Demonstration of Nitric Oxide on Asbestos and Silicon Carboide Fibers with a New Ultraviolet Spectrophotometric Assay

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Nitric oxide (NO) has a number of important functions in biological systems and may play a role in the toxicity of mineral fibers. We investigated whether NO might be present on the surface of mineral fibers and if crocidolite could adsorb NO from NO gas or cigarette smoke. NO was determined with a new gas chromatography–ultraviolet spectrophotometric technique after thermal desorption from the fiber surface and injection in a gas flow cell. NO was found in different amounts on chrysotile B, crocidolite, amosite, and silicon carbide whiskers. There was a strong correlation between the amount of NO and the specific surface area of these fibers (r=0.98). NO could not be demonstrated on rockwool fibers (man-made vitreous fiber(s) (MMVF)21 and MMVF22) or silicon nitride whiskers. NO on crocidolite, amosite, and silicon carbide whiskers was readily desorbed from the fibers at increased temperature, while NO on chrysotile B seemed to be more firmly adsorbed to the fiber and required a longer period of time to be desorbed. The amount of NO bound to crocidolite increased from 34 μg/g fiber to 85 and 474 μg/g after exposing the fibers to cigarette smoke and NO gas, respectively. These findings indicate that a) NO adsorbs to fiber surfaces, b) some fibers adsorb more NO than others, c) some fibers adsorb NO more strongly than others, and d) the amounts of NO on fibers may be increased after exposure of the fiber to cigarette smoke or other sources of NO. The biological significance of NO on mineral fibers remains to be investigated. — Environ Health Perspect 105(Suppl 8):1037–1040 (1997)

Key words: nitric oxide, asbestos, silicon carbide, UV spectrophotometry, cigarette smoke

Introduction

Nitric oxide (NO) has a number of important functions in biological systems, e.g., as endothelial relaxing factor and as a cell signaling and regulatory agent. NO may play a regulatory role both via adjuvant chemistry and redox actions. It may interact with protein kinases, which

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Abbreviations used: GC–UV, gas chromatography–ultraviolet spectrophotometric; MMVF, man-made vitreous fiber; NO, nitric oxide; UIICC, Union Internationale Contre le Cancer; UV, ultraviolet.

control function by covalent modification, i.e., phosphorylation, and with reactive oxygen species, which signal through both redox events and coordinate interactions with metals (1). NO is also important in the regulation of metalloproteins that affect gene expression (2) and it plays a key role in cellular iron metabolism by affecting the bifunctional iron regulatory protein by binding to its Fe–S clusters (2,3). NO is therefore believed to play an important part in pathological processes in which iron may participate (3). It has also been found that NO produced in macrophages in response to silica may affect the activation of nuclear factor-kB (4). NO may also modulate the activity of proteins that respond to stress (5) and/or genes that are related to cell proliferation and differentiation. For example, it has been suggested recently that NO induces the expression of c-jun protooncogene (6).

In addition to the above-mentioned functions, NO also reacts with superoxide anions to give peroxynitrite, a highly reactive oxidant (7). Thus, NO and peroxynitrite are potentially harmful agents that can induce oxidative DNA damage (5,8,9) and oxidative protein modifications (10,11). NO also decreases the activity of glutathione peroxidase in cultured lymphoma cells and macrophages (12). Crocidolite induces NO synthase and NO formation in cultured lung cells and NO may play a role in the mediation of fiber-induced oxidative DNA damage (13). Therefore, it is important to find out more about NO interaction with the surface of mineral fibers. In addition, since cigarette smoke (which increases the risk of asbestos-induced lung cancer) contains up to 600 μg NO per cigarette (14), the question arises whether NO from cigarette smoke might adsorb to asbestos fibers. In this study we investigated whether NO might be present on the surface of different mineral fibers and if crocidolite could adsorb NO from NO gas or cigarette smoke. NO was determined with a recently developed gas chromatography–ultraviolet spectrophotometric (GC–UV) technique after thermal desorption of NO adsorbed on the fiber surface.

