Effect of Thermal Treatment of Refractory Ceramic Fibres on the Induction of Cytotoxicity and Cell Transformation

MAURA TOMATIS, IV ANA FENOGLIO, ZOÉ ELIAS, ODILE POIROT and BICE FUBINI

1Dipartimento di Chimica IFM and Interdepartmental Center ‘G.Scansetti’ for Studies on Asbestos and Other Toxic Particulates, Università degli Studi di Torino, Via P.Giuria 7, I-10125 Torino, Italy;
2Laboratoire de Carcinogénèse In Vitro, Institut National de Recherche et de Sécurité, F-54500 Vandoeuvre-lès-Nancy, France

Refractory ceramic fibres (RCFs) are amorphous fibres made up of various metal oxides (mainly Al₂O₃ and SiO₂), used as high temperature insulation materials. We have examined the surface properties of three RCFs from the TIMA repository, RCF1, RCF3 and RCF4 (RCF1 heated at the expected working temperature), which showed different potentials for cytotoxicity and the induction of morphological transformation in Syrian hamster embryo cells, RCF4 being less active. The degree of surface hydrophilicity, evaluated by adsorption calorimetry, showed that RCF4 is less hydrophilic than RCF1, likely as a consequence of the prior thermal treatment. In order to investigate the effect of thermal treatment on the cellular response to RCFs we have heated (800°C for 24 h) the most active fibre (RCF3) in air until it was converted to a fully hydrophobic material. The cytotoxic (colony forming efficiency) and transforming potencies of heated and unheated RCF3, measured at concentrations between 1 and 16 µg/cm² of culture dish for 7 days, were then compared. Both cytotoxicity and potential to transform cells dramatically decreased when fibres were heated, indicating that hydrophobicity blunts the cellular response to RCF3. These results confirm that the state of the surface of RCFs modulates the biological response elicited and that a correlation exists between the degree of surface hydrophilicity and induction of cell damage and transformation. Thermal treatment, by lowering the surface affinity of RCFs for water, inhibits some cell–fibre interactions and decreases the extent of internalization of the fibres.

Keywords: colony forming efficiency; heat of adsorption; hydrophilicity/hydrophobicity; morphological transformation; refractory ceramic fibres

INTRODUCTION

Refractory ceramic fibres (RCFs) are amorphous fibres used as high temperature insulation materials, made up mainly of silica, alumina and various metal oxides. Epidemiological studies among workers employed in their manufacture have shown pleural plaques and obstructive respiratory diseases (Lemanster et al., 1998). Inhalation studies on rodents have shown the development of lung tumours (rats), pleural mesotheliomas (hamsters and rats) and interstitial fibrosis (Hesterberg et al., 1991; Bunn et al., 1993; Mast et al., 1995a,b). Not all fibres, however, are equally potent carcinogens, their potency depending upon different factors: dimension, chemical composition, surface reactivity and thermal history. Previous studies on the cytotoxic and transforming potencies of RCFs (TIMA) on Syrian hamster embryo (SHE) cells have shown different abilities of RCFs to induce cytotoxicity and morphological transformation (Elias et al., 2002). In particular, RCF4 is less cytotoxic and less transforming than RCF3 and RCF1. RCF4 and RCF1 have the same chemical composition, RCF4 being produced by heating RCF1 at the expected working temperature in order to simulate a used material. Thermal treatment may affect surface activity and/or the average size of the fibres. Scanning electron
microscopy (SEM) indicates that while the same size range is covered by RCF1 and RCF4, RCF4 is richer in short fibres, which may only partly account for the differences in the biological responses elicited. In order to establish the role played by surface hydrophilicity/hydrophobicity on the cytotoxicity and transforming potency of the fibres, we have measured the degree of hydrophilicity by means of adsorption calorimetry with water vapour as the adsorbate. RCF4, which is less cytotoxic and transforming, also turned out to be less hydrophilic, suggesting an influence of the degree of hydrophilicity/hydrophobicity on the overall pathogenicity of a given type of fibre. We then heated RCF3 fibres until they became mostly hydrophobic and compared the cytotoxic and transforming potencies of the heated fibres with the original ones.

