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Original Contribution

HYDROXYL RADICAL GENERATION BY COAL MINE DUST: POSSIBLE IMPLICATION TO COAL WORKERS' PNEUMOCONIOSIS (CWP)

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Abstract—Occupational exposure to coal mine dust causes coal workers' pneumoconiosis (CWP) and other pulmonary diseases by mechanisms that remain unclear. Because the hydroxyl radicals ('OH) may play an important role in the pathogenesis of CWP, we studied the potential role of bituminous coal mine dust samples for catalyzing the generation of 'OH from hydrogen peroxide (H_2O_2). These coal mine dusts evaluated represented two geographic areas with diversity in CWP prevalence. Electron spin resonance (ESR), with the aid of spin trapping techniques, was used to measure the 'OH radical generation. Bituminous coal mine dusts representing the Pittsburgh seam in the eastern United States and Blind Canyon seam in the mid-western United States were used together with a standard coal dust obtained from the National Institute of Standards and Technology, Gaithersburg, MD. All the coal mine dust samples generated varying levels of 'OH radicals from H_2O_2 in the presence of a 'OH spin trap 5,5,-dimethyl-1-pyrroline-N-oxide (DMPO). 'OH radical generation by the coal from H_2O_2 was effectively inhibited by deferoxamine and catalase, but only partially inhibited by superoxide dismutase. Metal chelators DETAPAC and EDTA enhanced the radical generation. These results indicated that the Fenton reaction is predominantly involved in the generation of 'OH radicals from H_2O_2. The 'OH-generating potential of all the coal dusts showed a positive correlation with the surface iron content of coal mine dusts. In addition, the potential to induce lipid peroxidation by the coal samples exhibited a good correlation with the available surface iron. Based on the results presented here, we propose that higher concentrations of surface iron in coal mine dust may be involved in the generation of increased levels of 'OH radicals and may play an important role in the development of CWP in different coal mining areas.

Keywords—Coal mine dust, Pneumoconiosis, Hydroxyl radicals, Hydrogen peroxide, Electron spin resonance, Free radicals

INTRODUCTION

Occupational exposure to coal mine dust leads to the development of a wide spectrum of pulmonary diseases of which coal workers' pneumoconiosis (CWP) continues to be a major concern.[1] Despite intensive research over several decades, the major factors contributing to the development of CWP remain unclear.[2,3] Coal mine dust is a complex mixture containing different proportions of minerals, trace metals, and organics with different grades of coal particulates.[4,5] In addition to the type of coal, variations in the coal seam and the mining methods affect the composition of coal mine dust.[6]

During the early part of this century, coal mine dust was considered innocuous, and pulmonary disease in coal miners was attributed to the presence of silica in coal mine dust. In 1928, however, Collis and Gilchrist[7] showed convincing evidence for the development of CWP in the lungs of coal trimmers who shoveled coal that contained little or no silica. It was also shown that graphite and carbon electrode workers exposed to carbonaceous materials free of silica develop pulmonary lesions morphologically similar to CWP.[7] Long-term epidemiologic studies on British coal miners revealed that progression of simple CWP correlated directly with the mean mass concentration of coal dust in the mining atmosphere.[8] Although these studies did not preclude the role of silica in the pathogenesis of CWP,[9] they implied that coal mine dust is an important factor in the development and progression of CWP. Furthermore, geographic variations in the CWP risk between collieries, even with comparable exposure and no correlation to quartz content, implied that coal mine dust plays a significant role in the development of CWP.[10–14]

We previously reported the presence of coal-based, carbon-centered free radicals in freshly fractured coal mine dust and in freeze-dried autopsied coal miners' lungs.[15–17] Although this type of coal stable radical

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entrapped in the coal mine dust might not be available for biologic reactions, the potential for oxygen radical generation during biologic reactions could be considered as a new parameter to predict the toxicity of different coal mine dusts. This hypothesis was considered plausible in light of new evidence suggesting that when oxygen radicals, generated by the inherent potential of minerals such as freshly fractured silica,[15,18-24] asbestos,[21,25-30] and other inorganic minerals,[21,33] are coupled with phagocytic events, they can overwhelm the antioxidant defenses to initiate cell injury and disease process.[21,34] This study was undertaken with the working hypothesis that oxygen radical generation by coal mine dust may play an important role in the development and progression of CWP. In an attempt to correlate such radical generating potential with prevalence data on CWP, we obtained coal mine dusts from two geographic areas representing a diverse prevalence of CWP. In addition, we evaluated coal mine samples from four different mining areas of the Pittsburgh seam to determine the differences within seams.

