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PROTECTION BY IRON AGAINST THE TOXIC EFFECTS OF QUARTZ

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INTRODUCTION

Inhalation of quartz (crystalline silica) can lead to the fibrosing lung disease, silicosis (Morgan and Seaton, 1984). Exposure to quartz alone is rare and most exposures occur through the presence of quartz in other dusts produced by activities such as mining, quarrying and sandblasting. Consequently, silicosis is often combined with mixed dust pneumoconiosis. A number of studies in iron-ore miners (Reichel *et al.*, 1977; Moore *et al.*, 1987) and in animals (Gross *et al.*, 1960) have indicated that iron can protect against the effects of quartz.

It is now believed that the characteristics of the surface of quartz particles determine their interaction with biological molecules and hence their toxicity. For example, the surface can generate a range of reactive oxygen species such as hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\cdot OH$), agents known to be toxic to cells and tissues (Fantone and Ward, 1984). The production of reactive oxygen species can be measured using electron spin resonance (ESR) techniques. The two main chemical activities of the silica surface are those involving siloxane bridges (Si–O–Si) and silanols (SiOH) (Iler, 1979). Silanol groups can form hydrogen bonds with cell membranes, thought to be the main site of quartz toxicity. These groups can also dissociate at physiological pH giving a net negative charge on the silicate surface which can lead to adsorption, or coordination, of organic and inorganic cations, such as iron and aluminium.

This binding of metals has been shown to reduce the toxicity of quartz (Nolan *et al.*, 1981). Paradoxically, the interaction of iron with H_2O_2 can also produce $\cdot OH$ radicals, extremely potent oxidants, through the Fenton reaction:

$$Fe^{2+} + H^2O^2 = Fe^{3+} + \cdot OH + OH^-$$

The H_2O_2 for this reaction can be produced at the quartz surface or be released from macrophages and neutrophils.

The objective of our study was to examine whether treating quartz particles with iron, using ferrous or ferric chloride solutions, or mixing quartz with carbonyl iron particles could alter the surface activity of quartz and the toxic and pulmonary inflammatory effects of quartz.

Table 1. Total cell and neutrophil numbers lavaged from lungs 7 days and 32 days post-instillation of quartz, carbonyl iron, or quartz/iron mixtures (geometric means) and tumour necrosis factor production in the presence of LPS by lavaged macrophages (geometric means and ranges)

Treatment	Total cells (millions) Day 7	Day 32	Neutrophils (millions) Day 7	Day 32	Tumour necrosis factor (units/10^6 AM)
Iron 1 mg	8.07	9.65	0.11	0.06	92 (55–156)
Quartz 0.08 mg	8.18	8.70	0.35	0.08	140 (58–340)
Quartz/Iron 0.08/0.92 mg	7.88	9.20	0.28	0.14	191 (113–322)
Iron 10 mg	6.37	7.60	0.61	0.04	166 (94–294)
Quartz 0.8 mg	24.60	44.66	9.01	19.06	976 (402–2373)
Quartz/Iron 0.8/9.2 mg	16.11	31.13	4.94	8.10	447 (253–792)
Iron 50 mg	12.58	10.68	1.77	0.72	384 (167–883)
Quartz 3.9 mg	47.12	123.36	30.19	65.76	562 (231–1366)
Quartz/Iron 3.9/46.1 mg	21.54	26.65	2.89	5.30	1331 (543–3261)
Control	3.82	4.42	0.01	0.02	61 (50–75)

