Role of Associated Mineral Fibres in Chrysotile Asbestos Health Effects: The Case of Balangeroite

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Received 3 February 2009; in final form 26 March 2009; published online 12 May 2009

Objectives: To evaluate the biodurability of balangeroite, present as contaminant of chrysotile asbestos in the Balangero mine, in order to have indication whether it might have been a confounding factor in the association of the mesothelioma cases reported among mine workers and employees.

Methods: The modifications taking place following incubation of the fibres in simulated phagolysosomal fluids have been measured on balangeroite, on one pure chrysotile sample (Val Malenco), on one chrysotile from Balangero with some associated balangeroite, and on two tremolite samples.

Results: The incubation modifies both chrysotile and balangeroite with substantial release in the medium of the metal ions which occupy the octahedral site in the mineral structure of the fibre while tremolite is virtually unaffected.

Conclusions: Considering the profound differences between the structure of balangeroite and amphiboles, previous results and observations on the poor ecopersistence of balangeroite, and the present data, we conclude that balangeroite traces may contribute to the overall toxicity of the airborne fibres in Balangero, but may not be compared to tremolite nor considered the sole responsible for the excess of mesothelioma found in Balangero.

Keywords: asbestiform minerals; balangeroite; biodurability; chrysotile; tremolite

INTRODUCTION

Chrysotile, the most abundant and widely employed asbestos, is often associated with minor amounts of tremolite, an amphibole asbestos. Both chrysotile and tremolite are widely recognized for their carcinogenicity (International Agency for Research on Cancer, 1977; WHO, 1986). Their relative potency in causing lung cancer and mesothelioma, however, is still under debate (Tweedale and McCulloch, 2004). Not all countries have banned the use of chrysotile asbestos yet and this form has not even been included in the Rotterdam convention (see details in Ogden, 2008).

The lesser potency of chrysotile is often related to its lower biopersistency. Indeed, people exposed to tremolite-contaminated chrysotile asbestos exhibit at autopsy an enrichment in the lung in tremolite by comparison to chrysotile, which has even been regarded as proof that mesotheliomas and lung cancers were caused by the associated tremolite and not by the massive doses of chrysotile which had entered their lungs day after day (Churg et al., 1984; McDonald and McDonald, 1997).

This unanswered question could be solved by accurate studies of tremolite-free chrysotile asbestos. A few authors have investigated this issue, but the work is confounded by the fact that chrysotile is often associated with other, less common, non-regulated asbestiform minerals such as carlosturanite and balangeroite (Compagnoni and Groppo, 2006), whose pathogenicity is mostly unknown. Recently, even such contaminants have been claimed to be responsible for mesothelioma cases reported in chrysotile-exposed workers, e.g. balangeroite (Yarborough, 2006;
Yarborough, 2007). To our knowledge, however, there are no studies reporting balangeroite in autopsies, biopsies, or sputum of people exposed to chrysotile in the Balangero mine.

Balangeroite is a fibrous mineral found mainly in the now disused chrysotile mine in Balangero (Italy) and in its surroundings (Compagnoni et al., 1983). Balangeroite is not an amphibole, as some misreported (Yarborough, 2006), nor belongs to the serpentine family, but has a gageite-like structure (Ferraris et al., 1987; Favero-Longo et al., 2009). Because of its occasional intergrowth with chrysotile (see Fig. 1), balangeroite abundance varies from one site to the other, so that any claimed percentage of it is just a coarse estimate. No one has ever been exposed to balangeroite alone nor is it easy to perform in vivo experimental tests with chrysotile-free balangeroite in sufficient amounts.

Many years ago, following a physico-chemical characterization from the standpoint of its potential toxicity, some of us suggested that balangeroite could contribute to the development of cancer in people exposed to airborne fibres during the activity of the Balangero mine (Astolfi et al., 1991). Later, in the context of a systematic study on the potential hazard associated with the various natural asbestiform minerals present in the Western Alps, we used a reasonably pure sample of balangeroite to assess, from a few cell-free and cellular tests, whether this type of fibre per se could be of some concern. Its surface reactivity, potential to release free radicals and to be cytotoxic to cells and activate them to release NO was similar to what had already been found for crocidolite (Gazzano et al., 2005; Turci et al., 2005) and for chrysotile (Gazzano, 2005; Turci, 2006) suggesting that it could be a hazard to human health. However, on the basis of these data only, there is no way to compare its carcinogenic potency to serpentine or amphibole asbestoses.

These results have now been evoked in the context of the higher carcinogenic potency of amphibole, and particularly tremolite, in the development of mesothelioma. It is suggested that, similarly to tremolite, balangeroite even if in small amounts could persist for longer periods of time than chrysotile in the lungs. In order to investigate this hypothesis, the biopersistence of balangeroite should be assessed, which is very difficult in the absence of in vivo tests. In our previous investigation, we limited our study to the simple use of the in vitro tests suggested for artificial vitreous fibres (Gamble’s solution, pH 7.4) which revealed a poor solubility of balangeroite indicative of a low biopersistence at neutral pH (Groppo et al., 2005).