As reported earlier for H_2O_2/silica reactions,[18-21] we used electron spin resonance (ESR) spin trapping as an indirect spectroscopic technique for monitoring $^{\bullet}OH$ radical generation by H_2O_2/coal dust mixtures. In this method,[35] the $^{\bullet}OH$ radical is detected by the ESR spectrum of the stable radical adduct formed as a result of the reaction of the $^{\bullet}OH$ radical with a spin trap molecule. For the radical detection, the spin trap used in this study was 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The characteristic 1:2:2:1 hyperfine quartet of the DMPO-OH adduct in conjunction with the use of $^{\bullet}OH$ radical scavengers serves as the fingerprint for the $^{\bullet}OH$ radical identification, whereas the signal intensity provides a measure of the quantity of the radicals generated.[35,38]

To investigate the chemical mechanism of $^{\bullet}OH$ generation by the coal mine dust, we measured surface iron and trace metals cobalt, chromium, copper, and zinc, which are known to influence the radical production. To address the question of whether the divergent potential for $^{\bullet}OH$ radical generation by the coal samples is correlated with any biologic toxicity, we investigated the lipid peroxidation potential induced by coal dust. The results obtained provide the first indirect evidence for $^{\bullet}OH$ radical generation by coal mine dust and its possible correlation to the prevalence of CWP.

MATERIALS AND METHODS

Chemicals

Catalase, diethyletriaminepentaacetic acid (DETAPAC), hydrogen peroxide (H_2O_2), and deferoxamine mesylate and superoxide dismutase (SOD) were purchased from Sigma Chemical Co., St. Louis, MO. The spin traps 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and phenyl-N-butylnitrone (PBN) were obtained from Aldrich Chemical Co., Milwaukee, WI. DMPO was purified prior to use with activated charcoal according to the procedure described by Buettner[35] until the DMPO solution became practically clear and free of nitroxide radical as monitored by ESR. The purified DMPO was used in experiments after filtration and was stored at −20°C in the dark.

Coal dust samples

Five bituminous coal mine samples obtained from two different geographic regions representing low prevalence of CWP (Utah, Blind Canyon seam) and moderate to severe degree of CWP prevalence (West Virginia, Pittsburgh seam) were investigated in this study for their potential for oxygen radical generation and lipid peroxidation. In addition, measurements of surface iron and transition metal concentrations were determined. Table 1 summarizes their geographic origin and other relevant chemical characteristics.

Coal Sample 1 was obtained from western coal fields in Utah representing high volatile, low sulfur bituminous coal and represented a geographic region with low prevalence of CWP. Samples 2, 3, 4, 5, and 6 were from geographic regions with moderately high prevalence of CWP. Sample 2 was obtained from Osage, WV, which represented the Pittsburgh seam, the largest coal production seam in the eastern United States. Sample 3 was collected from Arkwright, WV, representing the Pittsburgh seam 8. Sample 4, obtained from Blacksville, WV, coal mines, also represented a part of the Pittsburgh seam. (This coal is designated as a NIOSH standard coal sample.) This coal was micronized under nitrogen using a tungsten carbide-lined jet mill and then classified to < 5 μm size with the aid of an Accucut Particle Classifier (Donaldson-Majal Division, St. Paul, MN). Coal Sample 5 was purchased from the National Institute of Standards and Technology (NIST), Gaithersburg, MD. This NIST coal dust sample labelled as SRM 1632b was obtained by NIST from the Humphrey #7 mine of Consolidation Coal Co., Osage, WV. This coal is certified as bituminous coal representing the Pittsburgh seam. As stated in the NIST certification, this coal was blended in a stainless steel cone blender. Sample 6, designated as NIOSH $<$ 10 μm standard coal, was obtained from Blacksville, WV, coal mines. This coal was processed similarly to Sample 4 and then classified to < 10 μm size with an Accucut Particle Classifier.

All the coal mine dust samples except 4 and 6 were

Table 1. Description of Coal Samples, Geographic Origins, Coal Seams, and Chemical Characteristics

Coal Sample	Geographic Origin and Seam	Coal Type Carbon Content %	Silica Content %	Total Iron %	Cobalt μg/g	Copper μg/g	Chromium μg/g	Zinc μg/g
1 (PSOC-1554)	UT—Emery Blind Canyon	Bituminous	0.81	0.35	ND	52	5	ND
2	WV—Osage Pittsburgh	Bituminous	2.41	1.4	4	64	74	ND
3	WV—Arkwright Pittsburgh	Bituminous	0.75	0.43	ND	14	10	17
4 NIOSH < 5 μm	WV—Blacksville Pittsburgh	Bituminous 87	3.1	0.34	32	33	33	33
5 NIST SRM-1632b	WV—Osage Pittsburgh	Bituminous 78	3.2	0.76	22	6	11	12
6 NIOSH < 10 μm	WV—Blacksville Pittsburgh	Bituminous 87	3.1	0.42	27	27	27	27

ND = not detected.

sieved to < 28 μm size using a mechanical siever for 2 min.

