Cytotoxicity and Multinucleate Giant Cell Formation in Chinese Hamster Lung Fibroblast Caused by Crocidolite and Chrysotile

The mechanism of carcinogenic action of asbestos remains unclear but the physical properties of the fiber appear to be important in this process. Asbestos may cause multinucleate giant cell formation primarily by interfering with the normal course of mitosis. We evaluated the cytotoxicity and multinucleate giant cell formation induced by crocidolite and chrysotile in Chinese hamster lung fibroblast(V79 cell) with observation of phagocytic activities. Asbestos fibers were rapidly ingested by V79 cells and most fibers were inside the cells. Cytotoxicity was evaluated by observing inhibition of V79 cell proliferation with trypan blue exclusion test. For determination of frequency of multinucleate giant cells, the cells were treated with different doses of crocidolite or chrysotile for 72 hours. Crocidolite and chrysotile induced cytotoxicity in V79 cells in a dose-dependent manner. The pattern of inhibition of cell proliferation is similar for both types of fibers, but chrysotile was more potent at the highest level (20.0μg/ml) of fiber concentration. There was a good relationship (regression coefficient$_{crocidolite}$=0.02, P<0.01 ; regression coefficient$_{chrysotile}$=0.04, P<0.01) between the dose of both asbestos fibers and the frequency of multinucleate giant cells. Chrysotile was again more potent at inducing multinucleate giant cells in higher levels of fiber concentrations. We found that asbestos fibers were cytotoxic after phagocytosis and induced multinucleate giant cells by interfering mitosis. ***(JKMS 1997;12:99~104)***

Key Words : *Asbestos, Crocidolite, Chrysotile, Chinese hamster lung fibroblast, Phagocytosis, Cytotoxicity, Multinucleate giant cell*

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INTRODUCTION

Asbestos is now regarded as an established carcinogen based on sufficient evidence from epidemiological studies and animal experiments (1~5). However, despite numerous investigations, the mechanism of its carcinogenic action remains unclear (6, 7). Because asbestos treatment alone induces tumors and fiber dimensions appear to be important in this process (3, 8~10), asbestos may affect cells physically as well as chemically. Asbestos fibers are rapidly ingested by cultured cells (11). Once inside a cell after phagocytosis, asbestos is accumulated preferentially in the perinuclear region (7). As a result, asbestos fibers are frequently identified within the mitotic apparatus (12). These observations have led to the hypothesis that asbestos causes cytotoxicity directly by phagocytosis and induces multinucleate giant cell formation primarily by interfering with the normal course of mitosis.

This study was done to evaluate the cytotoxicity and multinucleate giant cell formation induced by crocidolite and chrysotile in Chinese hamster lung fibroblast with observation of phagocytic activities.

MATERIALS AND METHODS

Materials

Crocidolite and chrysotile were the UICC standard samples of USA. Average diameters of crocidolite and chrysotile were 0.35μm and 0.22μm, respectively and they showed the characteristics of a fiber with aspect ratio (length/diameter) more than 3 (Fig. 1A, 2A). We obtained Chinese hamster lung fibroblasts (V79 cells) from the American Type Culture Collection (Rockville, MD, USA). Gibco-BRL (Gaithersburg, MD, USA) was the source of Dulbecco's modified eagle medium (DMEM), phosphate buffered saline, fetal bovine serum, trypsin and trypan blue.

Exposure of asbestos fibers

V79 cells were maintained as monolayer in DMEM supplemented with 5% fetal bovine serum and cultured in a humidified atmosphere of 10% CO_2 at 37℃. The cells were treated with various concentrations of cro-