More recently, we have examined the ecopersistence of the three fibrous minerals, namely chrysotile, balangeroite, and tremolite, in order to predict if weathered fibres retain the toxic potential present in freshly extracted fibres (Favero-Longo et al., 2009). We found that balangeroite was much less ecopersistent than tremolite, with a behaviour closer to chrysotile than to amphiboles, which is in contrast with the previous finding of the low biopersistence reported at neutral pH.

In view of these new findings, as no data are yet available on the solubility of balangeroite at acidic pH, we decided to extend our study to the modifications experienced by balangeroite fibres in simulated body fluids, with a chemical composition closer to that really experienced by an inhaled fibre, i.e. a phagolysosomal simulant fluid (PSF) (Stefaniak et al., 2005; Liu et al., 2008). PSF incubation is a straightforward tool in evaluating potential hazard of a mineral, as macrophage activation and frustrated phagocytosis are currently considered one of the key factors in asbestos toxicity (Kamp et al., 1992; Mossman and Churg, 1998; Kamp and Weitzman, 1999).

We report these results here, together with an account of the characteristics of balangeroite, which makes it different from both amphibole and serpentine asbestoses.

We have considered four different samples from the Italian Western Alps: a commercial chrysotile \([\text{Mg}_3\text{Si}_2\text{O}_5\text{(OH)}_4]\) from the Balangero mine, contaminated by some balangeroite fibres, two tremolite samples \([\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}\text{(OH)}_2]\), one from the Susa valley and one from the Lanzo valley, and a fairly pure balangeroite sample \([\text{MgFeMn}_2\text{Si}_4\text{O}_{12}\text{OH}_{40}]\). In order to examine the behaviour
of a pure chrysotile, we have also included a sample of chrysotile from Val Malenco, Italian Central Alps, as in previous studies (Turci, 2006; Turci et al., 2007a).

**EXPERIMENTAL METHODS**

**Asbestos and asbestiform minerals**

Five samples from different asbestos-rich outcrops of the Italian Western and Central Alps were selected:

1. a commercial sample of chrysotile from the serpentinites of the disused asbestos mine of Balangero (Lanzo valley, Italian Western Alps). Calculated formula from scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis: (Mg2.92Fe0.08)[Si2O5](OH)4. Since in the Balangero mine chrysotile from long-fibre asbestos-bearing veins is often intergrown with balangeroite up to ~5 wt%, this sample is mixed with a certain amount of balangeroite, which can be promptly be distinguished from chrysotile by SEM-EDS evaluating the occurrence of Mn distinctive of balangeroite stoichiometry.

2. a field sample of the brown, Fe-rich asbestiform balangeroite (Compagnoni et al., 1983) from the serpentinites of the inactive asbestos mine of Balangero (Lanzo valley, Italian Western Alps). Calculated formula from SEM-EDS analysis: (Mg0.67Fe0.29Mn0.04)[Si4O12](OH)2. We have decided to normalize the data to the mass of fibres.

3. a relatively pure tremolite sample from the serpentinite outcrops of Jovenceaux-Oulx (Susa valley, Italian Western Alps). Calculated formula from SEM-EDS analysis: Ca2.00(Mg4.84Fe0.16)[Si8O22](OH)2. Since iron plays an important role in the pathogenicity of asbestos fibres (Kamp et al., 1992; Fubini and Mollo, 1995; Aljandali et al., 2001; Gazzano et al., 2007), the evaluation of biosoluble Fe ions should be performed. However, it was not possible to correctly estimate the iron release, in spite of the fact that balangeroite contains even more iron than silicon, because, in the experimental conditions adopted, any iron removed from the fibres would be precipitated as Fe(OH)3, thus not measurable in the supernatant. We may confidently estimate, however, on the basis of its stoichiometric composition that the magnesium extracted from balangeroite is an underestimate of the corresponding iron removed.

4. an iron-rich tremolite sample from Lanzo Valley (Italian Western Alps). Calculated formula from SEM-EDS analysis: Ca2.00(Mg4.72Fe0.28)[Si8O22](OH)2. The amount of ions released from the fibres after incubation in the PSF for 30 days are reported in Fig. 2. The data are expressed both as nanomoles of ions released per mass of fibres (Fig. 2A) and as percentage ions brought in solution normalized to the total amount of each ion in the mineral formula (Fig. 2B). It is not clear whether the mechanism of the ‘dissolution’ of fibres in body fluids is determined by the mass or by the surface of the fibres, most likely it is a complex mechanism which starts from the surface but also involves the bulk. For this reason, we have decided to normalize the data to the mass of the fibres.