ESR spin trapping measurements

ESR spin trap method was used to measure the hydroxyl (·OH) radical generation from mixtures of coal dust and hydrogen peroxide (H_2O_2). The spin trap used was 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) at 100 mM (final) concentration. Reaction mixtures were prepared to a final volume of 1 ml with PBS buffer containing 10 mg coal dust, 100 μl of 10 mM H_2O_2, and 100 μl of DMPO. The reaction was initiated by the addition of H_2O_2 and vortexed for 10 s to react with coal dust and was separated by filtration using a 0.45 μm nylon acrodisc filter-fitted syringe. This filtration step was found to be quite important because if the coal dust was not filtered, the strong ESR signal from its carbon-centered radicals swamped the much weaker signals from the trapped ·OH radicals.

All ESR measurements were made using a Varian E109 ESR spectrometer operating at the X-band (~9.4 GHz) frequencies. For the spin-trap measurements a 250 μl flat quartz cell was used with same sample volume. Care was taken in the orientation of the flat cell in the microwave cavity in all experiments. Control experiments were carried out without dust and without H_2O_2. The microwave power and magnetic field amplitudes were optimized for maximum signal intensity without compromising resolution. A typical value for the microwave power was found to be 50 mW, and that for the modulation amplitude was about 2 G. Other conditions are listed in the figure captions.

Surface iron measurements

Surface iron was measured in all the coal samples using a spectrophotometric method described by Roth et al.[36] Briefly, the procedure involved the treatment of 10 mg of coal with 5.5 ml of 0.3 M sodium citrate and 1 M sodium bicarbonate containing 100 mg sodium dithionate in a water bath at 80°C for 30 min. The treated samples were then centrifuged at 600 xg for 10 min, and the supernatant was transferred to volumetric flasks and diluted to 100 ml. Aliquots of the clear supernatant in duplicate were treated with 1 ml 10% hydroxylamine hydrochloride and 2 ml 0.5% 0-phenanthroline for 5 min and diluted to 100 ml. Absorbance of the samples in duplicate was measured at 508 nm and converted to mg of iron from a standard calibration graph obtained by treating analytical grade ferric ammonium sulfate.

Lipid peroxidation measurements

To measure the adverse biologic effect of oxidant generation, coal samples were evaluated for the potential to induce lipid peroxidation in a simulated biologic model using polyunsaturated linoleic acid (cis-9-cis-12-octadeca-dienoic acid). Thiobarbuturic acid reactive substance formed in the reaction mixture was measured according to the method of Hunter et al.[37] The reaction mixture in a total volume of 1 ml HEPES-buffered medium containing 140 mM NaCl, 5 mM KCl, 10 mM HEPES (pH 7.4), 10 mg coal, and 10 mg emulsified linoleic acid was incubated in capped scintillation vials for 1 h in a shaking water bath at 37°C. The reaction was terminated by the addition of 0.625 ml 40% trichloroacetic acid and 0.3 ml 5 N

hydrochloric acid. Vials were vortexed for 10 s and 0.625 ml 2% thiobarbuturic acid was added and mixed. The mixture was then transferred to glass tubes and heated for 20 min at 90°C. The tubes were cooled and centrifuged for 10 min at 600 g, and the absorbance of the supernatant was measured at 535 nm. Malondialdehyde standards were prepared and treated similarly and graphed for the direct conversion of thiobarbuturic acid produced by the coal samples.

RESULTS

Hydroxyl radical generation from H_2O_2 by coal dusts

All the six coal samples exhibited similar ESR signals, with differences only in the peak intensities when treated with H_2O_2 in the presence of spin trap DMPO. Because all the coal samples were qualitatively similar in the generation of $^{\bullet}OH$ radicals from H_2O_2, we opted to present data for Sample 4 (< 5 μm NIOSH standard), obtained from Blacksville, WV (Figs. 1, 2). Comparative data obtained for all the other coal samples are presented in the tables and figures and discussed only briefly.

Figure 1 shows typical ESR spectra obtained from spin trapping by DMPO of the short-lived oxy-radicals formed in mixtures of H_2O_2 and NIOSH standard coal dust Sample 4 of < 5 μm size. As controls, Figure 1a and Figure 1b show that only a barely detectable ESR signal was obtained from mixtures containing either 100 mM DMPO and 5 mg/ml of coal dust or from 100 mM DMPO and 10 mM H_2O_2. However, as presented in Figure 1c, a strong 1:2:2:1 quartet signal was observed when the same amounts of H_2O_2 and coal dust were mixed in the presence of 100 mM DMPO. This signal increased in intensity with increasing dust concentration, as may be noted in Figure 1d, which represents to an increase in dust concentration to 10 mg/ml. The 1:2:2:1 quartet spectrum of Figures 1c, d was analyzed to show hyperfine couplings of 15.0 G with one nitrogen and one hydrogen nucleus. These splitting constants are characteristic of DMPO-OH adduct,[35,38] indicating the possible generation of an $^{\bullet}OH$ radical in the reaction mixture. A confirmatory test for the $^{\bullet}OH$ radical generation was obtained by investigating the effect of ethanol (C_2H_5OH) as a competitive $^{\bullet}OH$ radical scavenger. As has been well documented,[35] ethanol competes with DMPO in reacting with $^{\bullet}OH$ radicals to yield the DMPO-C_2H_4OH radical adducts. The DMPO-C_2H_4OH adduct exhibits a distinctive six-line ESR spectrum.[35] The addition of 10% ethanol to the mixture of 100 mM DMPO, 10 mM H_2O_2, and 10 mg/ml, coal dust yielded a six line spectrum highlighted by arrows in Figure 1e. This six line spectrum yielded hyperfine