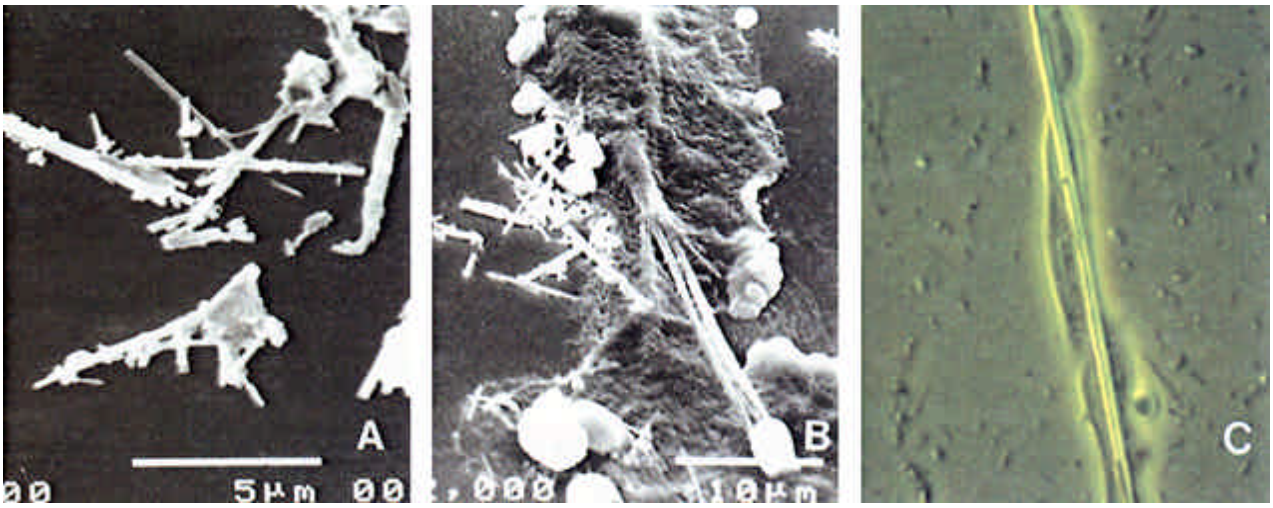

Fig. 1. Photograph of crocidolite fibers and phagocytosis by V79 cells. A : Scanning election microscopic finding of crocidolite fibers. B : Scanning electron microscopic finding of phagocytosis by a V79 cell. C : Inverted light microscopic finding of phagocytosis by V79 cells. They formed a structure resembling a string of beads.

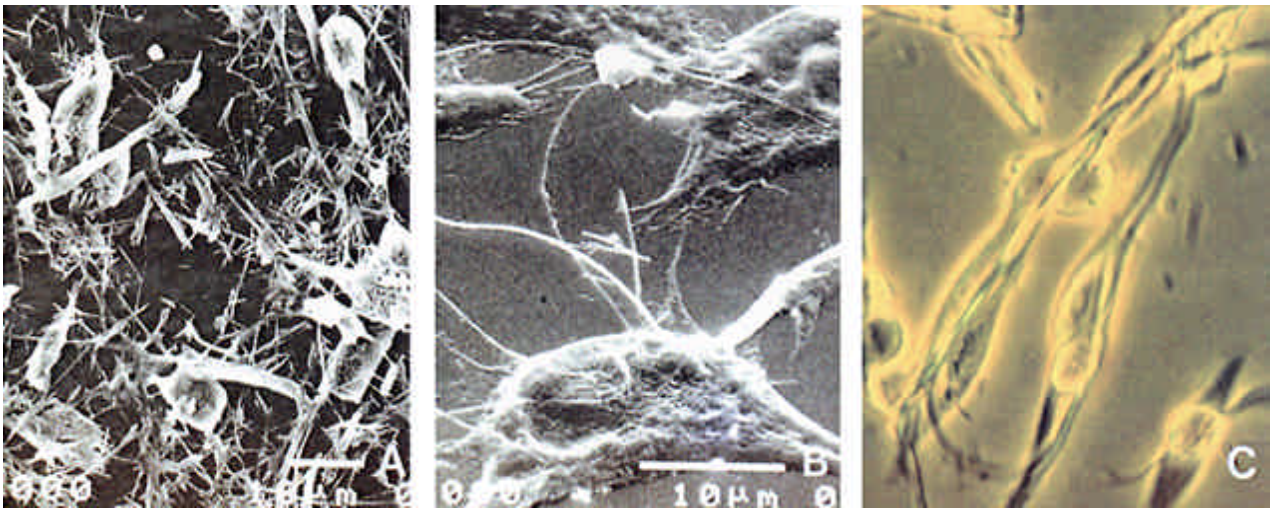

Fig. 2. Photograph of chrysotile fibers and phagocytosis by V79 cells. A : Scanning election microscopic finding of chrysotile fibers. B : Scanning electron microscopic finding of phagocytosis by V79 cells. C : Inverted light microscopic finding of phagocytosis by V79 cells. They also formed a structure resembling a string of beads.

cidolite and chrysotile (0.16 to 20.0μg/ml).