5. a pure chrysotile specimen, with limited iron contamination, from Val Malenco (Italian Central Internal Alps). Calculated formula from SEM-EDS analysis: (Mg2.93Fe0.07)[Si2O5](OH)4. Small bundles of each sample were separated from the hosting serpentinite rocks with Teflon tweezers and gently crushed in an agate mortar in order to further isolate the fibres and favour their good dispersion. The fibres were then washed with ultrapure MilliQ water.

**Incubation of minerals in PSF**

A 0.02 M potassium hydrogen phthalate (KHP)-buffered PSF was prepared according to Stefaniak et al. (2005) and the pH was adjusted to 4.5. Formaldehyde (0.1%) was added in order to prevent the growth of algae or bacteria. Ultrapure MilliQ (Millipore, Billerica, MA, USA) water was used to prepare the buffer. Fibres were incubated (3 mg ml⁻¹) in PSF and kept in the dark for 30 days at 37°C in unshered flasks. All incubations were performed twice."
the amount of magnesium, while the Si/Mg ratio is much 1.

In the case of the commercial chrysotile sample from Balangero, the contribution of balangeroite occurrence to total Mg and Si can be roughly estimated assuming that all Mn\(^{2+}\) measured by ICP-AES (data not reported) comes exclusively from balangeroite fibres and that balangeroite dissolves congruently (Favero-Longo et al., 2009). Reported Mg and Si amounts were recalculated from Mn/Mg and Mn/Si ratios deduced from balangeroite stoichiometry.

Three different patterns of dissolution are clearly evidenced: (i) both tremolite specimens are extremely stable and just release traces of the two ions in similar amounts (<0.5% of Si and Mg is removed during incubation); (ii) both chrysotile samples, as expected, reveal a large and selective loss of magnesium (~16 and 10% of the total Mg amount in the commercial sample and Val Malenco sample, respectively) and a lower, thus important, release of Si (~4% of the total in both samples) showing the well-known incongruent mechanism of dissolution (Luce et al., 1972; Thomassin et al., 1977; Schott et al., 1981; Turci et al., 2007a). The incongruence of dissolution (Mg > Si) is more pronounced for commercial chrysotile than chrysotile from Val Malenco, likely because, in the former sample, greater fragmentation and/or the occurrence of intergrown balangeroite fibres facilitate the access to the octahedral sheets; and (iii) balangeroite releases substantial amounts of both elements (~4% of the total) in nearly equal quantities, confirming previous findings (Favero-Longo et al., 2009).

**DISCUSSION**

**Crystal structure and stability in body fluids**

The three types of fibres examined have quite different crystal structures from one another (Fig. 3).

The crystal structure of ‘chrysotile’ results from the association of a tetrahedral silicate sheet of composition \((\text{Si}_2\text{O}_5)\) with an octahedral brucite sheet of composition \(\text{Mg}_3\text{O}_2\text{(OH)}_4\), in which iron can substitute for magnesium. The two sheets forming chrysotile fibres are bonded to form a 1:1 layer silicate; a slight misfit between the sheets causes curling to form concentric cylinders, with the brucite-like layer on the outside of the cylinder.

The structure of ‘balangeroite’ is based on an octahedral framework where channels are occupied by a chain of silicate tetrahedra. The chains of octahedra are grouped in three (walls) and four (bundles) rows running along the fibre axis.

The structure of ‘tremolite’ can be described in terms of a basic structural unit formed by a double tetrahedral chain (corner linked SiO\(_4\) tetrahedra) of composition \((\text{Si}_4\text{O}_{11})\). These silicate double chains share oxygen atoms with alternate layers of edge-sharing MO\(_6\) octahedra, where M stands for Mg\(^{2+}\) and Ca\(^{2+}\).

What makes a particle eco- and biopersistent, beside translocations, are the modifications experienced by the fibre upon contact with fluids and their dissolved components. Dissolution is seldom congruent, as it usually starts by removal of some of the metal ions in octahedral positions present loosely bound at the surface, with vacant coordination valencies. If the structure is tight, with no ion mobility nor access to internal positions, the dissolution will stop once surface ions are removed, as happens with tremolite. Alternatively, if the crystal structure allows ion mobility and access of the solvent and solute molecules to internal ions, dissolution may proceed, in some cases even up to partial disruption of the crystal framework. Acidic media and/or metal chelators usually assist such processes.

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*Fig. 2. Mineral dissolution in PSF with a pH of 4.5 after 30 days of incubation in a non-stirred system kept at 37°C in the dark. The amount of Mg (white bars) and Si (grey bars) brought in solution is reported as the number of ions normalized both by fibre mass (A) and by total quantity of each ion in the mineral formula (B).*
This is the case for both chrysotile and balangeroite, occurring, however, via different mechanisms.

