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Asbestos fibers promote iron oxidation and compete with apoferritin enzymatic activity

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ABSTRACT

Asbestos fibers interact with many different proteins and may affect either their structure or functions. The aim of this study was to determine whether ferritin absorbed onto fibers might modify its ferroxidase activity. By measuring apo-ferritin ferroxidase activity, data demonstrated that ferritin in the presence of fibers did not significantly modify this enzymatic activity. However, fibers in the absence of ferritin promoted ferrous iron oxidation. Evidence suggests that asbestos fibers may promote iron oxidation and subsequently affect cellular iron homeostatic mechanisms.

KEYWORDS

Asbestos; iron; ferritin

Introduction

Asbestos exposure is known to trigger chronic inflammation and cancer (Khandia and Munjal 2020; Lemen 2016; Liu, Cheresh, and Kamp 2013; Zolondick et al. 2021). Several investigators demonstrated that asbestos fibers interact with many different proteins and may affect either their structure or functions (Borelli et al. 2018; Carbone et al. 2019; Carey et al. 2021; Gaudino, Xue, and Yang 2020). Protein-asbestos fiber interaction is evident in the formation of ferruginous/asbestos bodies (AB), which consist of an asbestos fiber, coated with iron (Fe) containing proteins (Bardelli et al. 2017; Crovella et al. 2018). Borelli et al. (2007) showed the presence of ferritin in the AB coating material, and specific analysis suggested that ferritin may present a misfolded secondary structure. It is of interest that Pascolo et al. (2015) demonstrated the occurrence of a conformational change in the protein component of the AB coating. The high levels of β-sheet protein conformational structures were postulated to play a role in asbestos-induced damage.

Ferritin is a key factor in the complex process of Fe homeostasis. This protein stores cell Fe in a safe form (ferric) avoiding oxygen radical formation and, following ferritinophagy (Toyokuni et al. 2021) results in Fe availability. Asbestos exposure trigger significant cellular ferritin synthesis (Fang and Aust 1997). In vitro experiments, *in vivo* animal model and tissues of exposed subjects showed that respiratory cells exposed to asbestos undergo Fe overload despite an increased expression of divalent-metal transporter 1 (DMTI) and ferroport (FNP1) suggesting an imbalance between ferritin availability and free Fe (Ghio et al. 2009, 2016; Ghio, Pavlisko, and Roggli 2015; Wang et al. 2006). This paradoxical scenario, which suggests a functional Fe deficiency, in the presence of Fe overload, might be explained by assuming that the fiber-absorbed ferritin does not enable Fe to be available, and hence the cell paradoxically still requires more Fe. Eventually the fiber inside the cells may undergo AB formation, from which Fe release may be irreversibly impaired and sequestered from the cell environment. In this scenario, ferritin plays a key role, where (1) this protein is absorbed by the fibers, (2) contributes to AB formation, (3) is continuosly synthetized and (4) is also secreted in extracellular vesicles to initiate Fe overload and DNA damage in recipient cells (Ito et al. 2021; Toyokuni et al. 2021). The aim of this study was to examine whether under these particular conditions, ferroxidase enzymatic activity is maintained in order to avoid Fe-mediated damage.

Materials and methods

All chemical reagents used in this study were of analytical grade and purchased from Sigma-Aldrich unless otherwise stated. UICC asbestos was obtained from SPI supplies (Borelli et al. 2018). Wollastonite, as a non-asbestos silicate powder, was used as a control particulate and a kind gift of Bal-Co. SpA (Sassuolo, MO, Italy). Erionite was a kind gift from Michele Carbone, University of Hawaii. The ferroxidase activity of apoferritin was measured spectrophotometrically using a Perkin-Elmer at 5°C in 310 nm in HEPES buffer 20 mM at pH 7, containing 0.4 mM Fe^{+2} ammonium citrate (Ardini et al. 2018) and 10 mM $MgCl_2$, at 37°C. Iron (II) ammonium citrate was dissolved in Borate-Cacodylate buffer at pH 5. Apoferritin 100 µg/ml was incubated either in the absence or presence of fibers (15–50 µg/ml). After 10 min, iron (II) 0.4 mM was added and OD increase was measured for 4 min. Iron autooxidation was subtracted. To investigate whether or not oxidized Fe was taken up by the fibers, 1 ml complete HEPES buffer containing 0.1 mM iron (II) in 1 ml, either in the presence or absence of 50 µg chrysotile, incubated at 37°C for 40 min, to ensure the complete Fe oxidation, was centrifuged on Amicon Ultra 0.5 Filter Devices 3K at 4500 g for 30 min in a microcentrifuge. The filtrate was measured at 310 nm. Iron (III), holoferritin (50 µg/ml) and chrysotile alone (completely retained), were also processes as negative (iron) and positive (holoferritin and fibers) controls. The amount of Fe retained was calculated on the basis of the amount of Fe filtered, taking as 100% the OD310 nm of the starting iron (III) solution. Data were subjected to one-way ANOVA analysis and statistical significance determined using Student's t test or Tukey's multiple comparison. The criterion for significance was set at $p < 0.05$.