Observation of phagocytosis

The fiber suspensions were spread on the cultured cells which were grown on coverslips. The phagocytic processes were observed using phase contrast microscope and scanning electron microscope (SEM). SEM preparation was done by fixing the cells with methanol and air drying.

Cytotoxicity

Cytotoxicity was evaluated by observing inhibition of V79 cell proliferation. Four hours after cell plating on duplicated culture flasks, the cells were treated with different amounts of crocidolite or chrysotile. Following 24 hours of incubation, the cells were then detached after trypsination and the cell suspensions were mixed with 1 vol. of 0.2% trypan blue in PBS. The number of cells were counted in a hemocytometer.

Multinucleate giant cell formation

For determination of frequency of multinucleate giant

cells, the cells were treated with different doses of crocidolite or chrysotile for 72 hours. After incubation, the cells were fixed in methanol and stained in Giemsa. The frequency was counted in 4 fields(×400) of each flask of duplicated cultures.

Statistics

The mean and the standard deviation were calculated. The significance of the relationship between exposure doses and responses were judged by ANOVA with Tukey's multiple comparison test and regression analysis.

RESULTS

Both asbestos fibers were rapidly ingested by V79 cells. Although it is difficult to decide from phase-contrast microscopy whether fibers are inside cells or merely attached to their surface, scanning electron microscopy showed that most fibers were inside the cells. In many cases long asbestos fibers penetrated the cells (Fig. 1B, 2B). Several cells accumulated around a fiber and formed a structure resembling a string of beads. (Fig. 1C, 2C)

The results of the cell proliferation inhibition assay showed that crocidolite and chrysotile induce cytotoxicity in V79 cells significantly in a dose-dependent manner (regression coefficient$_{crocidolite}$ = − 0.37, $P<0.01$; regression coefficient$_{chrysotile}$ = − 0.60, $P<0.01$). The number of nonviable cells which were stained in the trypan blue viability test also increased with the higher doses of asbestos fibers (Table 1). However, the dose of asbestos that inhibited growth markedly had a little effect on trypan blue exclusion, suggesting that the majority of asbestos-exposed cells were still alive. The pattern of inhibition of cell proliferation is similar for both types of fibers but chrysotile is a more potent fiber with the lowest number of viable cells at the highest level of fiber concentration (Fig. 3).

Table 1. Inhibitory effect of crocidolite and chrysotile on V79 cell proliferation

Treatment	Cell count ($\times 10^4$/ml)[a]	Trypan blue exclusion (%)
Control	87.8 ± 6.4	97.9
Crocidolite 0.16 μg/ml	81.8 ± 8.3	91.4
Crocidolite 0.8 μg/ml	86.0 ± 6.9	94.9
Crocidolite 4.0 μg/ml	78.5 ± 8.1	87.8
Crocidolite 20.0 μg/ml	49.8 ± 7.1[b]	89.0
Chrysotile 0.16 μg/ml	96.8 ± 4.6	97.1
Chrysotile 0.8 μg/ml	86.3 ± 8.8	89.3
Chrysotile 4.0 μg/ml	76.8 ± 11.8[b]	85.5
Chrysotile 20.0 μg/ml	32.3 ± 4.4[b]	82.0

a. cell number for each flask of duplicated cultures(mean±SD)
b. statistically significant difference from contol by Tukey's multiple comparison test($P<0.05$)

The multinucleate giant cells found in cultures treated with asbestos changed greatly in morphology (Fig. 4). The number of nuclei in a single cell sometimes markedly increased to over 10 and the size of the cell dramatically grew to form a giant cell. There was a

