IMPACT OF COAL DUST IN GASTRIC CANCER AND DEFENSE MECHANISMS

FINAL REPORT

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Epidemiological studies suggest that there is an elevated incidence of gastric cancer mortality among coal miners (1). Although the actual etiology of gastric cancer in coal miners is not known, coal mine dust exposure has been suggested as a possible gastric cancer risk (2). Recently, we have hypothesized that coal mine dust is introduced into the stomach of coal miners through food ingestion, swallowing of air, or inhalation followed by lung clearance. Coal mine dust per se is either noncarcinogenic or weakly carcinogenic. However, carcinogenic compounds may be formed from coal dust in the stomach through enzymatic or nonenzymatic nitrosation processes or through other interactions with exogenous chemicals. These carcinogenic compounds may attack epithelial cells, induce precancerous lesions in the stomach and may be responsible for the increased gastric cancer incidence in coal miners (3). It has been documented that there is a good correlation between mutagenicity and carcinogenicity. Since somatic mutation may be responsible for the initiation of carcinogenesis, the hypothesis for gastric cancer in coal miners can be tested with the mutagenesis assay system.

Interferons are genetically inducible proteins of cellular origin in response to viral and nonviral microorganisms and other diverse substances (4). The interferon system is an important constituent part of the host's cellular defense mechanisms. In addition, the sensitive nature of the interferon induction process offers a useful assay, based on inhibition of interferon synthesis, for evaluating the carcinogenic potential of chemicals and particulates of public health concern. Using mammalian cell cultures, adverse activity on interferon induction has been reported with several known carcinogens and environmental agents (5,6). Additionally, such occupational-related dusts and particulates shown to affect detrimentally this biologic entity include: asbestos, coal, lignite fly ash and diesel emissions (7,8,9,10).

A compilation of recent experimental data revealed a close correlation between mutacarcinogens and their ability to inhibit interferon production (5).

The cytochrome P450 complex is also a cellular defense system that detoxifies xenobiotics by catalyzing the biotransformation of the toxic substances to less- or non-toxic compounds (11). In some cases, however, reactive intermediates are formed that bind to cellular components and cause cell damage (12). Cytochrome P450 activities can be induced or inhibited by a variety of chemical substances and this system has been recognized as an important component of environmental/occupational health studies (13).

Although cytochrome P450-mediated metabolism of xenobiotics and interferon-mediated resistance to infectious agents are distinct biological activities, correlations between them have been observed. Known inducers of interferon activity depress cytochrome P450 enzyme activities in a number of systems (14,15) and also inhibit induction of the P450 complex (16). Conversely, carcinogenic polycyclic aromatic hydrocarbons cause decreased antiviral activity (17) under conditions of metabolic activation (18).

The objectives of this project are: (A) to test the hypothesis that mutagenic and potential carcinogenic substances are generated from the reaction of swallowed coal dust and other exogenous factors (e.g. sodium nitrite, chewing tobacco, etc.) in the stomach environment and are risk factors for gastric cancer in coal miners, (B) to determine the effect of various extracted chemical components inherent to coal <u>per se</u> and those obtained by nitrosation on the interferon system, (C) to identify those components exhibiting adverse activity on the interferon induction process with those having mutagenic activity in an effort to correlate and elucidate underlying mechanisms that may be involved in the pathogenesis of gastric disease in relation to coal dust, and (D) to study the relationship between xenobiotic metabolism and antiviral activity in mice exposed to coal dust.

The detailed methodologies and results were described and/or discussed in the publications. Descriptions of the significant findings are as follows:

A. Genotoxicity assays

Using the Ames/Salmonella mutagenicity assay system (19), studies on the mutagenicity of different ranks of coal dust were conducted. Coal dust solvent extracts were either non-mutagenic or very weakly mutagenic with metabolic activation. However, high mutagenic activity was found when extracts of bituminous, subbituminous, and lignite coal dust were reacted with nitrite, the chemical often found in the stomach, under acidic conditions. Formation of mutagenic substances from coal dust extracts treated with nitrite was found to be dependent on acidic pH with the highest mutagenicity occurring at pH 3.2. The mutagenicity of coal dust plus nitrite was inhibited by ascorbic acid (Vitamin C), an effective nitrosation inhibitor, suggesting that mutagens formed from coal dust extracts might be due to nitrosation by nitrite generating mutagenic nitroso compounds. The mutagenic activity of coal dust extract plus nitrite was independent of metabolic activation and induced frameshift mutations. A fractionation study showed that only acidic and PNA fractions were mutagenic. The nitrosated coal dust extracts were further separated by thin-layer chromoatography. The mutagenic activity was found in two major peaks (Rf 0 and Rf 0.7). The activity in Rf 0.7 appeared to be higher than that in Rf 0. Studies with mammalian systems showed that nitrosated coal dust extracts induced gene mutations in mouse lymphoma cells, sister chromatid exchanges (SCE) in CHO cells and human peripheral lymphocytes (HPL) and chromosome aberrations in HPL. Extracts also caused SCE in bone marrow cells of mice in an <u>in vivo</u> study.

