of DPC in relation to exposure

	Total Mean (SE)	Male Mean (SE)	Female Mean (SE)
Population group			
Control	0.14 (0.006)	0.15 (0.008)	0.12 (0.008)
Exposed	0.21 (0.006)**	0.21 (0.011)**	0.20 (0.008)**
Sex			
Male	0.19 (0.006)**		
Female	0.16 (0.006)		
Smoking (NS)			
No	0.17 (0.006)	0.18 (0.010)	0.16 (0.007)
Yes	0.18 (0.006)	0.18 (0.009)	0.16 (0.009)
Age (NS)			
⩽43	0.17 (0.006)	0.18 (0.009)	0.16 (0.008)
>43	0.18 (0.006)	0.18 (0.009)	0.16 (0.007)
Education years (NS)			
1–12 years	0.18 (0.007)	0.19 (0.011)	0.17 (0.009)
13+ years	0.17 (0.005)	0.17 (0.009)	0.16 (0.007)
Origin (NS)			
Asia/Africa	0.17 (0.006)	0.18 (0.009)	0.16 (0.009)
Europe/America	0.18 (0.005)	0.19 (0.008)	0.16 (0.006)
Israel	0.17 (0.012)	0.17 (0.019)	0.17 (0.016)

p<0.01, least square mean from fitted model.
NS, non-significant; SE, standard error.

ELISA Kit (Cat no. QIA03), which is also a "sandwich" enzyme immunoassay, according to Oncogene Science protocol.

Occupational exposure to formaldehyde

We examined the concentration of FA in ambient air at various periods of a typical working day at the pathology departments by personal and field samples. Each measurement was for a mean time of 15 minutes. Based on these results the exposed group was divided into two subgroups:

- Low level of exposure: mean 0.4 ppm (range: 0.04–0.7 ppm). Professions included in this subgroup were laboratory assistants and technicians.
- High level of exposure: mean 2.24 (range 0.72–5.6 ppm). Professions included in this subgroup were physicians and hospital orderlies.

The threshold limit value for FA in Israel is 0.3 ppm.

Statistical analysis

Comparison of sex, smoking habits, origin, and education years among the study subgroups, exposed and unexposed, was assessed by the χ^2 test The difference in age between the two subgroups was estimated by the non-parametric Mann-Whitney U test.

Comparison of the adjusted means of DPC between the exposed and unexposed groups was assessed by analysis of variance for unbalanced design (general linear models procedure). Adjustment was made for sex, smoking, age, origin, and years of education.

In the exposed group, comparison of DPC between the two levels of exposure (low and high), and between the two periods of exposure (⩽16 years and >16 years) was estimated by the Mann-Whitney U test.

Comparison of the prevalence of high levels of p53 between the exposed and unexposed group was assessed by the χ^2 test.

The adjusted odds ratios (OR) and 95% confidence intervals (CI) for p53 >150 pg/ml by group exposure, sex, smoking, and age were assessed by logistic regression analysis. The association between p53 and mutant p53 was estimated by the Spearman rank correlation coefficient. Comparison of mutant p53 protein between those with pantropic p53 >150 pg/ml and those with p53 <150 pg/ml was estimated by the Mann-Withney U test. In the exposed group, the prevalence of p53 >150 pg/ml between those with high levels of DPC and those with low levels of DPCs was assessed by the χ^2 test, and the adjusted odds ratios and 95% confidence intervals for p53 >150 pg/ml in relation to DPC, sex, age, and smoking were assessed by logistic regression analysis. We found the median of DPC to be more appropriate than the mean, mainly because there is no established cut off point in the literature, and also because the distribution of DPC is asymmetrical. All data were analysed using the SAS statistical software package.

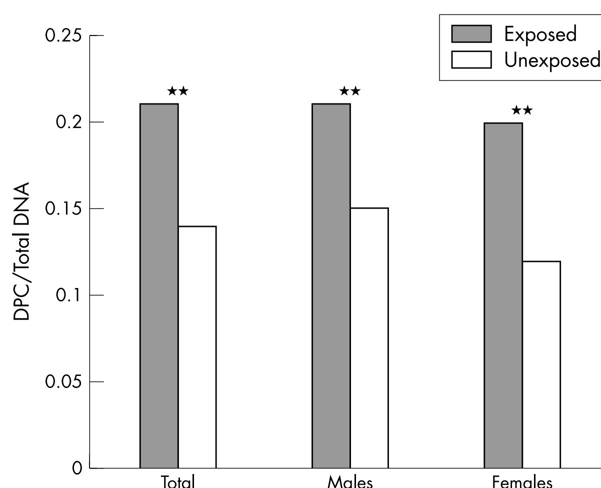

Figure 1 DNA protein crosslinks among groups in the study population. **p<0.01; adjusted mean to age, sex, smoking, education, origin.