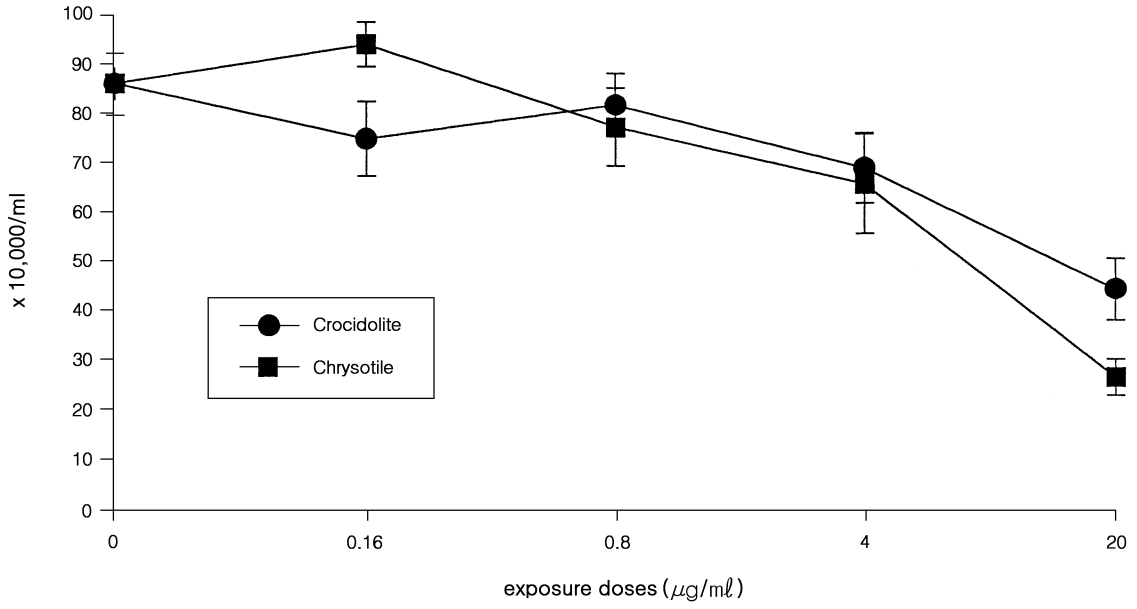

Fig. 3. Number of viable V79 cells 24 hours after exposure of crocidolite or chrysotile.

