Dissolution of Man-Made Vitreous Fibers in Rat Alveolar Macrophage Culture and Gamble’s Saline Solution: Influence of Different Media and Chemical Composition of the Fibers

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The effect of different chemical compositions of man-made vitreous fibers (MMVF) on their dissolution by alveolar macrophages (AM) in culture and in Gamble’s solution was studied. The fibers were exposed to cultured rat AMs, culture medium alone; or Gamble’s saline solution for 2, 4, or 8 days. The dissolution of the fibers was studied by measuring the amount of silicon (Si), iron (Fe), and aluminium (Al) in each medium. The AMs in culture dissolved Fe and Al from the fibers but the dissolution of Si was more marked in the cell culture medium without cells and in the Gamble’s solution. The dissolution of Si, Fe, and Al was different for different fibers, and increased as a function of time. The Fe and Al content of the fibers correlated negatively with the dissolution of Si by AMs from the MMVF, i.e., when the content of Fe and Al of the fibers increased the dissolution of Si decreased. These results suggest that the chemical composition of MMVFs has a marked effect on their dissolution. AMs seem to affect the dissolution of Fe and Al from the fibers. This suggests that in vitro models with cells in the media rather than only culture media or saline solutions would be preferable in dissolution studies of MMVFs. — Environ Health Perspect 102(Suppl 5):103–107 (1994)

Key words: dissolution, man-made vitreous fibers, rat, alveolar macrophage, Gamble’s, chemical composition

Introduction

Man-made vitreous fibers (MMVFs) are amorphous silicates manufactured from glass, rock, or other minerals by drawing, blowing or spinning the material into fibers. They are composed of oxides of Si, Al, Fe, alkaline and alkaline earths and other elements to add specific properties. The chemical composition of MMVF determines their chemical resistance and solubility in different environments (1–3). Biological activity of the fibers depends not only on their respirability, but also on their chemical durability and persistence (4–7). If the fiber dissolves rapidly, it is less likely to have long-term deleterious effects in the lungs (8).

Solubility studies of MMVFs have commonly been carried out in physiological saline solutions (9,10), where they gradually soften and subsequently slowly dissolve (11). Alveolar macrophages (AM) phagocytize inhaled fibers which then can be transported from the lungs within AMs (12–14). There is evidence that AMs can dissolve metal oxide particles (15–17) and probably also MMVFs.

The aim of the present study was to investigate the effects of different chemical compositions of MMVF on the dissolution of fibers by rat alveolar macrophage (AM) culture, culture medium alone, and Gamble’s saline solution.

Material and Methods

Chemicals

Hepes buffer (1M) and trypan blue (0.4%) were from Sigma Chemical Co. (St. Louis, MO). Triton X-100 was from Merck (FRG). EDTA was from Boehringer Mannheim GmbH (Penzberg, FRG). E1L silica standard (1000 ppm as SiO₂), spectrosil iron Standard solution (1000 ppm) and aluminium standard solution were from BDH Chemicals, Ltd (Dagenham, England). RPMI 1640 Medium with 25 mM of Hepes buffer without L-glutamine, trypsin solution (2.5%), L-glutamine 200 mM, penicillin-streptomycin solution (10000 IU/ml penicillin, 10,000 µg/ml streptomycin), Hanks balanced salt solution without calcium, magnesium and phenol red (HBSS) and fetal calf serum were from Gibco, Ltd. (Paisley, Scotland). Heparin 5000 IU/ml was from Medica (Helsinki, Finland).

Man-Made Vitreous Fibers

The fibers A (F), B, C, and G were prepared by conventional spinning techniques on a pilot line by Paroc Oy Ab Parainen, Finland. Neither binding material nor oil was added. Fibers D and E were normal rockwool samples received from Paroc Oy Ab. Sample H was commercially available ceramic fiber and the sample J commercially available glasswool. The very fine fibers were collected in a piece equipment, consisting of a rotating Vulcanized steel container, a separation chamber, and a filter system for collecting the airborne fibers. The majority (56–79%) of the collected fibers were less than 1 μm in diameter.