RESULTS

DNA–protein crosslinks

The amount of DPC is expressed as DPC/total DNA. Table 2 and fig 1 show the relation between occupational exposure and amount of DPC after adjustment for potential confounding variables.

The adjusted mean amount of DPC was significantly higher ($p < 0.01$) in the total exposed group (adjusted mean 0.21, SE 0.006) compared to that in the total unexposed group (adjusted mean 0.14, SE 0.006). It was also significantly higher in the exposed male (adjusted mean 0.21, SE 0.011) and female (adjusted mean 0.20, SE 0.008) subgroups compared to that of the unexposed subgroups (adjusted mean 0.15, SE 0.008 and adjusted mean 0.12, SE 0.008 respectively).

Table 3 Adjusted odds ratios and 95% confidence intervals for pantropic p53 >150 pg/ml—in relation to exposure, sex, age, and smoking

	Total	Male	Female
Group exposure			
Non-exposed	1.0	1.0	1.0
Exposed	1.6 (0.8 to 3.1) (NS)	2.0 (0.9 to 4.4)†	0.8 (0.2 to 2.7) (NS)
Sex			
Female	1.0		
Male	1.6 (0.9 to 3.2) (NS)		
Age			
≤43	1.0	1.0	1.0
>43	1.1 (0.6 to 2.0) (NS)	1.2 (0.6 to 2.6) (NS)	1.2 (0.5 to 2.7) (NS)
Smoking			
No	1.0	1.0	1.0
Yes	0.8 (0.5 to 1.3) (NS)	0.9 (0.6 to 1.6) (NS)	0.5 (0.2 to 1.2) (NS)

†p<0.10.
NS, non-significant.

Age, smoking habits, years of education, and origin were not significant confounders in this relation.

The mean amount of DPC shows an increase in relation to level of exposure (low level of exposure subgroups: total group 0.19, males 0.19, females 0.20; high level of exposure subgroups: total group 0.20, males 0.21, females 0.19), and median years of exposure (up to/or 16 years of exposure: total exposed group 0.19, males 0.20, females 0.19; above 16 years of exposure: total exposed group 0.20, males 0.21, females 0.20). However, these results were not statistically significant.

Pantropic (wild type+mutant) p53 protein, and mutant p53 protein expression

Based on our experience the limit of 150 pg/ml protein seemed reasonable for comparison between the exposed and the unexposed subgroups. We therefore divided the study groups into those who have pantropic p53 protein above or under 150 pg/ml. High levels of p53 (p53 >150 pg/ml) were more prevalent in the exposed group. p53 >150pg/ml was found in 44.1% of the exposed group compared to 36.3% of the unexposed group. The exposed males subgroup had a significantly higher proportion of p53 >150 pg/ml (54.8%) compared to the unexposed subgroup (36.5%) ($p < 0.05$). In the females subgroup the prevalence of p53 >150 pg/ml was higher among the exposed (38.8%) than among the unexposed (35.3%).

We also found that exposure to FA increases the risk of having pantropic p53 >150 pg/ml (OR 1.6, 95% CI 0.8 to 3.1), especially among males (OR 2.0, 95% CI 0.9 to 4.4) ($p < 0.1$). Age, sex, and smoking habits had no net effect on the risk of having pantropic p53 >150 pg/ml (table 3).

A significant positive correlation (rs = 0.75; $p < 0.01$) was found between pantropic p53 protein and mutant p53 protein, as well as between pantropic p53 protein >150 pg/ml and mutant p53 protein (rs = 0.6; $p < 0.05$). The mean level of mutant p53 protein was significantly higher ($p < 0.01$) among those with pantropic p53 >150 pg/ml (mean mutant p53 protein = 0.82 ng/ml, SE 0.022) than among those with pantropic p53 ≤ 150 ng/ml (mean mutant p53 protein = 0.71 ng/ml, SE 0.008).

