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On decreasing the carcinogenic activity of calcified chrysotile fibers

Хризотилдың кальцийленген талшығында канцерогенді белсенділікті төмендету мәселесі жайында

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Хризотил талшығын Mg портландцементмен өңдеген кезде жарым-жартылай Ca айнала бастаған. Осының нәтижесінде олар көп қабатты портландит $Ca(OH)_2$ жабылған, сонымен бірге қышқылдылығы, белсенді орталық мөлшері мен биологиялық белсенділігі өзгерді (мутагенділігі жойылған, макрофагтармен оттегінің белсенді формаларының туындауы анағұрлым төмен максимуммен және нативті хризотилмен салыстырғанда анағұрлым ұзақ фонға ие болды).

В статье рассмотрены результаты исследований, когда при обработке портландцементом волокон хризотила Mg частично заменялся на Ca, в результате чего они покрыты монослоем портландита $Ca(OH)_2$. При этом менялись кислотная сила, количество активных центров и биологическая активность (отсутствовала мутагенность, генерация активных форм кислорода макрофагами характеризовалась более низким максимумом и более длительным достижением фона по сравнению с нативным хризотилом).

Discovering carcinogenic properties of asbestos in cancer epidemiology and experimental studies resulted in a tough struggle between opponents and supporters of its continued industrial use. In some cases this struggle (and opposition in the first place) gets farther away from the reality and scientific facts and often acquires a commercial and political format.

The notion of asbestos is collective; it covers different fibrous minerals including crocidolite, amphibole asbestos banned long ago due to its high carcinogenicity, and chrysotile, the extent of carcinogenicity of which for humans is still debated. It is sufficient to recall findings of the recent Multicentre European study of the International Agency for Research on Cancer (IARC) that, on the whole, showed no risk for occupationally exposed persons [1]. The asbestos cement industry consuming about 80 % of all chrysotile has no unambiguous data either [2]. Risks for the population with environmental exposure to chrysotile are even less clear. Amphiboles can, as a rule, be traced in the studies showing a high carcinogenic risk. In general, there is no scientific evidence convincing enough to ban chrysotile whereas safety of its substitutes requires further investigation [1]. Meanwhile they believe that the denial of chrysotile has already led to negative consequences such as an increase in the number of car accidents in Western Europe and, probably, significantly accelerated the collapse of towers of the World Trade Center during the terrorist attack in the USA, September 11, 2001. At the same time the asbestos-free roofing material Onduline, widely promoted as «safe and environmentally friendly», contains high concentrations of benzo(a)pyrene [3] which is a Group 1 carcinogen according to IARC classification, i.e. the agent carcinogenic to humans, and is an indicator of the presence of other polycyclic hydrocarbons including blastomogenic ones.

Our previous studies showed [4] that chrysotile fibers treated with Portland cement were significantly different from natural fibers, had a different structure of the crystal lattice where Mg was partially substituted by Ca, and were covered with a monolayer of portlandite — $Ca(OH)_2$. The suggestion was made about the formation in this case of a new fibrous mineral possibly with new chemical, physical and biological properties [11]. The decreased cytotoxicity, mutagenicity and carcinogenicity of chrysotile fibers treated by acids and heat under pressure, which we found, may serve as an indirect confirmation of the latter; i.e. the effect on the crystal lattice of the fiber changes its properties [5, 6].

On the surface of curved and tubular fibers of chrysotile asbestos there are positively (prevailing) and negatively charged active centers [5] where biologically active molecules can be adsorbed and generated; other carcinogens such as polycyclic aromatic hydrocarbons (PAH) may also metabolize there [7, 8]. The former primarily include active oxygen radicals and NO-radicals with high biological aggressiveness. As mentioned above, when the chrysotile fiber is treated with hydration products of Portland cement a chemisorption of Ca ions takes place and a surface layer of portlandite forms [9]. Further studies showed that in this case the acid strength shifted to the right and the alkaline active centers prevailed whereas the native asbestos contains more acid centers. The evaluation of the acid strength and active centers on the «asbestos-cement» fiber yielded a hypothesis about a weaker biological aggressiveness of the latter as compared to natural asbestos [10]. The study of mutagenic activity in the micronuclear test of the bone marrow of mice confirmed this hypothesis. Chrysotile fibers covered with hydration products of Portland cement showed no mutagenic activity whereas untreated fibers did [10].