The fibers for the study were chosen based on their chemical composition (Table 1), particularly their Al and Fe content, because these elements markedly influence the dissolution of the fibers in aqueous solutions (18). The dimensions of the fibers were measured from SEM photographs magnified ×2600. A part of the
Man-made Vitreous Fibers in Rat Alveolar Macrophage (AM) Culture and in Cell Culture Medium

Adult male (273.7 ± 60.5 g) Han:Wistar rats (National Laboratory Animal Center, University of Kuopio, Finland) were used as the source of the AMs. Lavage of the cells was carried out by a modification of the method of Myrvik et al. (19) and Brain and Frank (20). Cell viability was never less than 95% (21). The AMs were resuspended in regular medium 1640 containing 100 IU/ml of penicillin, 100 μg/ml of streptomycin, 10% fetal calf serum (FCS) and 2 mM of l-glutamine and 10 IU/ml of heparin. The cell concentration was adjusted to 1 × 10^6 cells/ml, and the AMs were allowed to adhere to the culture wells for 1.5 hr in an incubator at 37°C in 5% CO₂/95% air mixture, then the medium in the wells was changed to remove the nonadherent cells. Fresh medium (2 ml) was added, and the culture wells were incubated overnight. Following incubation, the medium was removed, and fresh medium containing 200 μl/ml of MMVF was added to each well. Control wells contained only culture medium with mineral fibers. The wells were cultured for 2, 4, or 8 days. Fresh medium (1 ml) was added to the wells on day 4. For each time point, separate incubations were carried out.

After incubation, the medium was collected, and the cells were detached with a 0.25% trypsin solution containing 0.006% EDTA, lysed with 1% Triton X-100 solution, sonicated for 40 min, and filtered through a 0.45-μm Millipore filter using vacuum. Sr, Fe, and Al-concentrations in the filtrates were analyzed using a microcomputer-controlled atomic absorption spectrometer (AAS, Perkin-Elmer 2100) using a graphite furnace (Perkin-Elmer, HGA-700) technique. Three separate determinations were made to ensure the solubility of each fiber sample.

Man-made Vitreous Fibers in Gamble’s Saline Solution

The chemical composition of the Gamble’s solution (22) was modified according to Klingholz and Steinkopf (9). For determining the dissolution of fibers during continuous flow, a modification of an apparatus designed by Fürster (7) was used. A sample of 200 mg of each fiber was placed on the 0.45-μm pore Millipore filter paper and Gamble’s saline solution was passed through the sample using a peristaltic pump (Desaga PLG 132100, Heidelberg, Germany) at a flow rate of 3 to 5 ml/hr. The samples were kept at 37°C, and pH was adjusted to 7 with CO₂. The dissolution of the fibers was determined after 2, 4, and 8 days, with each fiber being tested twice. Two sample holders were included as controls in each battery of ten. The concentrations of Al, Fe, and Si in the effluents were measured with an atomic absorption spectrometer (AAS, Perkin-Elmer 5000).

Results

Man-made Vitreous Fibers in Rat Alveolar Macrophage Culture and in Cell Culture Medium

The amounts of silicon, iron and aluminum dissolved from MMVF in rat AM culture and culture medium were different with different fibers, and increased as a function of time (Figure 1A,B:Si; Figures 2A,B:Fe; and Figures 3A,B:Al).

More Si was dissolved in the culture medium than in the AM culture; the opposite was true for Fe and Al. The dissolution of Si was the greatest for the fiber C with a low Fe and Al content (Table 1), and the smallest for fibers D, E, and H, with high Fe or Al contents. The largest amounts of Fe were dissolved from the fibers B and E, which had relatively high Fe content, and the smallest from the fiber H, which had a low Fe content. The dissolution of Al was the highest from the fiber H with a high Al content, and much smaller from fibers C, G, and J, all with low Al content. Fibers D, E, and H seemed to show a negative correlation between their Fe and Al contents and the dissolution of Si from them.

Man-made Vitreous Fibers in Gamble’s Saline Solution

In the Gamble’s solution the dissolution of Si was highest from fiber C and lowest from fibers D, E, and H: in general it was higher in Gamble’s solution than in AM culture for fibers A, F, and G. For the other fibers it was lower or similar, while the dissolution of Fe was minimal. The dissolution of Al was higher from fibers D, E, and H, which all had high Al content, and low from all the others.

Discussion

The main thrust of these studies was to explore whether AMs can dissolve respirable-sized MMVF, and how the chemical composition of the fibers modifies their dissolution in different media. The results show that AMs play an active role in fiber dissolution but their effect seems to be complex. The dissolution profiles of the fibers exposed to AMs were different from those obtained with either Gamble’s solution or culture medium. The lower dissolution of Si by AMs could be explained partly by AMs preventing or slowing down the diffusion of elements by blocking the surface of the fibers. Another reason could be the lower pH in the AM cultures (15–17), which actually decreased during 2 to 3 days of incubation. In the controls, where the fibers were in culture medium alone, the pH increased, although the change of pH was smaller than in AM culture.