Studies were also conducted to determine the mutagenic effect of smokeless tobacco, the substance used by miners as a substitute of cigarette smoking,

and the interaction between tobacco snuff and coal dust in relation to mutagenicity. No mutagenic activity was found with tobacco snuff extract using the Ames/Salmonella assay system. However, mutagenic substances were formed from tobacco snuff extracts in an acidic environment. The mutagenic substances induced predominantly frameshift mutations and were direct-acting mutagens. The mutagenic activity of snuff was enhanced in the presence of coal dust extracts at low pH. The enhancement of snuff mutagenicity by coal dust extract was found only with polar solvent extracts of snuff. This acid-mediated mutagenicity was also observed in snuff-water extracts. The kinetic study of snuff mutagenicity as a function of acid-treatment time showed that snuff mutagenicity was obtained as early as 3 min and reached the plateau after 2 h treatment. These studies indicated that the stomach is an optimal environment for producing maximum mutagenicity of snuff. Formation of nitroso compounds and the mutagenic activity of snuff under acidic conditions was inhibited by ascorbic acid (a nitrosation inhibitor). The results indicate that the acid-mediated mutagenicity of snuff was probably due to nitrosation of snuff by nitrite under acidic conditions generating mutagenic nitroso compounds. Studies on the source of nitrite found in snuff showed that snuff contained bacteria which were able to reduce nitrate to nitrite and that the amount of nitrite in snuff extracts could be further increased by incubation of the extracts with the bacteria.

The mutagenicity of chewing tobacco was also studied with the Ames/Salmonella assay system. The mutagenic activity of chewing tobacco extracts (CTE) was observed only after being treated with nitrite under acidic conditions. Both organic solvent and water extracts of chewing tobacco showed mutagenic activities with or without metabolic activation only with nitrite

treatment. The nitrite-mediated mutagenicity of CTE also was dependent on acidic pHs with the highest mutagenicity noted at pH 2. A dose-related decrease in the mutagenicity of CTE plus nitrite by ascorbic acid was observed. The nitrite-mediated mutagenicity of CTE was proportional to the content of nitroso compounds generated in the reaction mixture, indicating that the nitrosation process might also be involved. It is conceivable, therefore, that the etiology of gastric cancer in coal miners may involve a number of factors. Results from this study imply that in addition to coal dust, tobacco snuff or chewing tobacco may also play a causal role in the elevated risk of gastric cancer in coal miners.

Antimutagenicity studies with chlorophyllin showed that chlorophyllin inhibits over 80% of the mutagenic activity of coal dust, tobacco snuff and chewing tobacco. These results indicate that chlorophyllin may be useful for the prevention of gastric cancer in coal miners.

B. Interferon - Mutagenesis Studies

The focus of interferon - mutagenesis research was directed to the effect of coal dust, extracts thereof, and nitrosated solvent extracts on a mammalian cellular defense mechanism, the interferon system, in conjunction with mutagenic activity of these substances using the Salmonella/microsome assay. Our studies revealed that coal dust per se in the amount from 1 to 5 mg inhibited viral induction of interferon by more than 60% in mammalian (LLC-MK_2) cell monolayers. Polar and non-polar solvent extractions of coal dust using either dichloromethane (DCM) or methanol + acetone (M+A) were significantly ($P < 0.05$) inhibitory for the interferon induction but only in the presence of a metabolic activating agent (rat liver S9). Without S9 activation, minimal inhibition of interferon induction occurred. It would appear, therefore, that the solvent extracted chemical compounds require

metabolic activation to form metabolites capable of adversely affecting the interferon system. When DCM and M+A coal extracts were combined in equal proportion, nitrosated with sodium nitrite and adjusted to pH 3.5, the nitrosated coal dust extract, depending on concentration, inhibited interferon induction by more than 70% in the presence of S9 and approximately 60% without S9 activation. Overall, this was greater than that by either DCM or M+A extracts alone.