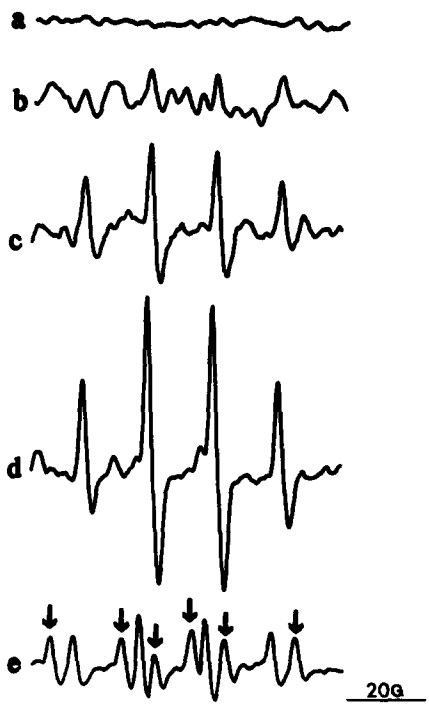

Fig. 1. Typical electron spin resonance spectra obtained from spin trapping of short-lived oxygen radicals formed in mixtures of coal mine dust and H_2O_2. (a) Spectrum obtained from 5 mg coal mine dust Sample 4 and 0.1 M DMPO. (b) Spectrum of 0.1 M DMPO and 10 mM H_2O_2. (c) Spectrum obtained with 5 mg coal mine dust Sample 4, 0.1 M DMPO, and 10 mM H_2O_2. Note the strong 1:2:2:1 quartet signal. (d) Spectrum obtained with 10 mg coal Sample 4. The 1:2:2:1 quartet spectrum in (c) and (d) showed hyperfine couplings of 15 G with one nitrogen and one hydrogen nucleus, indicating a characteristic $^{\bullet}OH$ radical adduct of DMPO-OH. (e) Spectrum obtained with 10% ethanol, 0.1 M DMPO, 10 mM H_2O_2, and 10 mg/ml coal dust Sample 4. A six line spectrum with hyperfine couplings of 15.8 G and 22.8 G, characteristic of DMPO-C_2H_4OH adduct was obtained, confirming $^{\bullet}OH$ radical generation.

couplings of A_N = 15.8 G and A_H = 22.8 G, which are produced by couplings for the DMPO adduct of the ethanolyl radical.[35] These results provide additional evidence for $^{\bullet}OH$ radical formation from the H_2O_2/coal dust mixtures.[35,38]

Dose dependence of coal mine dust for $^{\bullet}OH$ generation

The optimal concentration of NIOSH standard < 5 μm coal (Sample 4) required to generate a strong characteristic 1:2:2:1 quartet spectrum of DMPO-OH adduct was determined from a series of preliminary experiments using 1 mg to 100 mg dust. From these studies a concentration of 10 mg coal was selected as an adequate concentration to generate reasonable peak intensities. Figure 2 shows the dose dependence of $^{\bullet}OH$ radical generation by the coal dust Sample 4. Figures 2a–2e show the spectra obtained for 1, 5, 10, 20, and

Fig. 2. ESR spectra showing the dose dependence of ·OH radical generation from coal Sample 4. (a–e) show the spectra for 1, 5, 10, 20, and 40 coal with 10 mM H_2O_2 and 100 mM DMPO.

40 mg coal with 10 mM H_2O_2 and 100 mM DMPO. Dose dependence on radical generation using 10 mg coal and varying amounts of H_2O_2 (0.5 mM, 1 mM, 5 mM, 10 mM, 20 mM, 50 mM) showed that the optimum radical generation occurred at 10 mM H_2O_2 concentration (data not shown). Together, these results indicate that both H_2O_2 and coal dust are important in the mechanism of ·OH generation, thereby pointing to a Fenton type of reaction[39] as the underlying mechanism. Additional evidence in support of this conclusion was provided by the results obtained with other ·OH scavengers and chelators as discussed later.

