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 t e resealing of x-ray–induced single-strand breaks. Therefore, formaldehyde cou l 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*-nitrosodimethylamine (*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 DN \ protein cross-links and DNA singl 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 rat at which these cross-links were remove 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 in human bronchial epithelial cells and fibroblasts. [2-^{14}C]Thymidine μCi; 52 mCi/mmole) was added to growing cells for 3 days and then 1 to 2 days before the experiment. Formaldehyde (Fisher certified acs; by weight) was diluted to 5 mM in distilled water immediately before added to cells (5×10^4 to 10×10^4) in serum-free LHC-1 medium (8). treatment for 1 hour, the cells were rinsed twice with cold phosphate- saline (PBS). The cells were removed at 4°C with a rubber policeman into a solution of Dulbecco's Ca^{2+} and Mg^{2+}-free PBS containing 15 EDTA. The alkaline dilution technique was used to assess DNA damage. DNA single-strand breaks the cells were collected onto polycarbon- (2 μm pore size; Nucleopore), lysed with 2 percent sodium dodecyl (SDS) and 0.1M glycine, and 0.02M EDTA (pH 9.6); 2 ml of the same containing Proteinase K (0.5 mg/ml) was then put through each filter at min. EDTA (0.02M; acid form), 0.1 percent SDS, and tetrapropyl hydroxide were then added in the amount required to give a pH of eluted fractions were collected and assayed for radioactivity. The combi-

of the polycarbonate filters, Proteinase K digestion, and SDS in the eluting solution completely removed the DNA protein cross-linking of HCHO at concentrations up to 1 mM. An internal standard ([^{3}H]thymidine-labeled L1210 cells that received 300 rad at 4°C) was included in assay. An estimate of the frequency of single-strand breaks was quantitated from the relative elution (RE) of DNA which was derived $\log R_0 - \log R_F$, where R_0 and R_F represented the fraction of DNA retained on the filter in untreated and HCHO-treated cells, respectively. frequency of DNA single-strand breaks was calculated by comparing the RE when 27 percent of the tritiated internal standard DNA was on the filter (12 hours of elution) to that produced in L1210 cells after 300 rad of x-rays, assuming a single-strand break efficiency of 10^{-12} per rad per dalton. The abscissa represents a corrected time scale obtained from [^{3}H]thymidine-labeled L1210 cells that had received . (A) Fibroblasts. (B) Epithelial cells. Symbols: O, control cells; ●, cells exposed to 100 μM HCHO for 1 hour; □, cells exposed to 800 rad subsequent incubation; △, cells exposed to 800 rad and incubated for 1 hour in fresh medium at 37°C; ▲, cells exposed to 800 rad and in- for 1 hour in medium containing 100 μM HCHO.
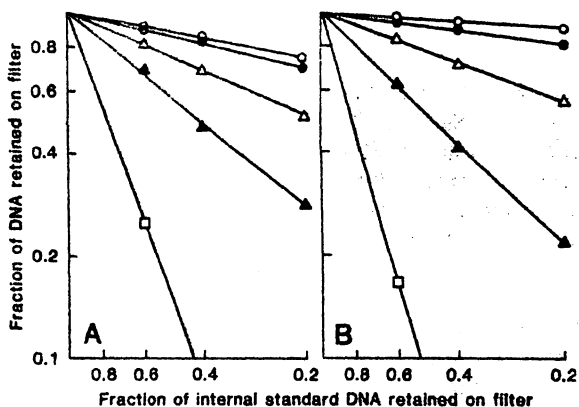
was dose-dependent. The removal of the single-strand breaks was also in the two cell types (data not .

relation between exposure to and the resealing of DNA single- breaks induced by x-rays is pre- in Fig. 1. We showed previously that epithelial cells and fibroblasts reseal x-ray–induced single- breaks at comparable rates. Ex- of cells to 800 rad resulted in a frequency of single-strand breaks rapid elution of DNA. Cells incu- for 1 hour after exposure to x-rays a substantial decrease in the number of breaks. Cells exposed to 100 HCHO showed insignificant num- of breaks, but the resealing rate of x-ray–induced breaks was substan- decreased in both cell types. Re- was also inhibited if the cells were with HCHO for 1 hour, then to x-rays, and then incubated in HO-free medium. Therefore, the ef- of HCHO persisted for at least 1

then bronchial cells were exposed to and HCHO simultaneously a syn- effect was observed (11). Signifi- potentiation of the toxicity of x-rays with 100 μM HCHO and 400 and with 300 μM HCHO and 200 and rad. Only slight toxicity was ob- in cells exposed to 100 and 300 HCHO alone (76 and 56 percent forming efficiency of untreated respectively).