Nitrosated coal dust extract was further extracted with horse serum, a protein to which some carcinogenic heavy metals and polynuclear aromatic hydrocarbons may be bound. A metal chelator, EDTA, was added to the serum extract to test the hypothesis that EDTA had chelated metals complexed with serum proteins. Both EDTA-treated and saline-treated horse serum extracts of nitrosated coal dust were fractionated on a Sephadex PD-10 column with a cutoff at 25,000 daltons. Collected fractions of which there were three for each extract, were assessed for their effect on viral interferon induction and mutagenic activity. Results revealed significant and equivalent inhibitory activity for interferon induction by all horse serum-EDTA and horse serum-saline fractions indicating that adverse chemical substances were complexed with serum proteins but they did not necessarily contain metals. Results were similar with or without S9 activation. Overall, the findings were comparable to those noted for mutagenic activity by these fractions.

Therefore, it would appear that nitrosated coal dust extract at acidic conditions contained substances highly mutagenic and inhibitory for interferon induction. Fractionation of nitrosated coal dust extract indicated that serum proteins were not complexed with heavy metals but may be complexed with mutagenic chemical compounds which are also capable of inhibiting a cellular defense mechanism, interferon induction. These findings add further

credibility to the belief that nitrosated coal dust may play a role in gastric carcinogenesis.

C. Cytochrome P450 Enzyme -- Virus Infection Studies

The specific activities of the two liver P-450 monooxygenases, 7-ethoxycoumarine deethylase (7ECdeEt'ase) and ethylmorphine demethylase (EMdeMe'ase), and NADPH cytochrome c reductase (NADPH c red'ase) from one-month-exposed animals were found to be affected by influenza virus infection of control mice. A property shared by EMdeMe'ase and NADPH c red'ase is the increased value on Day 4. 7ECdeEt'ase activity decreased after the initial time but appeared to return to the original value by 4 days after viral exposure. No changes in liver microsomal protein content were observed throughout the 8-day interval.

The effect of the 1-month preexposure to CD particulates on the influenza virus-induced alterations in enzyme activities was dependent on the activity. The decreased 7ECdEt'ase activity observed on Days 1-3 in control mice was not significantly changed as a result of exposure to CD. The virus-induced temporal pattern for EMdeMe'ase, on the other hand, was absent in the exposed mice. The Day 4 postinfection increase in NADPH c red'ase was also abolished by the 1-month preexposure to CD particulates. Protein content of liver microsomes was unaffected by the preexposure to CD. Without virus infection, no exposure-related changes in 7ECdeEt'ase, EMdeMe'ase, and NADPH cyto c red'ase activities were observed.

One out of the three activities exhibited a temporal pattern in control mice (6-month exposure to FA) following infection with influenza-virus. EMdeMe'ase specific activity decreased from an initial value of 17.6- to a value of 7.7 nmoles/min/mg protein at day-3 and returned to the original value

by day-4. Exposure to the CD particulates, however, abolished the influenza-virus-induced temporal pattern. This effect determined by a one-way analysis of variance, was confirmed when the data were analyzed by a two-way analysis of variance. No temporal patterns were observed with ECdeEt'ase or NADPH c red'ase activities following a 6-month exposure to FA or CD. The estimated enzyme specific activity values indicate an absence of exposure-related changes in 7ECdeEt'ase and NADPH c red'ase activities. EM'ase activity, however, exhibited a slight decrease (27%) as a result of the 6-month exposure to CD.

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bstract (Limit: 200 words)

In efforts to further define the role of exposure to coal dust in subsequent evelopment of gastric cancer, several studies have been performed and were highlighted n this report. In particular, consideration was given to the carcinogenic nature of oal dust once it has been introduced into the stomach of the miner either through nhalation, food ingestion or swallowing of air. Once in the stomach, the coal dust is cted upon by enzymatic or nonenzymatic nitrosation processes or through other nteractions with exogenous chemicals which may turn the noncarcinogenic coal dust into arcinogenic compounds. Studies on the mutagenicity of different ranks of coal dust ere conducted using the Ames Salmonella mutagenicity assay system. High mutagenic ctivity was found when extracts of bituminous, sub bituminous, and lignite coal dust ere reacted with nitrite, a chemical found in the stomach, under acidic conditions. tudies on the effect of coal dust and its extracts on the interferon system indicated nat nitrosated coal dust extracts at acidic conditions contain substances which are ighly mutagenic and inhibitory for interferon induction. Virus infection studies were erformed with the cytochrome-P-450 enzyme in efforts to understand the relationship etween xenobiotic metabolism and antiviral activity in mice exposed to coal dust.

ocument Analysis a. Descriptors

Identifiers/Open-Ended Terms

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