·OH *radical generation by coal mine dusts*

Having established that the standard NIOSH < 5 μm coal Sample 4 does have the potential to generate ·OH radicals, we investigated ·OH generation in the reaction of H_2O_2 with four other coal mine samples collected from bituminous coal mines in Utah and West Virginia. These mines represent diverse degrees of CWP prevalence and compositional differences in the coal dust. Figure 3 represents the relative potential of the six coal mine dusts in equal mass (10 mg/ml) for generating ·OH radicals from 10 mM H_2O_2. It is evident from these results that coal Samples 1 and 2 generate the least amount of ·OH radicals, and Samples 5 and 6 produce the largest amounts of ·OH radicals. All the coal mine dusts except the Osage coal mine dust showed significantly different ($p < .001$) ·OH radical generation.

Effects of scavengers and metal chelators on ·OH *generation in coal dust* H_2O_2 *mixtures*

To investigate the mechanism of ·OH generation from H_2O_2 by the coal dust, we studied effects of several metal ion chelators and oxy-radical scavengers to coal dust/H_2O_2 mixtures (Fig. 4). In Figure 4, the average intensity of the DMPO-OH peaks for the control system (coal + H_2O_2 + DMPO) was arbitrarily assigned as 100. It is evident from the data that ·OH radical scavengers in general decreased the DMPO-OH signal produced. The only exception was the metal chelator DETAPAC, which, in fact, caused a significant enhancement in the DMPO-OH signal (Fig. 4). EDTA also caused a significant enhancement in the DMPO-OH signal intensity (data not shown). DETAPAC is reported to enhance ·OH generation by complexing with iron and thereby favoring ·OH generation by H_2O_2 decomposition.[40] A major role for H_2O_2 in this ·OH generation was supported by the observation that catalase was the most effective scavenger in our experiments (Fig. 4). Similarly, the high efficiency of deferoxamine in diminishing the ·OH generation indicates a significant role for iron.[41] Together, these results imply that the dominant mode for ·OH generation is the iron-catalyzed decomposition of H_2O_2. However, the observed scavenging effect of SOD, albeit significantly smaller than that of catalase, demonstrates that the superoxide ($O_2^{\cdot -}$) radical must also be involved in the mechanism of ·OH generation by the coal dust/H_2O_2 system. The effect of metal chelators and scavengers were significantly different ($p < .001$) in comparison to controls.

Surface iron concentration and ·OH *radical generation*

To obtain additional data on the radical generation mechanism, we measured the total concentrations of silica, iron, cobalt, copper, and chromium in all the

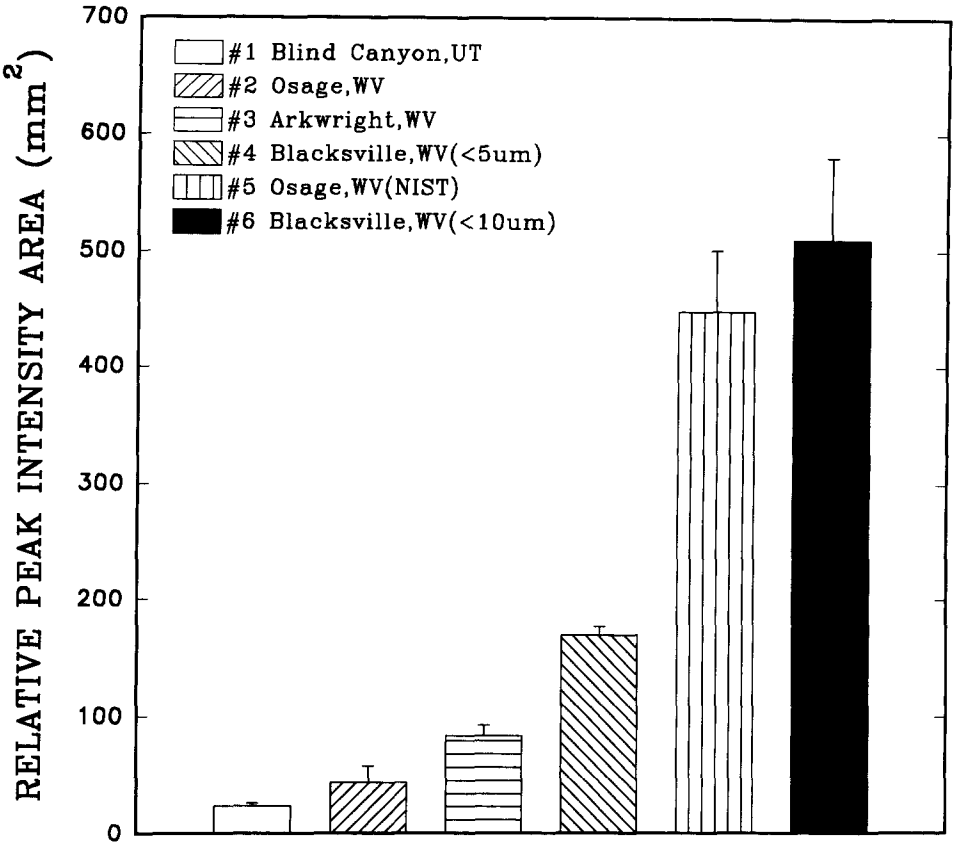

Fig. 3. Bar graph showing relative intensities of the DMPO-OH adduct formed by the reaction of equal mass (10 mg/ml) of six coal mine coal dust samples with 10 mM H_2O_2 and 100 mM DMPO as spin trap.

coal dust samples (Table 1). Plots of these data and the radical generation potential for all the coal dusts showed no apparent correlation (data not shown). We therefore measured the surface iron concentration of all the coal samples spectrophotometrically[36] as described in the earlier section. The results obtained are presented in Figure 5, indicating a positive correlation (r = 0.93) at 95% confidence between a dust's surface iron content and its $^{\bullet}OH$ generating potential.