Results

Figure 1a shows that a clear increment, (about 35%) statistically significant, was found when the apoferritin was incubated with 50 µg/ml chrysotile. Surprisingly, control experiments showed that the increment was completely accounted for the contribute of the fibers themselves: therefore, while ferroxidase activity of apoferritin in the presence of chrysotile seems to be unaffected, an activity comparable to that exerted by apoferritin was carried out by fibers alone. Figure 1bshows the iron oxidative activity of various types of asbestos and control fibers in the range 15-50 µg/ml. The rate of iron oxidation exerted by chrysotile seemed to be higher, but it was not significantly different from that of the other fiber types examined. This activity was asbestos specific, since other types of fibrous material (wollastonite and erionite), failed to exert any iron oxidative activity. Figure 1c demonstrates that a significant amount of iron (III) was retained on filter membrane, together with the chrysotile, while in the absence of fibers, only traces of metal were retained. As expected holoferritin was almost completely retained in the filter (more than 95%). The retained amount of Fe was calculated to be 0.544 nmoles/µg fiber.

Discussion

The absorption of ferritin on asbestos fibers might, in principle, modify ferroxidase activity, as described for other asbestos absorbed protein (Borelli et al. 2018). This process may markedly affect Fe homeostasis in exposed cells. By assaying the total ferroxidase activity of apoferritin in the presence of fibers a significant change was detected, which was attributed to the fiber and not apoferritin. Hence the fibers themselves appeared responsible for increased iron-oxidizing activity. All types of fibers showed an enhanced activity comparable to that of apoferritin but no significant difference between fibers. This activity appears to be independent on the presence of Fe in the fiber structure, since Fe-rich or Fe-poor fibers failed to exert any significant differences.

At present the mechanisms underlying the fiber ferroxidase activity are not known; however, it appears that the ferric iron is sequestered at least partially into the chrysotile fiber structure. It is conceivable that in the cell the fiber might compete with the enzyme for Fe oxidation and storage. Ghio et al. (1994) reported that chrysotile binds approximately 0.175 nmol of Fe(III)/µg), which is lower with respect to the 0.544 nmoles noted in this study. This discrepancy may be derived from incubation with Fe(II), which might be oxidized and