In AM culture, the dissolution of the fibers takes place both intra- and extracellularly, depending on the length of the fibers. Those of less than 20 μm are mainly dissolved intracellularly. There are, of course, many differences between intra- and extra-

| Fiber | A | B | C | D | E | F | G | H | J |
|-------|---|---|---|---|---|---|---|---|---|
| SiO₂  | 54.6| 50.4| 59.0| 44.4| 45.9| 54.6| 66.5| 52.3| 64.9|
| TiO₂  | 0.2| 0.3| 0.0| 1.0| 0.9| 0.2| 0.0| 0.0| 0.0|
| Al₂O₃ | 3.6| 3.3| 0.9| 13.6| 11.9| 3.6| 2.8| 46.9| 3.1|
| Fe₂O₃ | 1.5| 3.0| 0.3| 4.1| 1.5| 0.3| 0.1| 0.4| 0.1|
| MgO   | 4.9| 10.6| 4.6| 11.5| 8.9| 2.7| 0.0| 2.6| 0.0|
| CaO   | 31.2| 31.7| 35.5| 2.2| 31.5| 30.2| 14.5| 0.1| 7.6|
| Na₂O  | 0.6| 0.6| 0.1| 1.7| 1.6| 0.6| 0.2| 14.7| 0.7|
| K₂O   | 0.3| 0.5| 0.1| 0.5| 0.6| 0.3| 0.2| 0.0| 1.1|
| B₂O₃  | 0.0| 0.0| 0.0| 0.0| 0.0| 0.0| 5.4| 0.0| 5.0|
| P₂O₅  | 0.0| 0.0| 0.0| 0.0| 0.0| 0.0| 0.0| 0.0| 0.0|
| Surface area | 0.00133| 0.00075| 0.00104| 0.00132| 0.00075| 0.00064| 0.00075| 0.00130| 0.00130|
Figure 1. Dissolution of silicon from MMVF in alveolar macrophage culture (■), cell culture medium (□□□□) and Gamble's saline solution (△△△△) calculated proportional to the surface area of the fiber samples. Mean and standard deviation are given. Panel A shows Si dissolution for the fibers A, B, C, D, and E, and Panel B Si dissolution for the fibers F, G, H, and J.

Figure 2. Dissolution of iron from MMVF in alveolar macrophage culture (■), cell culture medium (□□□□) and Gamble's saline solution (△△△△) calculated proportional to the surface area of the fiber samples. Mean and standard deviation are given. Panel A shows Fe dissolution for the fibers A, B, C, D, and E, and Panel B for the fibers F, G, H, and J.
cellular milieu. Within the phagolysosomes of the AMs the pH is much lower than normal extracellular pH, which is near to pH 7 (15,17). Comparing rockwool samples (D and E) with the glasswool sample (J), the dissolution in AM culture is higher for glasswool in the extracellular medium, while rockwool dissolves more readily within the alveolar macrophages. Si dissolution from glasswool increases as pH increases. For rockwool the opposite is true, which explains why intracellular dissolution of fibers by AMs is probably more effective for rockwool than for glasswool. Moreover, Si forms stable insoluble silicates at low pH, whereas at higher pH, the formation of free silicon hydroxyx complexes increases (23).

The fiber samples, collected from the air after dusting of the raw material, provided the kind of airborne fibers that would exist in a real working environment. These were typically longer than 20 μm, but there were also shorter particles in the samples. Therefore, in the AM experiments, there could be both extracellular and intracellular dissolution, with AMs blocking the surface of the long fibers in the first case, and dissolving the short fibers within the phagolysosomes in the second case.

The dissolution of Si was the lowest for rockwool samples D and E and the ceramic fiber sample H, in all three types of solutions used (Figure 1), and this may be due to the high aluminium content in these fibers. The influence of different media on the dissolution of Fe and Al from the fibers is more marked for these ions than for Si (Figure 2, 3). The dissolution of Fe is minimal in Gamble’s saline solution, quite high in cell culture medium, and highest in the AM culture. This may be because there are different mechanisms due to the different chemistries of the media. Even stronger than the pH effect could be that caused by complex formation between Fe and Al, and chelating agents, such as amines and aminocarboxylic acids (23). Gamble’s solution does not contain any of those components. In the cell culture medium, the dissolution of Fe and Al may be reduced because of the formation of hydroxyl compounds, which precipitate at the surface of the fibers and thereby inhibit further dissolution. The increased dissolution of Fe and Al by AMs can have an important impact on the biopersistence of the fibers. Fe and Al stabilize and strengthen the glassy silicate network of the fiber (18), and their dissolution weakens the network and so makes further dissolution possible.