also investigated the mechanism of of unscheduled DNA synthe- by HCHO in confluent fibroblasts. effects of ultraviolet irradiation and

BPDE were studied in experiments with confluent fibroblasts. In the presence of hydroxyurea, an inhibitor of semiconser- vative DNA synthesis (12), HCHO alone caused no significant increase in un- scheduled DNA synthesis (Fig. 2). As expected, exposure to ultraviolet radia- tion and to BPDE caused significant amounts of such synthesis. The most interesting finding was that 100 μM HCHO did not inhibit the unscheduled DNA synthesis induced by ultraviolet radiation or BPDE. Substantially higher concentrations of HCHO, 600 and 1000 μM, were required to significantly inhib-

it such synthesis. In contrast, 100 μM HCHO inhibited the repair of x-ray– induced single-strand breaks. This find- ing indicates that the ligation step of excision repair may be preferentially sensitive to HCHO.

A number of mechanisms may be in- volved in the inhibition of DNA repair by HCHO. The high reactivity of the chemi- cal probably causes methylolation of chromatin or other proteins including enzymes critical to DNA repair process- es. The potentiating effect of HCHO on the cytotoxicity of x-rays may depend on interaction with enzymes critical for re-

Fig. 2. The effect of HCHO on unscheduled DNA synthesis induced by ultraviolet radia- tion and BPDE in confluent bronchial fibro- blasts. Cells (2×10^5) were inoculated into 60-mm^2 dishes in LHC-1 and medium 199 (1:1 by volume) and grown in the presence of [2-^{14}C]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 incu- bated with 10 mM hydroxyurea for 30 minutes to inhibit semiconservative DNA synthesis (11). With the hydroxyurea still present, [^{3}H]thymidine (4 μCi/ml, 80 Ci/mmole) was added and the cells were exposed to ultravio- let radiation (10 Jm^{-2}) or BPDE (5 μg/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 re- moved and the cells were washed five times with cold PBS, removed from the plate in PBS containing 15 mM EDTA with a rubber po- liceman, precipitated with 7.5 percent tri-

chloroacetic acid (TCA), and collected on 0.3 μm filters (Millipore PHWP). The filters were washed twice with cold 5 percent TCA and twice with ice-cold 95 percent ethanol. The precipitate on filters was heated at 70°C for 1 hour in 0.1N HCl, and the amount of unscheduled DNA synthesis was estimated from incorporation of [^{3}H]thymidine. The yield of DNA and the number of cells were quantitated from [^{14}C]thymidine incorporation as measured by spectros- copy. Symbols: ■, cells exposed to HCHO alone; ▲, cells exposed to BPDE (5 μg/ml); ●, cells exposed to ultraviolet radiation (10 Jm^{-2}).
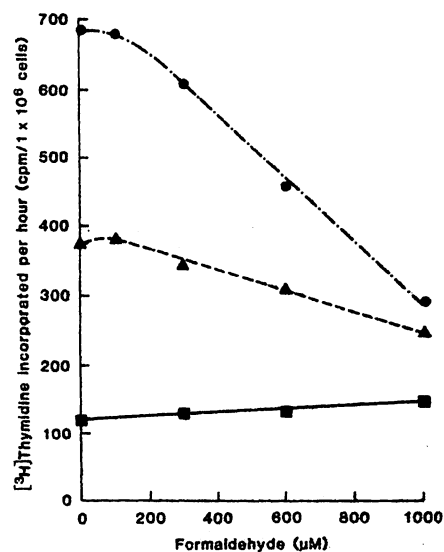
pair of x-ray–induced DNA damage. The comparatively low concentrations (100 μM) that decrease such repair are comparable to the effective levels of certain potent carbamylating and alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (13) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (14). Millimolar concentrations of dimethylsulfate 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 formate, 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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