Role of Iron and Surface Free Radical Activity of Silica in the Induction of Morphological Transformation of Syrian Hamster Embryo Cells

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Using various surface modified silica dusts we previously showed that the samples able to generate reactive oxygen species (ROS) induced transformation of Syrian hamster embryo (SHE) cells. The purpose of the present study was to determine in what measure trace iron on the surface of silica particles is involved in the cellular effects, through the formation of ROS. Min-U-Sil 5 quartz (Q) and an amorphous diatomite earth (DE), as well as the same samples pretreated with the Fe chelators ferrozine (fz, 3 mM) and desferrioxamine (dfx, 0.1 mM), were studied for: (i) morphological transformation; and (ii) aberrant mitotic division of SHE cells, which we reported as one of the possible mechanisms of silica-induced cell transformation. In order to determine the nature of the ROS involved in these effects, the antioxidants superoxide dismutase, catalase and mannitol were added to the treated cells. On a per weight basis, DE appeared more cytotoxic and slightly more transforming than Q. The suspensions of Q and DE preincubated in Fe chelators were significantly less transforming and, in particular Q particles, had reduced potency in inducing abnormal mitoses than the untreated samples. Although the amount of Fe mobilized from the silica particles by dfx was greater than that of Fe(II) mobilized by fz, a decrease in transforming potency was largely achieved after pretreatment of particles with fz. In the presence of antioxidants, both the transformation and mitotic aberration frequencies were significantly decreased. The results show that the iron associated with the surface of particles is implicated in the generation of ROS and is partly responsible for the transforming potency of silicas. This study confirms the role of free radical activity in the induction of cell transformation in vitro, suggesting that oxidative stress is one of the mechanisms of silica carcinogenesis.

Keywords: ROS; SHE cell transformation; silica; surface iron

INTRODUCTION

Occupational exposure to some silicas is known to be associated with silicosis and other respiratory disorders. More recently, from epidemiological and experimental data on crystalline silica, the International Agency for Research on Cancer concluded that there is now sufficient evidence for the carcinogenicity to humans of inhaled quartz and cristobalite by occupational exposure (IARC, 1997). The mechanisms of silica-induced carcinogenesis are only partially understood. Recent studies have indicated that silica carcinogenicity may be related to the generation of reactive oxygen species (ROS) directly from the silica surface and/or from inflammatory cells (IARC, 1997; Saffiotti, 1997; Donaldson and Borm, 1998; Fubini, 1998; Shi et al., 1998).

Using various surface modified silica dusts we previously showed that the particles able to generate ROS induce Syrian hamster embryo (SHE) cell transformation (Elias et al., 2000) and reported a direct relationship between the transforming potency of the silica particles and their hydroxyl (HO·) yield (Fubini et al., 2001).

The purpose of the present study was to determine in what measure iron on the surface of silica particles is involved in the cellular effects, through the formation of ROS. Min-U-Sil quartz and an amorphous
diatomaceous earth, as well as the same samples pretreated with iron chelators, were studied for the induction of morphological transformation and cell division aberrations in SHE cells. In order to determine the nature of the ROS, the antioxidant superoxide dismutase (SOD), catalase and mannitol were added to the treated cells.

MATERIALS AND METHODS

Silica samples: physico-chemical characteristics and surface properties

Three samples were studied: Min-U-Sil quartz (Q) (US Silica Corp., Berkeley Springs, WV), an amorphous diatomaceous earth (DE) (Sigma, St Louis, MO) and an amorphous vitreous synthetic sample (V) (Spectrosil). Their structure was confirmed by X-ray diffractometry. The specific surface area of the particles (BET N2 adsorption method) was 5 m²/g for Q and DE and 13 m²/g for V. Iron impurities (ICP spectrometry) in the Q, DE and V samples were 0.05, 0.03 and 0.006%, respectively. The surface hydrophilicity, measured microcalorimetrically by the heat of water vapour adsorption (Fubini, 1988), was higher for the DE sample than that for the Q sample, whereas the V particles were only partially hydrophilic.

Silicon-based radicals (ESR spectroscopy) (Fubini et al., 1989) were detected on the surface of Q particles. The release of HO· radicals by reaction with H₂O₂ (EPR spectroscopy using the spin trapping technique) (Fubini et al., 1995) was recorded for the Q and DE particles.

Iron-depleted silica particles

Suspensions of Q and DE (8 mg/ml pure water) were preincubated for 24 h in: (i) phosphate-buffered saline (PBS); (ii) 3 mM ferrozine (fz) (Sigma); (iii) 0.1 mM desferrioxamine (dfx) (Sigma). After centrifugation for 15 min at 3000 g, the sedimented particles were used to treat cell cultures and the supernatants were kept for iron measurement (SAAET method). A parallel fz-treated particle pellet received 0.1 mM desferrioxamine for 24 h. The supernatant and pellet were used in the same way. The release of HO radicals by the Q and DE particles preincubated in PBS and fz + dfx was determined by ESR.

