Formaldehyde Damage to DNA and Inhibition DNA Repair in Human Bronchial Cells

Abstract. Cultured bronchial epithelial and fibroblastic cells from humans were used to study DNA damage and toxicity caused by formaldehyde. Formaldehyde caused the formation of cross-links between DNA and proteins, caused single-strand breaks in DNA, and inhibited the resealing of single-strand breaks produced by ionizing radiation. Formaldehyde also inhibited the unscheduled DNA synthesis that occurs after exposure of cells to ultraviolet irradiation or to benzo[a]pyrene diolepoxide but at doses substantially higher than those required to inhibit the resealing of x-ray-induced single-strand breaks. Therefore, formaldehyde could exert its mutagenic and carcinogenic effects by both damaging DNA and inhibiting DNA repair.

Formaldehyde (HCHO) is a highly reactive chemical (1) that is genotoxic in several species (2) including the rat, in which it is a respiratory carcinogen (3). The chemical is a common environmental pollutant occurring in tobacco smoke, consumer products, many occupational settings, and gasoline and diesel exhaust (4). It is also formed endogenously in the cell during demethylation reactions and during the metabolism of N-nitosodimethylamine (5) and certain drugs (6). Exposure to HCHO has a variety of pathobiological consequences [for reviews, see (2, 4)].

The potential carcinogenic hazard of HCHO in the human respiratory tract is currently being debated (7). Since its effects on the respiratory tract are essentially unknown, we evaluated its ability to kill and to damage the DNA of human bronchial cells grown as clones in a serum-free medium as described (8). We compared the effects of HCHO on epithelial cells and fibroblasts that originated from the same bronchial specimen, and determined whether HCHO could interfere with the repair of DNA that had been damaged by other genotoxic agents. The repair mechanisms studied were (i) the rejoining of x-ray-induced DNA single-strand breaks and (ii) the unscheduled DNA synthesis induced by ultraviolet radiation or (+)-(7β,8α)-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE). We also investigated the direct effects of HCHO on DNA including the formation of DNA-protein cross-links and DNA single-strand breaks.

Cells exposed to HCHO developed both DNA protein cross-links and single-strand breaks. Using methods previously described (9), we found that the number of DNA protein cross-links induced by 100 μM HCHO in epithelial cells and fibroblasts was similar (0.65 and 0.83 unit, respectively), and that the frequency of these cross-links was proportional to the concentration of HCHO. The rate at which these cross-links were removed was also similar in the two cell types; the half-removal time was approximately 2 hours (data not shown). This is consistent with results obtained in mouse L1210 cells (10). Formaldehyde-induced DNA single-strand breaks were also detected after removal of the cross-links with proteinase K. The frequency of DNA single-strand breaks induced by 500 μM HCHO was 4.2 per 10^10 daltons in epithelial and 3.5 per 10^10 daltons in fibroblastic cell
Effect of HCHO on the repair of x-ray-induced DNA single-strand breaks in human bronchial epithelial cells and fibroblasts. [2-\textsuperscript{3}H]Thymidine (Ci; 22 mCi/mmmole) was added to growing cells for 3 days and then replaced with medium containing 0.1% of the final concentration of HCHO before the experiment. Formaldehyde (Fisher certified acs;) was added to 5 mM in distilled water immediately before treatment with HCHO at concentrations up to 1 mM. A internal standard ([\textsuperscript{3}H]thymidine-labeled L1210 cells that received 300 rad at 4°C) was added to each test tube. An estimate of the frequency of single-strand breaks was quantitated from the relative elution (RE) of DNA which was derived from the curve of log RE vs. log Rf, where Rf, and Rf, represented the fraction of DNA retained on the filter in untreated and HCHO-treated cells, respectively.

To quantify the frequency of single-strand breaks, we used an apparatus that measured the RE of DNA in the eluting solution completely removed the DNA protein cross-linking HCHO at concentrations up to 1 mM. An internal standard ([\textsuperscript{3}H]thymidine-labeled L1210 cells that received 300 rad at 4°C) was added to each test tube. An estimate of the frequency of single-strand breaks was quantitated from the relative elution (RE) of DNA which was derived from the curve of log RE vs. log Rf, where Rf, and Rf, represented the fraction of DNA retained on the filter in untreated and HCHO-treated cells, respectively.