Materials and Methods

Fibers

The following fibers were examined: crocidolite, chrysotile B, amosite (all Union Internationale Contre le Cancer [UIICC]), silicon carbide whiskers (SCW 15 from Tateho Chemical, Tokyo, Japan), silicon nitride whiskers (UBE Industries, Tokyo, Japan) and two rockwool fibers (man-made vitreous fiber (MMVF)21 and MMVF22, Thermal Insulation Manufacturers Association standard). The specific surface area was determined by nitrogen adsorption technique. The areas of chrysotile B, crocidolite, and amosite were taken from the literature (15); those of silicon carbide and nitride whiskers were supplied by the manufacturer (Table 1). Treatment of crocidolite with NO or cigarette smoke was in a 50-ml plastic syringe in which 10 to 30 mg fibers was exposed to either 40 ml 10% NO gas in N2 (Air Liquide Gas AB, Kungsängen, Sweden) or cigarette smoke from a commercial filter cigarette. (The cigarette was lit and 40 ml smoke was drawn into the syringe.) The fibers were mixed at room temperature by shaking the syringe for 30 sec then transferred from the syringe to small glass tubes for NO analysis as described below.
Table 1. Nitric oxide on different mineral fibers.

| Fiber     | Nitric oxide, mg/g fiber | Surface area, m²/g fiber |
|-----------|--------------------------|--------------------------|
| Crocidolite | 34                       | 8.3*                     |
| Chrysotile B | 103                      | 26.8*                    |
| Amosite    | 15                       | 5.7*                     |
| MMVF22     | ND                       | NA                       |
| MMVF22     | ND                       | NA                       |
| Silicon carbide whiskers | 25                   | 4.9*                     |
| Silicon nitride whiskers | ND                  | 2.2b                     |
| Crocidolite, NO treated | 474                   | —                        |
| Crocidolite, smoke treated | 85                   | —                        |

Abbreviations: NA, no data available; ND, not detectable. *Data from Timbrell (15). †Data from the manufacturer (Tateho Chemical). For comparison, the specific surface area of the different fibers is listed.

**Determination of Nitric Oxide**

To determine NO, we used a new GC-UV instrument (INSCAN GC/UV 175 spectrophotometer, INSCAN AB, Järfälla, Sweden) that consists of a small gas chromatograph (built into a gas flow cell) connected to a diode array spectrophotometer and a simple thermal desorption oven (Figure 1). The fibers (10–15 mg) were packed into small glass tubes that were placed in the desorption oven and flushed with N₂. The flow valve was then closed and the compounds on the fiber surface desorbed at 190°C for 1 min in N₂. The flow was then turned on and the desorbed compounds were led into a GC column and separated before analysis with an ultraviolet (UV) diode array spectrophotometer. The GC was carried out using a carrier gas flow rate of 25 ml/min and a separation column of 80 × 1.5 mm maintained at room temperature and packed with 10 μm Hypersil WP C8 and silicon DCQF-1 as a stationary phase (Alltech, Deerfield, IL). The length of each chromatogram was 60 sec; retention time of NO was 4 sec. The typical and specific UV spectrum of NO, with its eight narrow peaks, is shown in Figure 2. Because the measurement takes place in a vapor phase of N₂, it is possible to register UV spectra between 180 to 230 nm where the highest absorptivities and the most important spectral details of many compounds are found. The identification limit, based on three spectral peaks between 200 to 230 nm, was about 10 ng. Standard curves showed linearity from 20 ng to 20 μg. We previously used this GC-UV technique to study organic solvents in ambient air (16,17) and isoprene in human breath (18).

**Results and Discussion**

As shown in Figure 3 and Table 1, considerable amounts of NO were found on chrysotile B, crocidolite, amosite, and silicon carbide whiskers. The highest NO content was found on chrysotile B. It should be noted that chrysotile B has a larger specific surface area than crocidolite, amosite, and silicon carbide whiskers. As illustrated in Table 1, there was a strong correlation between NO on the surface and the specific surface areas of these four fibers ($r = 0.98$), which indicates that surface area is an important factor for the adsorption of NO to mineral fibers. NO was not found on silicon nitride or on the two rock wool samples.