Lipid peroxidation

Because all the coal dust samples exhibited an ability to generate $^{\bullet}OH$ radicals, we evaluated their lipid peroxidation potential as a marker of cellular damage. The lipid peroxidation potential varied significantly among the coal mine dust samples. Comparison of this lipid peroxidation potential with surface iron concentration showed a good correlation (r = 0.93) for all the coal mine dusts (Fig. 6). Because $^{\bullet}OH$ radical generation is enhanced from the coal mine dust by the addition of iron and totally inhibited by treatment with deferoxamine, we investigated whether lipid peroxidation would be enhanced by exogenous iron. Results presented in Figure 7 show that the lipid peroxidation potential of all the coal mine dust is enhanced considerably by the addition of iron, providing additional support for the proposed iron-mediated mechanism of lipid peroxidation by coal mine dusts.

DISCUSSION

A major goal of this study was to evaluate coal dusts for their ability to generate oxygen-centered radicals from H_2O_2, and to correlate their lipid peroxidation potential with surface iron and $^{\bullet}OH$ generation. This investigation was considered important, because earlier studies have reported that toxic inorganic dusts such as silica[15,18-24] and asbestos[21-30] do catalyze $^{\bullet}OH$ radical generation from H_2O_2, and those radicals are associated with cell injury. It has also been proposed that silica exerts its toxic effects through oxygen-radical initiated chain reactions leading to lipid peroxidation.[15,18-24] Because silica is generally considered a dominant contributor to coal mine dust's cytotoxic and fibrogenic potential, it was deemed important to establish whether some essentially silica-free coal dusts could also exhibit oxygen-radical generating potential.

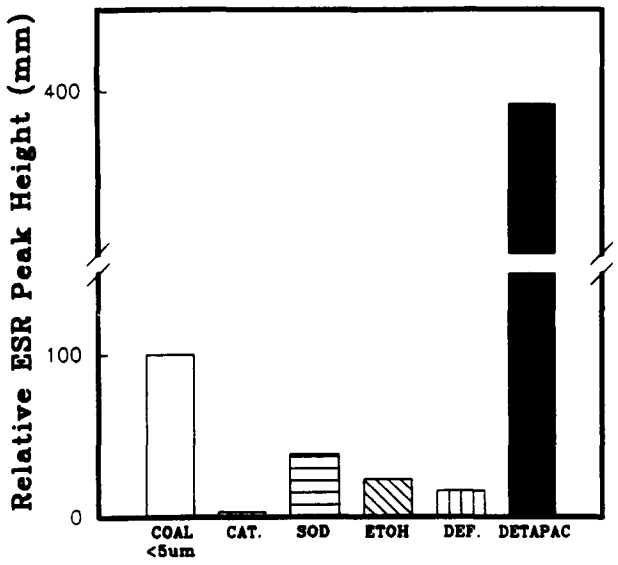

Fig. 4. A bar graph representation of the effects of various free radical scavengers and chelators on the amount of 'OH radicals generated from 10 mM H_2O_2 by 10 mg coal dust Sample 4. The amount of DMPO-OH adduct generated by the coal sample without the addition of scavengers or chelators was arbitrarily assigned a value of 100.

This study demonstrates that bituminous coal mine dusts containing silica from 0.75% to 3.2% obtained from different geographic regions and different mining areas of the same coal seams have the potential to generate 'OH radicals from H_2O_2 irrespective of silica concentration. In addition, we find that the potential to induce lipid peroxidation exhibited a good correlation with available surface iron and 'OH radical generation by the coal mine dusts. The oxygen-radical generating potential of the coal dusts investigated here appears to be greater than that of silica on, at least, a mass basis.