Colony-forming efficiency (CE) and transformation assay

The bioassay procedure was performed as previously described (Elias et al., 2000). The culture medium, Dulbecco’s minimal essential medium, pH 7.0 (Gibco), was supplemented with 20% fetal calf serum. Samples of 300 SHE target cells/60 mm dish were seeded onto the feeder cells and treatment took place 24 h later, for 7 days. In some experiments, 12.5–50 µg/ml SOD, 10–40 µg/ml catalase or 10–40 mM mannitol (Sigma) was added immediately before cell treatment.

Cell division aberration assay

Mitotic SHE cells from the cultures exposed to silica samples at concentrations between 2.25 and 34 µg/cm² for 24 h (~1 cell cycle) were examined by: (i) the differential chromosome/spindle staining technique; (ii) indirect immunofluorescence staining, performed as previously described (Béna et al., 2000).

Statistical analyses were performed by Fisher’s exact test at the 95% confidence limit.

RESULTS

Cytotoxic and morphological transformation of SHE cells

The untreated Q sample appeared non-cytotoxic at concentrations up to 60 µg/cm², which decreased the relative CE by 40%. DE particles were more cytotoxic, reducing the relative CE by 40% at a concentration of 30 µg/cm². A concentration-dependent increase in transformation frequency (TF) was induced by both samples, starting from the lowest concentration tested of 3.5 µg/cm². The TF induced by DE appeared slightly above that induced by the Q particles (e.g. 3.4 versus 1.9% at 60 µg/cm², respectively). The V sample was neither cytotoxic nor transforming up to the highest concentration tested, 30 µg/cm².

The suspensions of Q and DE preincubated in iron chelators significantly decreased their transforming potency (Fig. 1a). At a concentration of 30 µg/cm² the Q sample pretreated with fz followed by dfx was 2.8-fold less transforming than the untreated Q sample (TF of 0.25% compared with 0.7%). The fz + dfx pretreated DE particles showed a 1.8-fold less transforming potency than the untreated ones (TF of 0.33% compared with 0.6% at the treatment concentration of 15 µg/cm²). Although the amount of Fe mobilized from particles by dfx was greater than that of Fe(II) mobilized by fz (Fig. 1b), the decrease in TF of both samples was mostly achieved after the pretreatment only with the Fe(II) chelator fz. A strong decrease in HO production induced by the Q and DE particles pretreated with fz + dfx was recorded.

Co-treatment of SHE cells with Q (30 µg/cm²) or DE particles (15 µg/cm²) and the antioxidant SOD, catalase and mannitol at three concentrations decreased the TF in an antioxidant concentration-dependent manner. Catalase at 40 µg/ml almost abolished Q- and DE-induced transformation. Mannitol was also a strong inhibitor, whereas SOD at the highest concentration of 50 µg/ml decreased the TF induced by Q and DE by 3.7- and 2.5-fold, respectively.
Cell division aberrations

A significant concentration-dependent increase in the frequency of abnormal mitoses was induced by both the untreated Q and DE samples. Mitotic spindle disturbances, mono- and multi-polar mitoses and some chromosome lagging were the most frequently observed.

The iron-depleted Q particles showed a decreased ability to induce aberrant mitoses when compared with the effect of the untreated sample at the same concentrations, but the difference was statistically significant ($P < 0.05$) only at one concentration (9 $\mu$g/cm$^2$). The $f'c + dfx$ treated DE particles appeared scarcely less active than the untreated particles in the induction of abnormal mitoses (differential staining).

The frequencies of aberrant mitoses in the SHE cells co-treated with Q or DE at three concentrations and an antioxidant are shown in Fig. 2. For both particles the frequency of abnormal mitoses was significantly decreased in the presence of any of the antioxidants.

**DISCUSSION**

Our study shows that iron associated with the particle surface is partly responsible for the trans-
forming potency of silicas. This is supported by the observation that removal of iron from the particles by chelators significantly decreases the ability to induce morphological transformation of SHE cells. Iron present on the surface of particles as an impurity, mostly in the Fe(III) state, will undergo the Haber–Weiss cycle and catalyse the formation of ROS, in particular of hydroxyl radicals. Thus, through the generation of ROS at the surface of particles, iron may be an important factor in the pathological effects of silica.

Taking into account that the treatment with iron chelators decreased but did not suppress the HO· yield of quartz particles, additional reactive sites (e.g. silicon based) could also be involved in the generation of ROS at the surface of crystalline silica particles. The inhibitory effects of antioxidants on the induction of cell transformation and mitotic aberrations shown in this study provide additional evidence on the involvement of ROS in silica carcinogenicity.

Fig. 2. Effect of antioxidants on (a) the aberrant mitotic spindles induced by Q (immunostaining) and (b) the abnormal chromosome arrangement induced by DE (differential staining). Treatment with Q (+) and DE (+) alone. Co-treatment with 50 µg/ml SOD (star), 40 µg/ml catalase (filled circles) and 40 mM mannitol (open triangle). (*) Statistical significance between treated and control cultures; (+) start of significance between treated and co-treated cultures at P < 0.05.

REFERENCES


Iron and surface free radical activity of silica