MATERIALS AND METHODS

Samples of DQ12 quartz were treated with iron using ferrous or ferric chloride dissolved in distilled water to 1M concentrations. The presence of iron on quartz particles treated with iron chlorides was confirmed using an iron staining kit (Sigma, Poole, U.K.). For some experiments quartz particles were mixed with particles of elemental iron (carbonyl iron; Sigma). Treated and untreated quartz and quartz/iron mixtures were instilled at several doses into the lungs of specific pathogen-free (SPF) male Wistar rats which were lavaged 7 or 32 days later. The recovered cell types were enumerated and alveolar macrophages (AM) were cultured for the production of the inflammatory cytokine tumour necrosis factor alpha (TNFα) which was measured using the L929 cell bioassay (Flick and Gifford, 1984). *In vitro* experiments examined the effects of iron coating on (a) toxicity to macrophages, as lactate dehydrogenase (LDH) release, and their release of H_2O_2 and superoxide dismutase and (b) on radical activity on the surface of quartz particles and ·OH generation by quartz particles. Additionally, some comparisons between iron coating and aluminium lactate coating were made. Results were analysed using analysis of variance and regression methods using the statistical software of Genstat 5.1 and Minitab, release 8.2. Data from the instillation experiments were log-transformed prior to analysis.

RESULTS

The effect of iron particles on the pulmonary response to quartz

In the series of experiments with rat lungs instilled with various doses of quartz, the relatively non-pathogenic dust carbonyl iron, or quartz mixed with various proportions of particulate iron, inflammation (as total cell numbers and numbers of neutrophil leukocytes) increased with dose of quartz in both the quartz alone and quartz/iron mixtures (Table 1). At the lower doses (e.g. 1 mg of iron alone or iron mixed with 8% quartz) there was little difference in response between the treatments. With higher doses and the same percentage quartz mixture, it was

evident that iron was reducing the strong response to quartz. Particulate iron alone produced very little response. Additional, experiments with lower ratios of iron to quartz and a range of doses showed no evidence of an iron-protective effect. It was clear that the ratio of iron to quartz (e.g. 12 to 1 weight for weight in Table 1) had to be sufficiently high for the effect to occur.

Histological examination of lungs from additional rats, 60 days following treatment with the same dusts, confirmed the lavage cell results, with quartz on its own proving to be the most pathogenic dust with evidence of lipoproteinosis and fibrosis.

Production of tumour necrosis factor (TNFα) by cells recovered in lavage

Alveolar macrophages lavaged from dust-treated lungs were cultured overnight with or without 10 μg ml^{-1} LPS (endotoxin) and their production of TNFα assayed. In the absence of LPS, there were no significant differences between the treatments for any dose at any time point (data not shown). In the presence of LPS there was considerably greater production of TNFα. Although results from the 32 day experiments tended to be higher than those of the other time points, there was no significant effect of time and results have been pooled across the time points for display in Table 1 as estimated geometric means. TNFα production increased with dose for iron and quartz/iron, but not for quartz alone where the middle dose gave the highest results. TNFα values for macrophages from the iron treatments were significantly lower than those from the quartz/iron treated group.

The effect of coating quartz with iron or aluminium on the pulmonary cellular response

The effect on the pulmonary leukocyte response of coating quartz particles with ferrous or ferric iron was studied. Aluminium lactate-treated quartz was also included as aluminium has previously been shown by us to reduce quartz toxicity in the lung (Brown *et al.*, 1989). An additional sample of quartz was subjected to the same coating procedure using distilled water in place of the metal salts. Rat lungs were lavaged 7 days or 32 days following intratracheal instillation of 1 mg amounts of the quartz dusts. Geometric means of total neutrophil counts, pooled from all experiments, are shown in Fig. 1. The strong neutrophil response to quartz was significantly reduced only by the aluminium lactate treatment, pre-treatment with iron had no effect. Similar statistically significant reductions were seen with aluminium treatment for total, macrophage and lymphocyte numbers (data not shown). No effect of iron coating was seen in additional experiments using quartz at lower doses.

Hydrogen peroxide production and superoxide dismutase activity by alveolar macrophages exposed in vitro *to untreated quartz or to quartz coated with iron or aluminium*

H_2O_2 production by alveolar macrophages incubated for 20 min with 0.5 mg ml^{-1} untreated quartz or quartz particles coated with iron or aluminium is summarised in Table 2. H_2O_2 levels were greatest with the water (control) and aluminium lactate treatments and lowest with Fe^{2+}-treated quartz.