Measurements of the effects of radical scavengers and metal ion chelators (Fig. 4) indicate the probable chemical mechanism underlying the oxygen-radical generation. It is known that chelation of iron can either reduce or increase catalytic generation of the 'OH by Fenton reaction depending on the chelator. Chelators that block all of iron's coordination sites, like deferoxamine, can effectively inhibit 'OH generation.[40,41] On the other hand, chelators that leave some of the iron's coordination sites open, like EDTA and DETAPAC, can actually enhance 'OH generation.[40] The total inhibition of 'OH radical generation from H_2O_2 and coal dust by catalase points to the dominant role of the Fenton reaction.[39,42]

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^- \quad (1)$$

In addition, the scavenging effect of SOD, albeit weaker than that of catalase or deferoxamine, indicates a minor role for the superoxide ($O_2^{\bullet -}$) radical as an intermediate for 'OH generation, through the Haber–Weiss cycle.[43]

$$O_2^{\bullet -} + Fe(III) \rightarrow Fe(II) + O_2 \quad (2)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^- \quad (3)$$

$$\text{Net } O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^- \quad (4)$$

This conclusion is supported by the observation of a significant enhancement of 'OH generation on the addition of DETAPAC, which is known to favor the Fenton reaction (Equation 1) over the Haber–Weiss step (Equation 4). Furthermore, this result is consistent with the findings that the amount of radicals generated was in approximate proportion to a sample's surface iron content (Fig. 5).

In comparison with our earlier studies with silica[18–22] (Min-U-Sil), some of the coal mine dust samples produced a stronger 'OH signal. Earlier studies by Zalma et al.[30] using DMPO as a spin trap in a similar system reported that minerals without ferrous iron or a surface

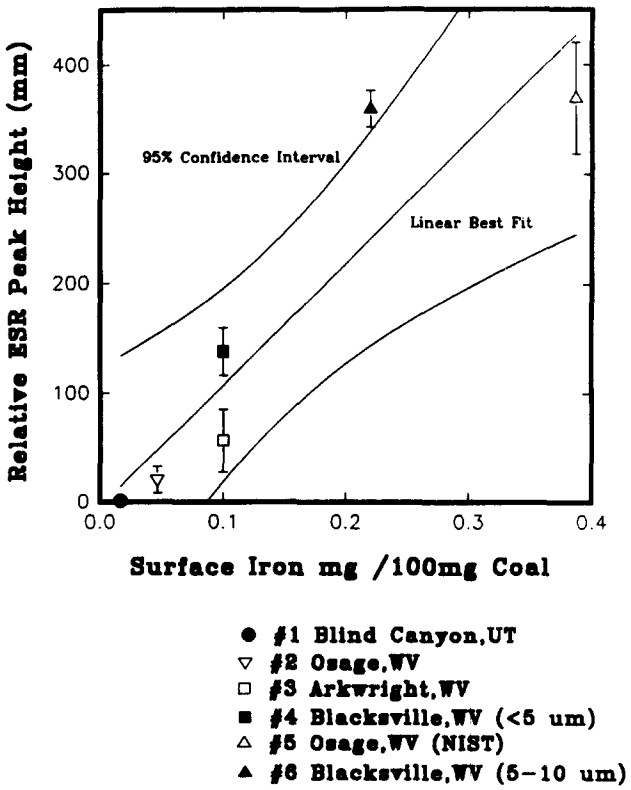

Fig. 5. Plot of 'OH radical generation potential versus surface iron concentration of coal dust samples. The data indicate a good correlation between ESR peak heights and surface iron content.

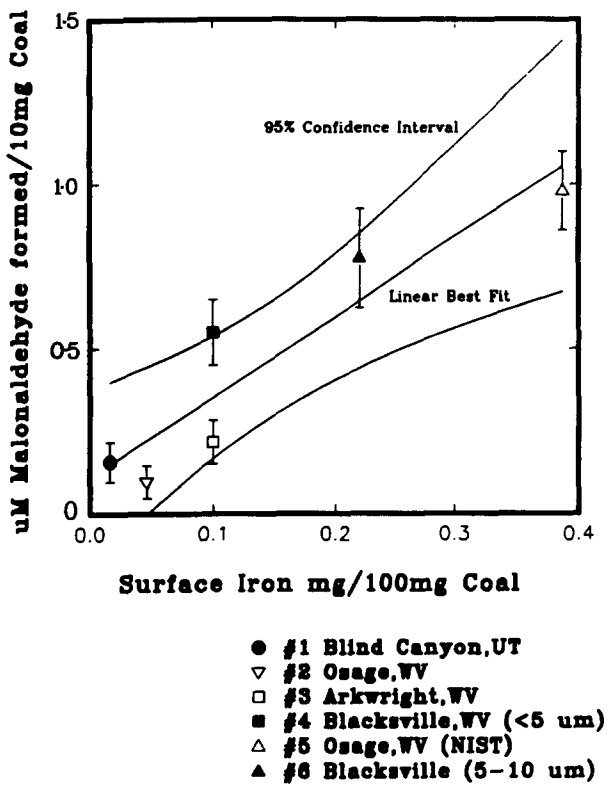

Fig. 6. Potential for lipid peroxidation versus the concentration of surface iron for six coal dust samples.

chemistry did not produce ·OH spin adduct. Our earlier studies on crystalline silica[18–22] support this conclusion, because we have shown that surface chemistry, altered by grinding, results in an increased generation of ·OH radicals. In addition, we have observed that grinding the NIST coal with an average diameter of 250 μm for 5 min in an agate mortar did cause a substantial increase (30–50%) in ·OH radical generation. ·OH radical generation by crystalline silica and coal can be substantially blocked by catalase and deferoxamine. It is, therefore, clear that both the iron content and surface chemistry play a major role in the mechanism of ·OH generation by coal mine dust.