When we examined the relation between the median of DPC (namely 0.187) of the exposed group and pantropic p53 protein, we found that pantropic p53 >150pg/ml tended to be more common among workers with the DPC amount above the median level than among those with the amount of DPC below the median. This difference reached statistical significance in the total group and in the females subgroup. In the exposed group a significantly higher proportion of p53 >150 pg/ml was found among workers with DPC >0.187 (55.7%) than among workers with DPC ≤0.187 (33.3%) ($p < 0.01$). Among males, 63.64% of those with higher DPC had p53 >150pg/ml, while only 44.4% of those with lower DPC had p53 >150 pg/ml. In the females subgroup the prevalence of p53 >150 pg/ml in those with DPC above the median (51.3%) was significantly higher than among those with DPC below the median (28.57%) ($p < 0.05$). The risk for pantropic p53protein >150 pg/ml was determined mainly by DPC. Those workers with DPC above the median level have a significantly high risk of having pantropic p53 >150 pg/ml. Adjusted ORs of 2.5 (95% CI 1.2 to 5.4) among the total population and 2.8 among the females (95 % CI 1.1 to 7.1) were both significantly higher ($p < 0.05$). In both cases age and smoking had no net effect on pantropic p53 protein (table 4).

Table 4 Adjusted odds ratios and 95% confidence intervals for pantropic p53 >150 pg/ml—in relation to DPC, sex, age, and smoking in the exposed group

	Total	Male	Female
DPC			
≤0.187	1.0	1.0	1.0
>0.187	2.5 (1.2 to 5.4)*	1.9 (0.5 to 7.2) (NS)	2.8 (1.1 to 7.1) *
Sex			
Female	1.0		
Male	2.1 (0.9 to 5.0) (NS)		
Age			
≤46	1.0	1.0	1.0
>46	1.3 (0.6 to 2.8) (NS)	1.1 (0.3 to 4.2) (NS)	1.3 (0.5 to 3.4) (NS)
Smoking			
No	1.0	1.0	1.0
Yes	0.7 (0.4 to 1.3) (NS)	1.4 (0.6 to 3.3) (NS)	0.5 (0.2 to 1.04) (NS)

*p<0.05.
NS, non-significant.

DISCUSSION

The main results in our study are the finding of significantly higher levels of DPC in the exposed workers compared with unexposed workers. Exposure to FA increases the risk of having a higher level of pantropic p53. A significant positive correlation was found between the increase of pantropic p53 protein and mutant p53 protein. The risk of having higher levels of pantropic p53 was determined mainly by levels of DPC.[8 24 25]

No data have yet been reported on DPC formation in humans following in vivo exposure until the publication of our pilot[15 16] and present studies. In both studies, by using the assay described (K-SDS), we were able to detect DPC in workers exposed to FA.

Studies of animals and humans have shown that FA undergoes rapid biotransformation immediately after absorption to formate, which is partially incorporated via normal metabolic pathways into the one-carbon pool of the body, or further oxidised to carbon dioxide. Therefore, no increase in its concentration in tissue or blood can be detected even moments after exposure.[26 27] In addition, none of its metabolites are specific enough to be used as biomarkers of internal dose.

Although the concentration of FA in blood does not increase after exposure to FA, due mainly to rapid metabolism in the erythrocytes, FA may escape metabolism and succeed in reacting with macromolecules.[9] FA is genotoxic and mutagenic. The primary genetic effect of FA would appear to be the formation of DPC. The general structure of FA induced DPC is: histone–NH–CH_2–NH–DNA.

The above data can explain our finding of a high DPC amount in peripheral blood lymphocytes of workers exposed to FA. The latter are in contact with many tissues, including those that have a very rich blood supply and are exposed directly to FA, and are thus able to provide an integrated measure.

These facts strengthen the suggestion that measuring DPC can be regarded as surrogate for the dose of FA at critical target sites, and can enhance exposure assessment. Based on our results, we concluded that exposure in vivo to formaldehyde causes the formation of DPC in human peripheral white blood lymphocytes, and that the assay we used is sensitive enough to discriminate between exposed and unexposed workers.