Fibers A, B, C, D, and E were studied in one experiment and fibers F, G, H, and J in another. The chemical composition of fibers A and F were identical they were prepared by identical methods (see Material and Methods), but their surface areas differed from each other (Table 1). These fiber samples were intended to be used as standards in comparing the two different experiments; unfortunately, they were not optimal for this purpose.

The results of the present study are in agreement with those of Lundborg et al. (15-17), which indicate that AMs can also dissolve metal oxide particles in vitro. The differences in dissolutions in AM culture compared with that in the culture medium and in Gamble’s solution, indicate the importance of also using in vitro cell culture methods for in vitro assessment of the solubility of MMVF.
REFERENCES

1. Environmental Health Criteria 77. Man-Made Mineral Fibres. Geneva:WHO, 1988.
2. IARC EURO Reports and Studies 81. Biological Effects of Man-Made Mineral Fibres. Lyon:International Agency for Research on Cancer, 1988.
3. Wheeler CS. Exposure to man-made mineral fibres: a summary of current animal data. Toxicol Indus Health 6:293–307 (1990).
4. Port F. Some aspects on the dosimetry of the carcinogenic potency of asbestos and other fibrous dusts. Staub-Reinhalt Luft 38:486–490 (1978).
5. Port F. Problems in defining carcinogenic fibres. Ann Occup Hgy 31:799–802 (1987).
6. Port F, Schlipkötter H-W, Roller M, Rippe RM, Germann P-P, Mohr U, Bellmann B. Carcinogenicity of glass fibres with different durability. Zbl Hgy 189:563–566 (1990).
7. Förster H. The behavior of mineral fibres in physiological solutions. In: Biological Effects of Man-made Mineral Fibers, Vol 2. Copenhagen:World Health Organization, 1984; 27–59.
8. Potter RM, Mattson SM. Glass fiber dissolution in a physiological saline solution. Glastech Ber 64:16–28 (1991).
9. Klingholz R, Steinkopf B. The reactions of MMMF in a physiological model fluid and in water. In: Biological Effects of Man-made Mineral Fibers, Vol 2. Copenhagen:World Health Organization, 1984; 60–86.
10. Leineweber JP. Solubility of fibres in vitro and in vivo: In: Biological Effects of Man-made Mineral Fibers, Vol 2. Copenhagen:World Health Organization, 1984; 87–101.
11. Spurný KR. Measurement and analysis of chemically changed mineral fibres after experiments in vitro and in vivo. Environ Health Perspect 51:343–355 (1983).
12. Lundborg M, Holma B. In vitro phagocytosis of fungal spores by rabbit lung macrophages. Sabouraudia 10:152–156 (1972).
13. Camner P, Hellström PA, Lundborg M. Alveolar macrophages and 5μm particles coated with different metals. In vitro studies. Arch Environ Health 19:211–213 (1974).
14. Bernstein DM, Drew RT, Kushner M. The solubility of man-made mineral fibres in the lung in contrast to natural fibres. Soz und Prävent 30:52 (1985).
15. Lundborg M, Lind B, Camner P. Ability of rabbit alveolar macrophage to dissolve metals. Exp Lung Res 7:11–22 (1984).
16. Lundborg M, Eklund A, Lind B, Camner P. Dissolution of metals by human and rabbit alveolar macrophages. Br J Ind Med 42:642–645 (1985).
17. Marafante E, Lundborg M, Vahter M, Camner P. Dissolution of two arsenic compounds by rabbit alveolar macrophages in vitro. Fundam Appl Toxicol 8:382–388 (1987).
18. Scholte H, Glass. Natur, Structur und Eigenschaften. Berlin:Springer-Verlag, 1988; 318–321.
19. Myrvik QN, Leake ES, Fariis B. Studies on pulmonary alveolar macrophages from a normal rabbit: a technique to procure them in high state of purity. J Immunol 86:128–132 (1961).
20. Brain JD, Frank RN. Recovery of free cells from rat lungs by repeated washings. J Appl Physiol 25:63–69 (1968).
21. Phillips, RH J. Dye exclusion test for cell viability. In: Tissue Culture Methods and Applications (Kruse PR, Patterson EK, eds). New York:Academic Press, 1973; 406–408.
22. Gamble JL. Excerpts from chemical anatomy, physiology and pathology of extracellular fluid, chart 2-a. Cambridge, MA, 1952.
23. Ringbom A. Complexation in Analytical Chemistry. New York:Wiley Interscience, 1968.