**MATERIALS AND METHODS**

**Samples**

The three different types of RCF samples considered (TIMA, USA) included kaolin-based ceramic fibres (RCF1), high purity fibres (RCF3) and ‘after service’ RCF1 that had previously been heated at 1300°C for 24 h, then stored in air (RCF4) (Mast et al., 1995a).

**Heated fibres**

Thermal treatment of RCF3 was performed by heating the fibres in air at 800°C for 24 h. The treatment does not significantly change the specific surface (evaluated by the BET method) and the average dimensions (determined by SEM).

**Heat of adsorption of water**

The heat of adsorption was measured by means of a Tian-Calvet microcalorimeter (Setaram) connected to a volumetric apparatus, which allows simultaneous measurement of the adsorbed amount (uptake \( n \)) and the equilibrium pressure (\( p \)) for small increments in the water vapour in contact with the fibres. Subsequent doses of the adsorbate were admitted to the sample following a technique previously described (Fubini et al., 1992). A typical adsorption sequence comprised three runs: (i) dosing successive amounts of water vapour onto the sample up to a defined pressure, typically 10 torr (Ads I); (ii) desorption at 30°C under vacuum; (iii) readsorption (Ads II) for evaluation of the fraction of adsorbate which is reversibly held on the surface.

**Cell cultures and treatment**

SHE cell cultures were established from individual 13 day gestation foetuses. The culture medium was Dulbecco’s minimal essential medium (DMEM) (Gibco), pH 7.0, supplemented with 20% preselected, heat-inactivated, foetal calf serum (Hyclone) and 2 mM L-glutamine (Gibco) (complete medium). The cells were incubated at 37°C in 10% CO₂. In order to preserve their physicochemical characteristics, the test fibres were not submitted to a preliminary sterilization treatment. The stock suspensions in sterile tri-distilled water were prepared immediately before cell treatment. Serial dilutions were made in culture medium without serum and the treatment dilutions were made in complete medium. At least three concentrations for each sample were tested in each experiment.

**Colony-forming efficiency and transformation assay**

X-irradiated SHE feeder cells were seeded at 6 × 10⁴ cells/60 mm dish in 2 ml of complete medium and the next day 300 target SHE cells in 2 ml of complete medium/dish were seeded onto the feeder cells. Treatment took place 24 h later, for 7 days. At least 10 dishes per point were used. Each dish received 4 ml of treatment dilution or complete medium (negative control). After 7 days incubation the dishes were washed (Hank’s phosphate-buffered saline; Flow Laboratories) and the colonies fixed (absolute methanol), stained (10% Giemsa) and counted under a stereo microscope. For each treatment concentration and control the colony-forming efficiency (CE) was calculated by dividing the total number of colonies by the total number of target cells seeded. The relative CE [i.e. (CE of the treated cells/CE of concurrent control) × 100] was used to determine the cytotoxicity of a treatment.

The identification of morphologically transformed (MT) colonies was conducted by examination of each colony at 12–50× magnification under a stereo microscope. The criteria described by Pienta et al. (1981) were used to define a MT colony. For each sample concentration the MT frequency was calculated by dividing the number of transformed colonies by the total number of colonies and multiplying by 100. The data presented are the combined results of three experiments.