It is possible that the main reason Casanova and colleagues[28 29] failed to detect DPC in tissues or organs of rats remote from the immediate contact with FA, is because they used an assay that lacked the sensitivity to detect DPC at concentrations of FA below 2.0–0.3 ppm. Using our methods, we were able to detect DPC at concentrations <0.001 mM, which is closer to the real life condition.[15]

Beal and Ulsamer[30] reviewed 84 articles relating to the adverse health effect in animals and humans of subchronic exposures to FA and concluded that animal data revealed a qualitative relation between FA absorption and hepatotoxicity. Later, Woutersen and colleagues[31] came to the same conclusion. Migliore and colleagues[32] showed that oral administration of FA to rats caused the induction of micronuclei and molecular anomalies in cells of the stomach, duodenum, ileum, and colon. These data suggest that FA not only causes nuclear damage at the site of application (the stomach), but is also able to reach other segments of the gastrointestinal tract. Oral administration of FA to rats in drinking water significantly increased the incidence of leukaemia and gastrointestinal tumours compared with controls.[33] Chang and colleagues[34] and others[35] showed that significant concentrations of ^{14}C are localised in the nasal mucosa and in other well perused tissue, such as bone marrow, liver, and kidney, following exposure by inhalation of rats to ^{14}C-FA. Industrial cohorts[36–38] showed possible toxic effects of FA in these tissues (leukaemia and lymphatic/haematopoietic cancers), as did rats after exposure to FA by oral administration.[33]

According to data reported above there is evidence of potentially harmful effects on an organ or tissue that is remote from the first point of contact with FA, namely the nasal mucosa.

The reason the main effect is in the nasal cavity after inhalation of FA by rats is complex. Rats are obligatory nose breathers; FA is a highly reactive molecule; and high concentrations of detoxifying enzymes (such as FA dehydrogenase) in the nasal mucosa cause rapid metabolism of inhaled FA. In humans, where respiration is both oral and nasal, the site and degree of toxicity are different from those in rodents. That is, humans are subject to higher incidences of cancers in the nasopharynx, nasal cavities, and paranasal sinuses, associated with occupational exposures in industry, while embalmers, anatomists, and other professionals are at higher risk for possible brain tumours.[9]

The studies of Sugiyama and colleagues,[39] and Tsapakos and colleagues,[40] who reported that DPC can persist long after removal of the crosslinking agent, probably due to poor repair capacity, and the finding of Quievryn and colleagues[41] that human peripheral blood lymphocytes lost DPC at a significantly slower rate (~50% longer than that in cultured human cells) are contrary to previous studies that reported a relatively rapid loss of FA induced DPC from cultured cells.[28 42–44] Quievryn and colleagues also found that removal of DPC involved a novel and less efficient repair pathway, that appears to act through the proteolytic degradation of crosslinked protein and not nucleotide excision repair.[41] Formation of DNA histone crosslinks was shown to be strongly correlated with tumour incidence at the target sites.[45 46] These facts render DPC as an important lesion in the carcinogenesis process of FA.

Based on the above evidence, it can be suggested that DPC may remain present as an obstacle during DNA replication and may possibly lead to a loss of genetic material that could, among other things, inactivate tumour 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 that is also known to be a crosslinking agent.[47 48]

FA induces squamous cell carcinoma in the nasal passage of rats following chronic inhalation.[49 50] Using DNA sequence analysis, Recio and colleagues[51] examined for mutation the DNA of the tumour suppressor gene p53 from 11 primary FA induced tumours from the nasal passages of F344 rats. Point mutations in the p53 cDNA were found in five of the 11 tumours.[52] All of the mutated p53 codons identified in the FA induced rat squamous cell carcinoma (SCC) have been described as mutated in a variety of human cancers: one of the mutated codons (rat codon 271, human codon 273) is considered a p53 mutational "hot spot" for human cancer. The proportion of human respiratory tract cancer (SCC) that exhibit p53 alterations is similar to that found in FA induced rat SCC.[53–57] Therefore, p53 mutation is a common molecular event in the development of human SCC of the respiratory tract. Mutation of p53 occurs in human skin SCC[58] and at a high frequency (27/32, 84%) in human oesophageal SCC.[59] Since p53 mutation occurs during the development of many human SCC, these data indicate that the development of certain human and rat SCC share a common molecular change. Although alterations of tumour suppressor genes occur in a number of human cancers, they do not occur at a high frequency among many rodent tumours.[60] Mutation of the p53 tumour suppressor gene is the most common genetic alteration found in human cancers.[61] The cellular p53 protein is involved in a number of biological functions, including gene transcription, DNA synthesis and repair, and apoptosis.[62] Mutations of the p53 gene in human cancer can provide a selective advantage for clonal expansion and progression of preneoplastic and neoplastic cells. The finding of a high