This study indicates the usefulness of establishing new parameters for characterizing the coal mine dust for mineral inclusions, surface iron content, oxygen radical generation, and lipid peroxidation potential. In addition, from the point of view of dust control, it is important to identify the source of this iron. Whether iron available for the ·OH radical generation is a part of the coal structure or a separate entity of minerals present in the coal mine dust is not known. It is known that coals do contain many iron-containing minerals, chief among them being pyrite (FeS_2), and the various clay minerals in which iron can substitutionally replace aluminum. The ·OH radical generating properties of FeS_2 was investigated earlier,[33] wherein it was shown that FeS_2 by itself does not generate any significant amount of ·OH radicals because the iron is nominally in the +4 oxidation state, and not in +3 or +2 states that are involved in the radical generation mechanism. However, weathered FeS_2 particles can catalyze ·OH radical generation from H_2O_2 probably because of the formation of a sulfate ($FeSO_4$) layer at the particle surface.[33] Thus, the amount of ·OH radicals generated decreases on grinding FeS_2 particles.[33] In contrast, in this study, we noted that as reported earlier for silica,[18,19] the ·OH radical signal increased upon grinding the dust particles utilizing mortar and pestle (data not shown), thereby arguing against a major role for FeS_2 particles. Another explanation might be that perhaps the iron is released into the coal dust by the jet milling and blending in stainless steel apparatus as described for the processing of NIOSH and NIST standard samples. This is evident from the available iron concentrations, lipid peroxidation potential, and relative ESR peak intensities obtained for ·OH radical generation by these three standard coal dust samples. We hypothesize that iron is embedded in the solid bulk of coal as part of a mineral such as FeS_2 in a loose physical bond, and when coal is fractured, these particles are liberated in the coal mine dust for interaction.

Recent studies reported from France suggest that the reactive oxygen species generated by inhaled coal mine dust may be related to the development of emphysema.[44] In this study, Huang et al.[44] hypothesized that emphysema in coal workers may be related to the production of reactive oxygen species generated by

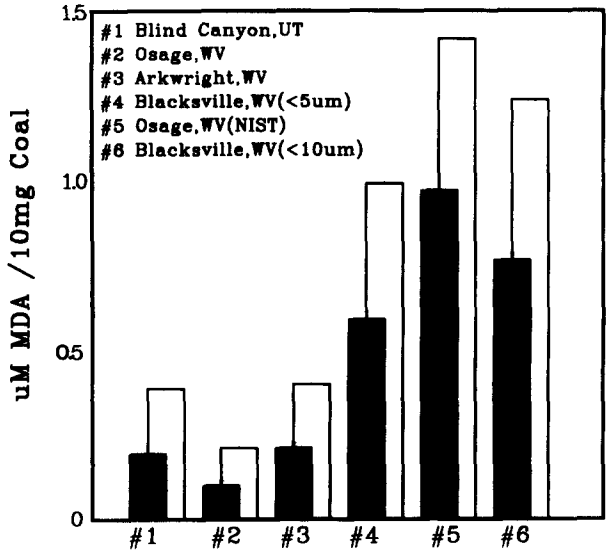

Fig. 7. Enhanced potential for lipid peroxidation in six coal mine dust samples by the addition of iron.

the inhaled coal. In support of this hypothesis, they presented evidence on the inactivation of α 1-antitrypsin by coal aqueous solutions and identified the active component to be primarily ferrous sulfate. These authors proposed that evaluation of Fe^{2+} in the coal mine dust can be used as a parameter to predict the incidence of emphysema in coal miners. Our results present evidence that CWP occurring with diverse prevalence rates may result from the potential of the coal mine dust to generate oxygen radicals, which, in turn, is indirectly related to the iron content of the coal. Increased potential of coal mine dust to generate oxygen radicals in combination with the respiratory burst and generation of reactive oxygen intermediates may impair the defense of the lung and induce injury.

Although additional work is needed to establish the source of iron in coal mine dust, this study indicates that the iron content of a coal dust sample is a measure of the dust's potential for cellular injury through $^{\cdot}OH$ generation. This conclusion is further supported by our observation that the extent of lipid peroxidation by the coal dust had a fairly good correlation with the amount of surface iron as shown in Figure 6. Based on these results, we postulate that higher concentrations of surface iron in coal mine dust may be involved in the generation increased level of $^{\cdot}OH$ radicals, increased cellular injury, and unexplained prevalence of CWP in different coal mining areas.

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