Fiber carcinogenesis, including the asbestos one, is considered by most researchers to be non-genotoxic, i.e. cell transformation is not induced directly by the fiber but indirectly, through biologically active compounds generated by the fiber which affect the genetic apparatus of the target cell and cause mutation. However, in some cases a direct, «mechanical» effect of fibers on chromosomes and spindle during cell mitosis, which can also lead to mutation, are not excluded. It is regarded that asbestos has both inducing (causing mutation) and promoting effects; in particular it promotes the increase in the pool of mutated cells [7, 11–16].

In the organism of mammals both synthesis and destruction of active free radicals constantly occur. These free radicals include molecules or their fragments having one or more unpaired electrons on the outer orbital. They can be both neutral and positively or negatively charged. Because of their structure they are highly reactive. Active radicals include, in the first place, oxygen and NO-radicals. Generation of oxygen radicals is as follows:

I $O_2 + \bar{e} \rightarrow O_2^-$ (superoxide anion)

II $O_2^- + +2H^+ + \bar{e} \rightarrow H_2O_2$

III $H_2O_2 + \bar{e} \rightarrow OH^- + OH^-$ (hydroxyl radical)

Iron in its active form (Fe^{2+}) acts as a catalyst of these processes; it is also formed by means of the reaction of Fe^{3+} with an active oxygen radical superoxide anion (O_2^-)

I $Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$.

The hydroxyl radical is formed in the Fe^{2+}-dependent reaction (Fenton reaction).

II $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$.

Destruction of the hydroxyl radical ($OH^{\cdot}$) occurs in the reaction with Fe^{2+}

III $OH^{\cdot} + Fe^{2+} \rightarrow Fe^{3+} + OH^{-}$,

And superoxide anion — with Fe^{3+} (IV).

Apart from oxygen and NO-radicals a Fe-dependent peroxidation of lipids on cell membranes and in liposomes takes place in the organism, thus forming biologically active alkoxyl radicals. Active radicals play an important role in initiation and damage of many intracellular signal paths, secretion of different growth mediators, cytokines, in cell proliferation and apoptosis. They induce the DNA damage, its completeness and reparation as well as point mutations. A specific circulation of active radicals, particularly oxygen radicals, in the organism may, therefore, lead to both positive (protective) and negative consequences. It is probable that the law of «dose – time – effect» works here, too.

Nowadays active oxygen radicals, and the OH-radical in the first place, are thought to play the major role in the mechanism of the carcinogenic effect of asbestos [7, 12, 13, 17–22]. They are mainly formed from macrophages, but they can be also generated on the surface and inside target cells. Iron, the catalyst of these processes, can be a part of the crystal lattice of the fiber (amphibole) or be present in the form of admixtures on its surface (chrysotile).

When fibers affect cells, the latter experience an oxygen burst that leads to a sharp increase in the number of active radicals of oxygen [7, 21, 24], which cause an «oxidative stress» in target cells (here, lung epithelium and mesothelium) leading also to the damage of their genetic apparatus [7, 14, 21, 23, 24].

In contrast to long-living peroxides of lipids (alkoxyl radicals), the excess formation of which may be also caused by asbestos fibers, oxygen radicals live a very short life lasting nanoseconds. The radius of their effect is very small. For instance, the radius of effect of the OH-radical is about 100 nm, i.e. it affects only neighboring target cells. Here, naturally, it is both the number of cells and the number of oxygen radicals that matter. When discussing the role of active radicals, one should not forget about a complex effect of fibers on the target cell, about the importance of fibers' sticking to it (for this the presence of specific proteins such as fibro- and vitronectin, intergrins, and of their receptors on the cell membrane is necessary [25]), about the ability of activated macrophages to secrete a large number of various compounds that can activate and inhibit carcinogenesis [26, 27].