frequency of A:T base pair point mutations as part of the FA mutational spectrum in a human cell line exposed to FA[63] is inconsistent with the presence of point mutations occurring only at G:C base pair at p53 in FA induced SCC. The lack of p53 point mutations at A:T base pair in FA induced SCC suggests an indirect mechanism of genotoxicity resulting from FA exposure and not a direct effect of FA on the cellular genome.

Accumulation of p53 protein has been observed in many human tumours.[20 64–68] Increased levels of mutant p53 protein were detected in the serum of asbestos exposed patients with lung cancers with known intracellular alterations in p53 as well as in the serum of asbestos exposed patients who subsequently developed lung cancer.[22 23] Other studies have shown a strong statistically significant correlation between serum levels of p53 protein as determined by the pantropic and mutant specific ELISAs and the amount of p53 protein in corresponding lung tumour tissue as determined by immunohistochemistry.[69]

Furthermore, tissue studies also suggested that changes in p53 may occur early in the carcinogenic process in some cases of lung cancer. For example, in one study, p53 gene mutation with concomitant accumulation of the mutant p53 protein was detected in a squamous cell carcinoma of the lung and in the adjacent dysplastic epithelium.[68] Increased amounts of serum p53 were identifiable years prior to the development of clinically detectable cancer.[70] This is also consistent with studies of other cancers. For example, plasma p53 levels were increased in patients with colon cancer and in patients with premalignant polyps.[71]

Over expression of mutant p53 may be detected in vivo via the identification of increased amounts of the protein in the sera of some persons exposed to environmental carcinogens before the detection of clinical disease.[23] Brand-Rauf and colleagues[72–74] have reported increased levels of several oncoproteins in healthy workers with occupational exposure to numerous chemicals. They concluded that serum oncoproteins might be useful biomarkers for detecting the early effect of carcinogens in molecular epidemiological studies. In the study of Hanaoka and colleagues,[75] the workers who had had a long duration of exposure to hexavalent chromium compounds and who were thus presumed to be at high risk for lung cancer, showed high levels of serum pantropic p53 proteins. If exposure duration actually reflects the degree of exposure (that is, an increased opportunity for gene damage), this finding would suggest that the level of circulating p53 proteins is an early effect marker and thus is a possible indicator of cancer risk. Mutations of tumour suppressor genes are considered to play important roles in the process of multistage carcinogenesis.[76–79] These studies strengthen our finding of p53 protein increase in the serum of the exposed worker.

The relation between DPC and mutation has not yet been examined in the same cell types in vitro and in humans. Craft and colleagues,[80] in their in vitro studies on human lymphoblasts, found that the shape curve for induction of mutation and DPC were qualitatively similar. Merk and Speit[42] did not find a relation between DPC and mutation at the HPRT locus using V79 Chinese hamster cells, as they did not find gene mutation at the HPRT locus following exposure to FA. These results are in contrast to other in vitro studies which found induction of HPRT mutation by FA in Chinese hamster cell lines[63] and in V79 cells.[81] It has been proposed that FA might induce deletation mutation,[12 51 52] and the negative result of Merk and Speit[42] might be due to difficulties in the test used.

Although laboratory studies (in vitro and animal studies in vivo) provide a basis for extrapolation to humans, the final conclusion must be based on human studies. Our results strengthen the in vitro and animal studies,[51 52 80] and our results suggest that DPC and mutation in p53 may represent steps in FA carcinogenesis and a possible causal relation between DPC and mutation in p53. These biomarkers can be applied in the assessment of the development of cancer due to FA exposure.

Main messages

- DPC and increased p53 protein may represent steps in FA carcinogenicity and a possible causal relation between DPC and mutation in p53.
- Our finding may represent biological plausibility for the increased risk of cancer found in epidemiological studies.

Policy implication

- DPC can be applied in the follow up programme (secondary prevention) of workers exposed to FA in order to identify high risk populations.

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