**RESULTS**

**Heat of adsorption of water on the original fibres**

Figure 1 shows the volumetric isotherms uptake (\( \mu \text{mol/m}² \)) versus equilibrium pressure (torr) and differential heat (kJ/mol) versus uptake (\( \mu \text{mol/m}² \)) for adsorption of water vapour on the three RCFs for Ads I. The adsorption capacity of the three RCFs (Fig. 1a) was as follows: RCF1 > RCF3 > RCF4. The amount of water vapour adsorbed on the RCF4 surface was about half that adsorbed on RCF1: 11 and 20 \( \mu \text{mol/m}² \), respectively, at an equilibrium pressure of 5 torr. Furthermore, while half of the water vapour adsorbed on RCF1 is irreversibly fixed at the surface, all water adsorbed on RCF4 may be removed by simple evacuation (data obtained from both Ads I
and Ads II; not reported for brevity). On all samples the differential heat, i.e. energy of interaction (Fig. 1b), decreases with increasing coverage down to a plateau value. Such behaviour indicates heterogeneity of the surface sites which interact with water: on RCF1 and RCF3 the energy of interaction varies from 110 (strongest surface sites) to 65 kJ/mol. On the RCF4 surface the energy of interaction is lower (90–40 kJ/mol). These data indicate that RCF1 and RCF3 are substantially more hydrophilic than RCF4.

Thermal treatment of RCF3 at 800°C dramatically decreased the amount of water vapour adsorbed (Fig. 2a): the heated fibres adsorbed only ~10% of the water vapour adsorbed by the original ones and no irreversible uptake of water took place on the surface of the modified fibres (data not reported for brevity). After heating the energy of adsorption decreased to ~20% of the original value (Fig. 2b): the plateau energy of interaction of water vapour attained at high coverage was 60 kJ/mol on RCF3, but only 15 kJ/mol on heated RCF3, indicating full hydrophobicity.

Cytotoxicity

The cytotoxic effects of heated RCF3, compared with the originals fibres, are reported in Fig. 2c. Both fibres reduced colony formation in a dose-dependent manner, but after thermal treatment cytotoxicity decreased dramatically: at 15 µg/cm² RCF3 was highly cytotoxic, reducing the relative clonning efficiency to 2%, whereas heated RCF3 decreased relative clonning efficiency only 20%.

Morphological transformation

Treatment with original and modified RCF3 induced a linear concentration-dependent increase in the frequency of MT of SHE cells (Fig. 2d), but their potencies were different. RCF3 appeared much more potent than heated RCF3 at all concentrations tested. The transformation frequency with RCF3 was 4.4% at a concentration of 15 µg/cm², whereas the transformation frequency with heated RCF3 was only 0.5% at the same concentration.

DISCUSSION

The adsorption of water vapour onto the surface of fibres is due to two main processes: (i) adsorption via hydrogen bonding on the surface silanols (SiOH); (ii) coordination of the molecular water onto exposed metal cations. The first process is fully reversible under the experimental conditions adopted for adsorption (30°C); the second one may be ether reversible or irreversible, depending upon the charge density of the cation. Thermal treatments lower the amount of silanols present at the surface, following progressive condensation into stable unreactive siloxane bridges (Si–O–Si) (Fubini, 1998). Heating also favours the embedding of metal ions. RCF4 has been exposed, after heating, to the atmosphere for several years. Prolonged contact with water vapour present in the atmosphere may have partly reconverted the siloxane bridges, generated by heating, into silanols, which may explain the partial hydrophilicity of RCF4. Conversely, heated RCF3 was used a few days after heating, which is consistent with their total hydrophobicity.

In both cases the decrease in surface hydrophilicity caused a reduction in the cytotoxic and transforming potencies. A possible explanation is a reduction in fibre phagocytosis when the surface becomes hydrophobic. It appears that free silanol groups must be present at the fibre surface of asbestos fibres (Brown et al., 1990, 1991; Sara et al., 1990) for interaction with the cell membrane, cell–fibre binding and, consequently, phagocytosis of the fibres. More generally, if
MT of cells is caused by signalling pathways activated by direct contact between fibres and cells, it may well be that, in the absence of surface silanols, such contacts are unlikely to occur.

CONCLUSIONS

Prolonged thermal treatment transforms RCFs into hydrophobic materials which have lost the intrinsic potential of the original fibres to damage and transform cells. This result suggests that heated fibres are less pathogenic than unheated ones when inhaled.

REFERENCES