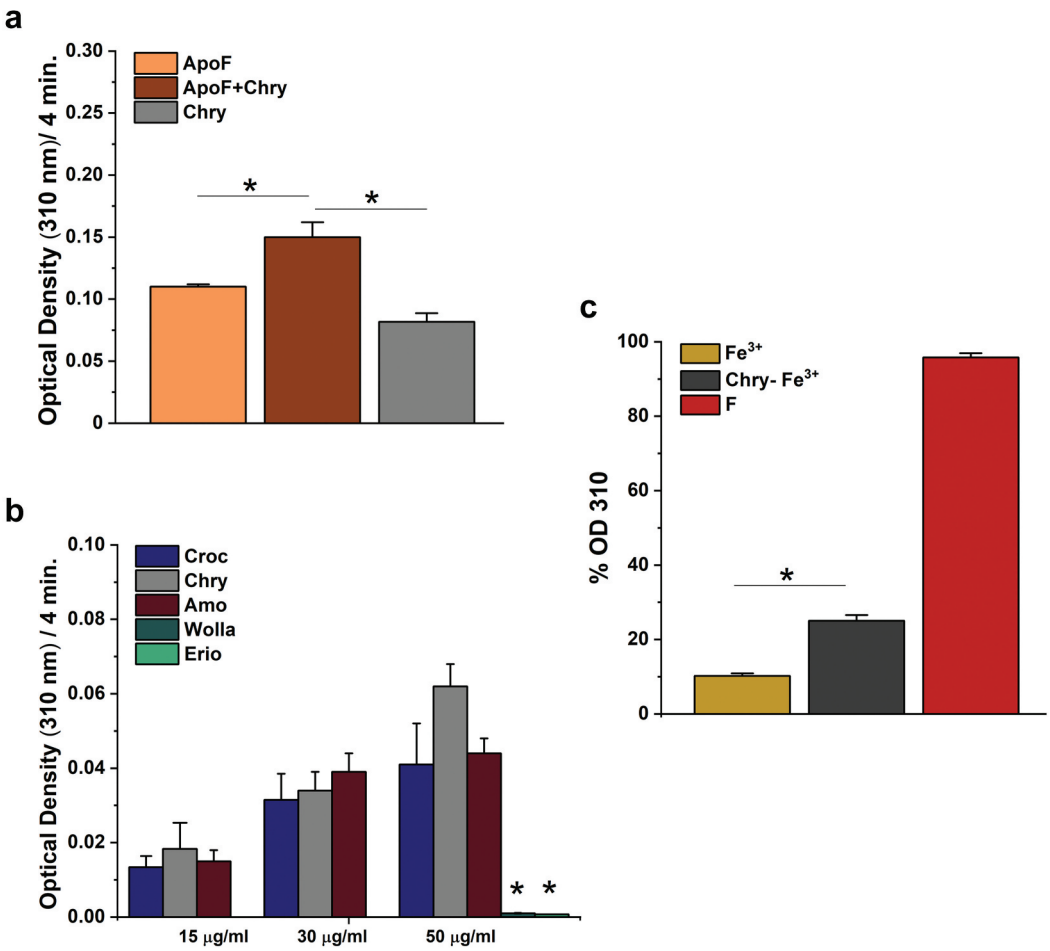

Figure 1. **a)** ferroxidase activity of apo-ferritin in absence and presence of chrysotile. Values are expressed as the mean of OD increment for 4 min evaluated in 5 different experiments ± SE. The iron auto-oxidation was subtracted. **b)** dose response curve of Fe oxidation carried out by various type of asbestos fibers. The iron auto-oxidation was subtracted. Values are expressed as the mean of OD increment for 4 min evaluated in 5 different experiments ± SE. **c)** Amount of iron retained following centrifugation on Amicon Filter Devices 3K. The filtrate was measured at 310 nm. Iron (III) (0,1 mM), ferritin (50 μg/ml) and chrysotile alone (50 μg/ml, completely retained), were also processes as negative and positive controls. The amount of Fe retained was calculated on the basis of the amount of metal filtered, taking as 100% the OD310 nm of the starting iron (III) solution. Values from three experiments are expressed as mean % ± SE. Chry= chrysotile; Croc =crocidolite; Amo = amosite; Wolla = wollastonite (50 μg/ml); Erio = erionite (50 μg/ml); F=holo-ferritin; F=holo-ferritin. * Significant from control $p<0.05$.

subsequently incorporated into the chrysotile fiber. Our findings suggest that oxidized Fe is hindered from entry into the ferritin shell, which requires metallic chaperon involvement (Toyokuni et al. 2021). It is postulated that during cell-fiber interaction, in competition with apoferritin, Fe (II) may be oxidized and sequestered by the fibers and made unavailable for the cell; at the same time also ferritin is absorbed by the chrysotile fibers. Subsequently, and depending upon the fiber load, cells might experience Fe deficiency. The decrease of Fe availability may stimulate new apoferritin synthesis and more metal uptake, as shown by Ghio et al. (2016) in lung tissue of patients with asbestosis and in cell cultures (Ghio, Pavlisko, and Roggli 2015). A vicious cycle is triggered and maintained until the ferritin and Fe absorbing capacity of the fibers lasts. The first cell type which interacts with inhaled asbestos fibers is the alveolar macrophage (Toyokuni 2019). This cell undergoes Fe overload and increased ferritin synthesis upon asbestos exposure (Ghio, Churg, and Roggli 2004; Ghio, Pavlisko, and Roggli 2015; Ito et al. 2020, 2021) and exhibits an increased expression of molecules involved in Fe uptake. These macrophages are characterized by a high turnover due to the high level of ferroptotic cell death (Ito et al. 2021). Within these cells AB are formed and the secretion

of extracellular vesicles containing Fe-loaded ferritin is triggered (Ito et al. 2021) which might induce persistent metal overload in bystander responsive target cells, creating tumor cells promoting conditions. In this complex scenario various events play a key role contemporaneously including ferritin absorption, ferritin ferroxidase activity, new ferritin synthesis, increase of Fe uptake, AB formation, fiber Fe uptake, Fe release from fibers, formation and secretion of extracellular vesicles.

Conclusions

Our data support a role for a new tile in this mosaic, that is, the Fe oxidative capacity of asbestos fibers. The relative extent and relationship between these processes might decide if a tumor, chronic inflammation or fibrosis will result from asbestos exposure.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings in this are available from the corresponding author [GZ] upon reasonable request

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