As part of the body's defence system against oxidant injury, cells can produce the

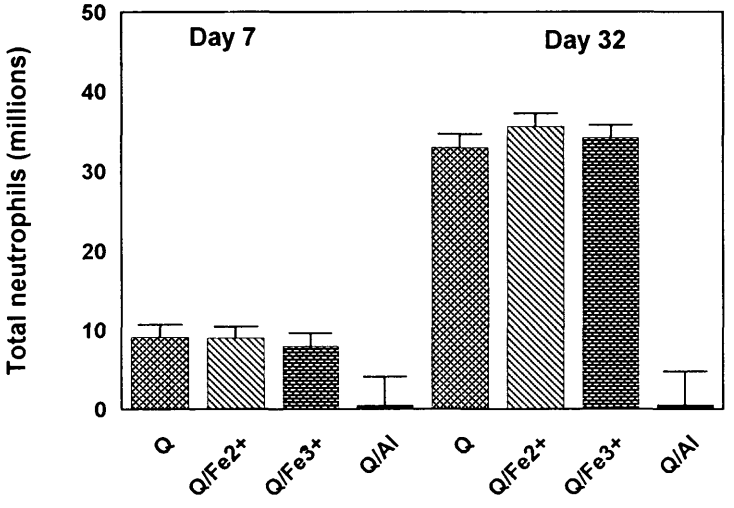

Fig. 1. Neutrophil numbers in lavage 7 and 32 days post-instillation of 1 mg metal-treated quartz (geometric mean and s.d.).

enzyme superoxide dismutase (SOD) which converts superoxide to oxygen and hydrogen peroxide. Accordingly, we assessed the levels of SOD in alveolar macrophages incubated for 1 h with 0.5 mg untreated quartz or quartz pre-treated with iron or aluminium. Both the intracellular and extracellular (supernatant) concentrations of SOD were assayed. Compared to untreated (no dust) macrophages, only untreated quartz caused a significant ($P < 0.05$) drop in the intracellular level of SOD (Table 2). All the quartz dusts caused some release of SOD extracellularly into the culture supernatant (Table 2), but only aluminium treated quartz produced a significantly lower concentration than untreated quartz ($P < 0.05$).

Toxicity of coated and uncoated quartz to alveolar macrophages

Lactate dehydrogenase (LDH) is a cytosol enzyme whose release from cells provides a reliable measure of cytotoxicity. We used this assay to identify any differences in cell injury between the treated and untreated quartz samples. Alveolar macrophages were incubated for 1 h with 1 mg ml^{-1} untreated quartz or quartz pre-treated with ferrous or ferric iron or aluminium lactate. All quartz

Table 2. *In vitro* treatment of alveolar macrophages with metal-coated quartz: superoxide dismutase levels, hydrogen peroxide production and lactate dehydrogenase release (mean and standard error)

Treatment	Superoxide dismutase Intracellular	Superoxide dismutase Extracellular	H_2O_2 nmol/ 10^6 AM	LDH units/ 10^6 AM
None	111.5 (4.6)	1.5 (0.9)	0.33 (0.02)	59.8 (1.4)
Quartz	92.7 (6.8)	39.7 (4.2)	0.38 (0.22)	113.8 (1.7)
Quartz/water	96.8 (7.0)	24.1 (7.4)	1.09 (0.05)	101.4 (2.3)
Quartz/$Fe2^+$	103.5 (7.2)	19.6 (8.2)	0.01 (0.00)	113.0 (2.6)
Quartz/$Fe3^+$	101.4 (4.8)	31.1 (4.8)	0.42 (0.17)	110.0 (1.1)
Quartz/aluminium	106.6 (4.6)	16.9 (8.6)	1.44 (0.15)	99.4 (3.2)