The frequency of single-strand breaks was calculated by comparing the RE when 27 percent of the tritiated internal standard DNA was removed from the solution (12 hours of elution) to that produced in L1210 cells after 300 rad of x-rays, assuming a single-strand break efficiency of 10\textsuperscript{-15} per rad per dalton. The absorbance represents a corrected time scale obtained from [\textsuperscript{3}H]thymidine-labeled L1210 cells that had received (A) Fibroblasts. (B) Epithelial cells. Symbols: ○, control cells; ●, cells exposed to 100 mM HCHO for 1 hour; □, cells exposed to 800 rad after reseal in a fresh medium at 37°C; ▲, cells exposed to 800 rad and then incubated for 1 hour in fresh medium at 37°C; △, cells exposed to 800 rad and incubated for 1 hour in medium containing 100 mM HCHO.

The effect of HCHO on the repair of x-ray-induced single-strand breaks was studied in experiments with confluent fibroblasts. In the presence of hydroxyurea, an inhibitor of semiconservative DNA synthesis (12), HCHO alone caused no significant increase in unscheduled DNA synthesis (Fig. 2). As expected, exposure to ultraviolet radiation and to BPDE caused significant amounts of such synthesis. The most interesting finding was that 100 mM HCHO did not inhibit the unscheduled DNA synthesis induced by ultraviolet radiation or BPDE. Substantially higher concentrations of HCHO, 600 and 1000 mM, were required to significantly inhibit such synthesis. In contrast, 100 mM HCHO inhibited the repair of x-ray-induced single-strand breaks. This finding indicates that the ligation step of excision repair may be preferentially sensitive to HCHO.

A number of mechanisms may be involved in the inhibition of DNA repair by HCHO. The high reactivity of the chemical probably causes methylation of chromatin or other proteins including enzymes critical to DNA repair processes. The potentiating effect of HCHO on the cytotoxicity of x-rays may depend on interaction with enzymes critical for repair.

Fig. 2. The effect of HCHO on unscheduled DNA synthesis induced by ultraviolet radiation and BPDE in confluent bronchial fibroblasts. Cells (2 \times 10	extsuperscript{4}) were incubated into 60-mm	extsuperscript{2} dishes in LHC-1 and medium 199 (1:1 by volume) and grown in the presence of [\textsuperscript{3}H]thymidine for 48 hours to uniformly label the cellular DNA. The dishes were left for another 48 hours until the cells had just reached confluency. The cells were then incubated with 10 mM hydroxyurea for 30 minutes to inhibit semiconservative DNA synthesis (11). With the hydroxyurea still present, [\textsuperscript{3}H]thymidine (4 \muCi/ml, 80 Cl/mmol) was added and the cells were exposed to ultraviolet radiation (10 J/m	extsuperscript{2}) or BPDE (5 \mug/ml) added from a stock solution of 5 mg/ml in tetrahydrofuran and then incubated for 1 hour at 37°C. The medium was subsequently removed and the cells were washed five times with cold PBS, removed from the plate in PBS containing 15 mM EDTA with a rubber policeman, precipitated with 7.5% trichloroacetic acid (TCA), and collected on 0.3 \muE filters (Millipore PHWP). The filters were washed twice with cold 5% TCA and twice with ice-cold 95% ethanol. The precipitate on filters was heated at 70°C for 1 hour in 0.1 N HCl, and the amount of unscheduled DNA synthesis was estimated from incorporation of [\textsuperscript{3}H]thymidine. The yield of DNA and the number of cells were quantitated from [\textsuperscript{3}H]thymidine incorporation as measured by spectroscopy. Symbols: ●, cells exposed to HCHO alone; ▲, cells exposed to BPDE (5 \mug/ml); ○, cells exposed to ultraviolet radiation (10 J/m	extsuperscript{2}).
pair of x-ray-induced DNA damage. The comparatively low concentrations (100 μM) that decrease such repair are comparable to the effective levels of certain potent carbamylating and alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (13) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (14). Millimolar concentrations of dimethylsulphate are required to inhibit excision repair of ultraviolet radiation-induced DNA damage (15).

The critical factors determining the concentration of HCHO that can be safely handled by the cell remain to be identified. Exogenous HCHO will readily react with respiratory mucus and the exterior surface of the target cells so that only a small fraction of the HCHO reaches the nucleus. Since HCHO is formed endogenously in the cell during demethylation reactions, cells must maintain pathways for its detoxification. Many xenobiotics are also metabolized by demethylation reactions. Formaldehyde is metabolized to formaldehyde, but this agent did not cause the formation of DNA single-strand breaks or affect the repair of such breaks induced by x-rays (data not shown).

Since HCHO damages DNA, inhibits DNA repair, and potentiates the cytotoxicity of x-rays in human bronchial epithelial cells, and since the HCHO may act in concert with physical and chemical agents to produce toxic, mutagenic, and carcinogenic effects (11), we suggest that the mutagenic and carcinogenic effects of this chemical alone or in combination with other agents should be further investigated.

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References and Notes