Chrysotile looses the brucitic layer rolled up with the silicate one, often retaining its fibrous shape even if most of the brucitic layer has been released. A partial hydrolysis of the silicate layer deprived of its octahedral counterpart accounts for the presence of silicon atoms in the supernatant, as a silicate anion. This process, widely described (Luce et al., 1972; Thomassin et al., 1977) and recently reported by some of us (Turci et al., 2007b) may proceed until the disruption of the fibre only under very strong conditions such as a prolonged incubation in solution of strong acidic chelators and with an association of strong chelators and ultrasound. However, in more mild conditions such as that experienced in the environment and in the body, the process may proceed involving a few external layers and modifying the nature of the fibre surface, hence its reactivity (Favero-Longo et al., 2005; Turci et al., 2007a). This is the case in our experiment with PSF at pH 4.5, with some components which may act as chelators. The iron present in chrysotile, which substitutes for magnesium, will follow the fate of the brucitic layer or may even be one of the first ions to be extracted by the chelators.

Balangeroite, with opposing behaviour to chrysotile, has more metal ions than silicon; it may, in fact, be regarded as a sort of complex iron oxide containing, within its crystal structure, patches of a silicate framework. Thus, it offers to the surrounding fluid a large number of octahedrally coordinated cations which, unlike amphiboles, may be easily removed. Magnesium and iron are released and, as a consequence, also the silicate patches become loosely bound and pass into solution. This was found in an extensive investigation of such mechanisms mimicking the environmental forces acting on the fibres (Favero-Longo et al., 2009).

The present results appear to suggest that a similar behaviour may occur within the body.

As a general conclusion, we may confidently state that while the tremolite does not appear to be attacked by the fluid, both chrysotile and balangeroite are modified appreciably, which suggests, for both, a much lower biopersistence than amphiboles.

Other evidence of the difference between balangeroite and tremolite can be drawn from the previous work performed within our Interdepartmental Centre on asbestos environmental hazard in the Western Alps (Fornero, 2005; Groppo, 2005; Turci, 2006) as follows:

- Tremolite, but not balangeroite, is found airborne or in the flowing water close to chrysotile-bearing serpentinites in the Italian Western Alps.
- Sentinel animals may contribute to evaluation of asbestos environmental hazard. In the lung of cows living in the Lanzo Valley, the tremolite/chrysotile ratio in cows’ lungs was higher than what found airborne in the breeding area, confirming what reported for humans. Significantly, no balangeroite was ever found in the lung of sentinel animals (Belluso et al., 2006).
- Opposite to tremolite, the kinetics of ion release in balangeroite do not reach any plateau during a simulated bio-weathering process (Favero-Longo et al., 2009), suggesting an ongoing process continuing with time.

**CONCLUSIONS**

We are fully aware that to state the toxicity of a fibre in the absence of human studies, some in vivo tests have to be associated to in vitro data, the latter being only predictive of a potential hazard. Therefore, we do not think that the present data may add much to the knowledge on the hazard to be associated to the exposure to balangeroite or to chrysotile.
largely contaminated by balangeroite. Nevertheless, we feel that, on the basis of the general knowledge previously acquired on this asbestiform mineral and of the additional experimental results, we may rule out the idea that the balangeroite occasionally associated to chrysotile might be largely responsible for the health effects found in humans, e.g. among the people exposed in Balangero (Mirabelli et al., 2008), as claimed in some epidemiological surveys and discussions (Yarborough, 2006; Case and McDonald, 2008).

The role of tremolite contaminant in overwhelming the effect of chrysotile in the development of mesothelioma is still a very controversial question, while the majority of the scientific community agrees that it cannot apply to lung cancer. However, such hypotheses are based on acceptable chemical and mineralogical grounds as, indeed, tremolite appears very persistent in both the environment and body fluids. In contrast, balangeroite has a crystal structure different from amphiboles and exhibits an ecopersistence and a durability in body fluids of the same order of magnitude of chrysotile and was ever detected in exposed workers. Under such circumstances, it may slightly contribute to the overall toxicity, but cannot be considered responsible for the excess of mesothelioma found in Balangero in past and more recent studies (Piolatto et al., 1990; Silvestri et al., 2001; Mirabelli et al., 2008).

FUNDING

Regione Piemonte, Environmental Section.

Acknowledgements—Many of the statements on the presence and behaviour of balangeroite in the Western Alps are the result of a collective work of many scientists within ‘G. Scansetti’ Centre involved in the project ‘Asbestos Hazard in the Western Alps’ committed by Regione Piemonte, whose financial help is here acknowledged. R.C. acknowledges Prof. Alain Baronnet, Paul Cézanne University (Aix-Marseille III), and Centre de Recherche en Matière Condensée et Nanosciences, Marseille, France, for the Transmission electron microphotography microphotography.

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