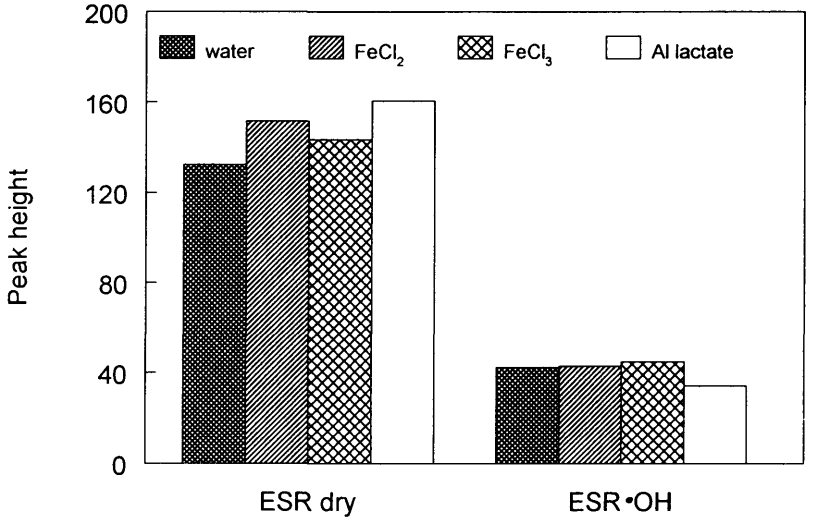

Fig. 2. Electron spin resonance (ESR) measurements as peak heights from 50 mg dry quartz samples and ·OH radical activity from 10 mg quartz samples in the presence of DMPO and H_2O_2 (mean of 3 experiments).

samples, treated or untreated, caused LDH release from macrophages (Table 2). There was no difference between untreated quartz and ferrous or ferric-treated quartz.

Measurement of radicals on the surface of quartz particles treated with iron or aluminium

Electron spin resonance (ESR) measurements were made on three batches of quartz treated with water, ferrous or ferric chloride, or aluminium lactate. All the treated quartz samples produced surface radical activity but there were no significant differences between the treatments (Fig. 2; data pooled from the three batches). To determine generation of ·OH, measurements were also carried out in liquid in the presence of the spin trap DMPO and hydrogen peroxide but there were no significant differences between the treatments (Fig. 2).

DISCUSSION

We have shown that particulate iron can reduce the inflammatory effects of quartz in the rat lung and modulate TNF production by alveolar macrophages provided that the ratio of iron to quartz is sufficiently high, 11.8–1 in our experiments. Coating quartz with ferrous or ferric iron did not affect the cellular response in marked contrast to the protective effects of aluminium in rat lungs, previously shown by our laboratory (Brown *et al.*, 1989) and in the lungs of sheep (Begin *et al.*, 1986). Nolan *et al.* (1981) reported the ameliorating effects of treating the quartz surface with ferric chloride, aluminium chloride, zinc chloride, or polyvinyl-pyridine-N-oxide (PVPNO, a hydrogen-bonding polymer) on the lysis by quartz of red blood cells. The reason for the lack of effect of iron coating, as

opposed to aluminium treatment, in our *in vivo* assay is not known. Aluminium may be bound more strongly than iron and be better able to resist leaching from the quartz surface within the body. Geometrical considerations would indicate that ions with a similar charge to radius ratio to that of silicon, and a similar preference for tetrahedral sites, would fit more readily into the quartz surface. Aluminium^{3+} and, to a lesser extent, Fe^{3+} can both replace silicon in silicate minerals. Fe^{2+} being larger and with less charge would not fill tetrahedral sites or be held as strongly by electrostatic forces (Iler, 1979). We found metal binding had no effect on radical production at the quartz surface. Radical activity is normally greatest with freshly ground quartz surfaces (Shi *et al.*, 1988) and we did not investigate the effects of grinding in our assay.

The reason why particulate, elemental iron was able to protect against the inflammatory effects of quartz is not clear. It is unlikely that the iron is interacting directly with the quartz surface and thus it may be acting indirectly by reacting with and neutralising reactive oxygen species.

In conclusion, this study confirms previous work showing (a) the protective effect of modifying the quartz surface with aluminium (Brown *et al.*, 1989) and (b) that particulate iron can also reduce the pulmonary toxicity of quartz (Gross *et al.*, 1960). Accordingly, in industries with significant mineral dust exposures that include quartz and iron, inhaled dust may be less harmful than would be predicted merely from its quartz content.

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