As to the origin of NO, we have no detectable NO or NO₂ in our laboratory atmosphere, so we have no reason to believe that NO was adsorbed to the fibers in the laboratory. At the moment we can offer no conclusive explanation as to the source of NO on the fibers. The asbestos fibers were UICC standard samples prepared about 30 years ago and the silicon carbide carbide fiber came directly from the manufacturer in Japan. For lack of more extensive data we prefer not to speculate on the origin of NO.

Figure 3 also illustrates that NO on crocidolite, amosite, and silicon carbide was readily desorbed at increased temperature whereas NO on chrysotile B seemed more firmly adsorbed to the fiber and required more time at 190°C to be desorbed. It is reasonable to assume that this difference is a result of the different surface properties of the fibers. Chrysotile is made up of sheets of silica tetrahedra rolled into concentric tubes with an external hydrophilic surface of magnesium hydroxide. The amphiboles crocidolite and amosite have a different structure that consists of double chains of silicate tetrahedra linked by hydrated metal cations with...
ionizable silanol groups on the surface (19). It appears that NO adsorbs more firmly to the magnesium hydroxide of chrysotile than to the silanol groups on the surface of amphiboles. It should also be considered that chrysotile, unlike most other mineral fibers, has a basic surface and a positive zeta potential in solution; this suggests chemisorption of NO.

Many investigators have suggested that cigarette smoke may add synergistically to the toxic and pathologic effects of asbestos fibers. Cigarette smoke contains large amounts of NOs (14), which suggests the possibility that NO from cigarette smoke might adsorb to asbestos fibers and increase their toxicity. As shown in Table 1, treatment of crocidolite with cigarette smoke increased the fiber-bound NO from 34 to 85 μg NO/g fiber. A more than 10-fold increase of fiber-bound NO was found on crocidolite treated with pure 10% NO (from 34 to 474 g NO/g fiber [Table 1]). Thus, it seems that NO in cigarette smoke may adsorb to the surface of asbestos fibers.

The biological implications of the presence of NO on fiber surfaces remain to be investigated. For lack of adequate data, one might only speculate that if NO is released from fiber surfaces, it could increase thiol nitrosylation and also increase NO binding to metals and metalloproteins. These are events that affect the regulation and/or action of a number of important proteins in the cell (1). However, it is also possible that NO, which takes part in the normal regulation of protein function, might be absorbed onto fibers, and that the ensuing intracellular NO depletion could affect gene regulation and expression.

Thus, it is unclear whether the presence of NO increases or decreases the general toxicity of a fiber. NO inactivates glutathione peroxidase, which could lead to accumulation of peroxides and increased toxicity (12). Moreover, if fiber-bound NO reacts with superoxide anions, one would expect the formation of highly reactive peroxynitrite and nitrosylation of proteins and DNA bases (5,9). On the other hand, it is also possible that fiber-bound NO could decrease the toxic effect. For example, oxygen radicals play an important role in toxic effects of mineral fibers (20,21). It is possible that an absorption of NO to fiber surfaces might reduce the number of electron donating sites and the formation of oxidants such as superoxide anions or hydroxyl radicals. This way, NO would act in a manner similar to the iron chelator desferroxamine, which reduces the toxic effects of asbestos. Alternatively, NO may also function as an antioxidant through the formation of NO–iron adducts, thus reducing the availability of ferrous ions and thereby the production of reactive oxygen species (21). It has also been demonstrated that NO reduces the toxic effect of hydrogen peroxide or xantine/xantine oxidase (23), and that it increases the intracellular glutathione level (24) in cultured lung fibroblasts.

In summary, we provide evidence to indicate that a) NO adsorbs to fiber surfaces, b) some fibers adsorb more NO than others, c) some fibers adsorb NO more strongly than others, and d) the amounts of NO on fibers may be increased after exposure of the fiber to cigarette smoke or other sources of NO. The GC–UV technique described appears useful for studies of NO on the surface of mineral fibers and particles, although it remains to be clarified whether the thermal desorption is entirely quantitative. In addition, the biological significance of NO on mineral fibers remains to be investigated.

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