Taking into account all mentioned above, the evaluation of the ability of asbestos to generate formation of active oxygen radicals by a cell may serve as an indicator of one of the types of its biological activity. Using the acknowledged experimental model and method [28] we studied the available samples [10]. As it has been expected [29], native chrysotile activated macrophages rather quickly (the peak was observed in 4 minutes) and increased the number of active oxygen radicals (maximum 26×10^3 impulses) generated (when luminol was used — of OH-radicals), which, however, quickly (in 19 minutes) dropped to background values. The fibers taken from asbestos-cement were significantly less active, and the increase in the number of impulses was smooth. The peak was observed in 15–17 minutes, the maximum was 17×10^3 impulses, and the drop to background values lasted longer (27 minutes).

Thus, in this particular case also we found proof of our hypothesis [10] based on physical and chemical studies that properties of the surface of a fiber play an important role in its ability to induce a biological effect. «Screening» the surface of a chrysotile fibril with hydration products of Portland cement «eliminates» its mutagenicity and significantly reduces the potency to activated macrophages and generate biologically active molecules (in this case — active oxygen radicals). It was demonstrated [30] that asbestos also activated the generation of these compounds in the mesothelium, which is a known target for this type of carcinogenesis (mesothelioma). This does not happen when fibroblasts are affected. Moreover, if asbestos fibers cause damage of some signal paths and of the cell cycle in fibroblasts killing them, then the same was not observed in mesothelial cells. This might partially explain the «riddle» of the absence of connective tissue neoplasms in people and experimental animals exposed to asbestos.

The obtained results have a large scientific and practical importance. They broaden our knowledge and capabilities in studying the mechanism of fibrous carcinogenesis and show that the investigated biological aggressiveness of the fiber from asbestos-cement is much lower than that of asbestos, which is important since, as it has been mentioned, the major part of all asbestos mined is used for the production of asbestos-cement. These data confirm the hypothesis [27] that the crystal lattice of the fiber treated with Portland cement is changed so much that one can speak about a new fibrous mineral with new properties.

A weaker but a longer ability of asbestos-cement fibers to generate oxygen radicals by macrophages indirectly relates to one of the important problems of occupational pathology and hygiene — the extent of hazard of the intermittent effect. According to our data [31], a long-term effect of low doses of asbestos dust is

more dangerous than a short-term effect of a high concentration. It should be noted, however, that this has been demonstrated in experimental conditions using a method, not very adequate to such works. But the problem remains. Fibrous carcinogenesis is significantly different from other types of blastomogenesis, and the occurring pathology and its mechanism are significantly different from that caused by non-fibrous dusts. At the same time, the distinctive feature of «carcinogenic» fibers is their fibrogenicity where active radicals, and oxygen ones in the first place, play important, if not the main role [20].

Is it the evidence of the link between asbestosis and cancer? Etiologically yes; in both cases we, probably, speak about one and the same inducing factors; but pathogenetically — no, as the mechanisms of developing fibrosis and cancer or mesothelioma are different. The cell DNA damage, disruption of the processes of its reparation and mutation play an important role in the latter. The target cells are also different. It should be noted, however, that the chronic inflammation that occurs in both cases can contribute to the endpoint but by different ways [7, 20]. All mentioned above allows one to assume that the effect of intermittent dust exposures for the development of fibrosis and cancer may be different and it is not correct to extrapolate regularities from the fist to the second one. In this connection, the conclusion about a lower biological activity of asbestos-cement fibers in inducing the generation of oxygen radicals by macrophages must be considered correct. Yet, is it enough to speak about its smaller carcinogenicity? Formally — yes as oxygen radicals are of key importance in asbestos carcinogenesis and the carcinogenesis induced by asbestos substitutes. At the same time, it is well-known that quite a number of non-fibrous and non-carcinogenic dusts stimulate the generation of oxygen radicals by macrophages rather actively. At this, peaks of chemiluminescence under effect of those dusts are similar to those caused by asbestos when doses are almost equal [11, 29].

Obviously, not belittling the importance of oxygen radicals in fibrous carcinogenesis, it is essential to think about the importance other factors and fiber properties. Probably this still makes researchers speak about the «inscrutability» of the mechanism of this type of blastomogenesis. It is not by chance that there are so many hypotheses on this issue [7].

The necessity of continuation and broadening of studies in this direction is obvious. Means of decreasing the carcinogenic risk of asbestos dust for humans mostly depend upon the understanding of the mechanism of its carcinogenic effect on the organism and, in the first place, of the transforming effect on the cell.

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