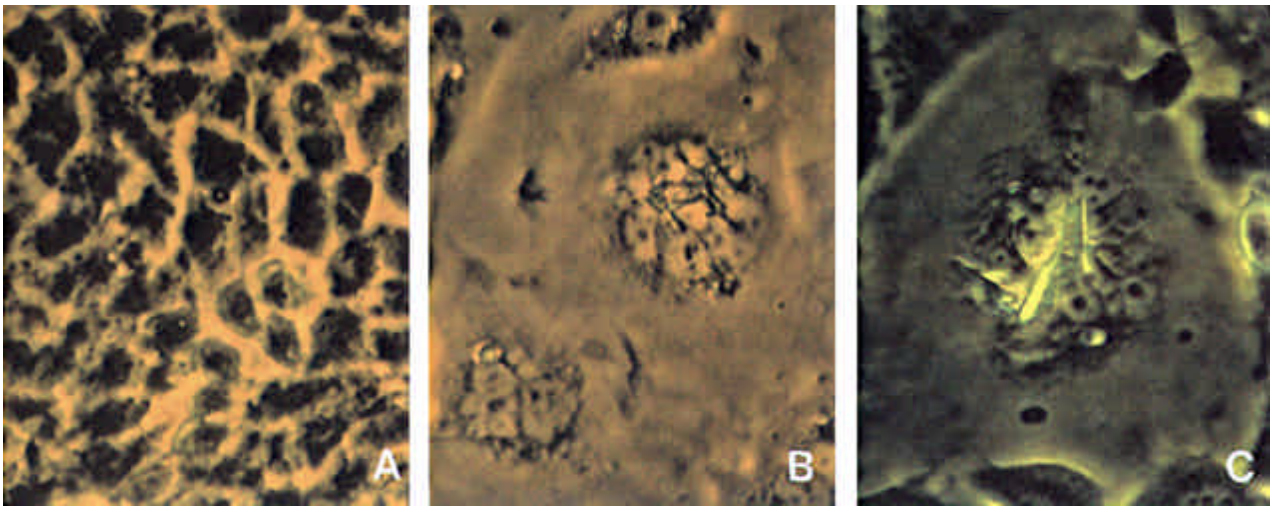

Fig. 4. Inverted light microscopic findings of multinucleate giant cells induced by asbestos fibers. A : Control cells. B : Crocidolite treated cells. C : Chrysotile treated cells. The size of the cells grew greatly and the number of nuclei increased. They also contained the asbestos fibers in the perinuclear region.

statistically significant relationship between the dose of both asbestos fibers and the frequency of multinucleate cells (regression coefficient$_{crocidolite}$=0.02, P<0.01 ; regression coefficient$_{chrysotile}$=0.04, P<0.01). When the cells were exposed to a lower dose (0.16μg/ml), there was a slight increase over the background in crocidolite treated cells, but no change in chrysotile treated cells. Higher exposure doses of both fibers resulted in higher yields of multinucleate giant cell and chrysotile was again more potent fiber to induce multinucleate giant cells in those fiber concentrations (Fig. 5).

DISCUSSION

The results obtained in the present study confirm previous observations about the cytotoxicity of asbestos fibers to cultured cells (9, 13). V79 cells cultured in the

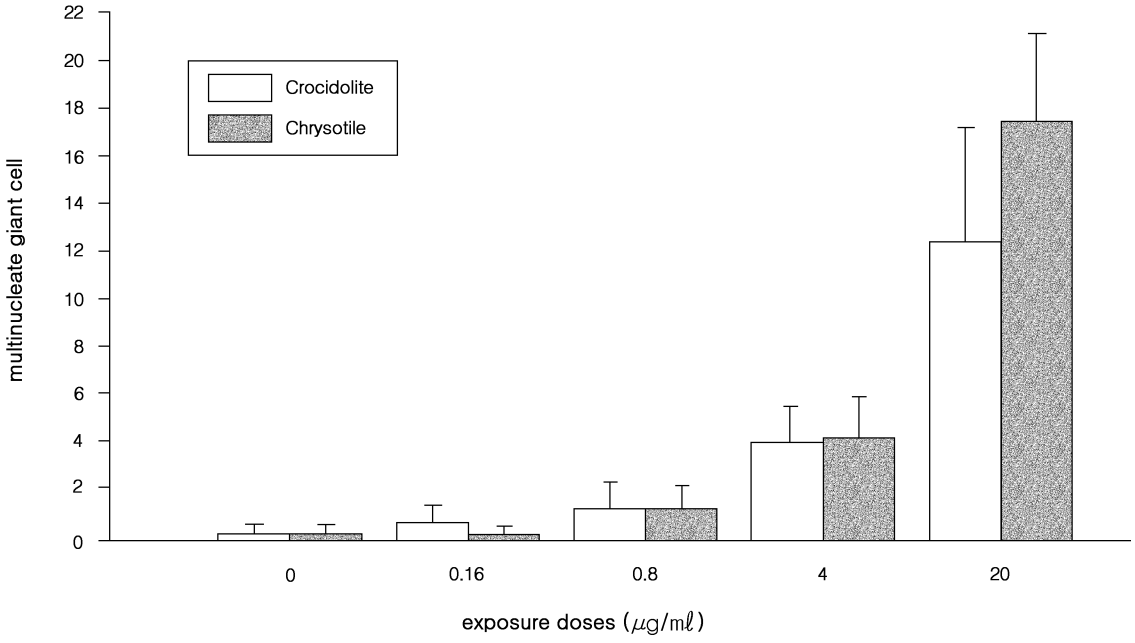

Fig. 5. Multinucleate giant cell formation induced by crocidolite or chrysotile after 72 hours of exposure.

presence of asbestos fibers displayed the inhibition of cell proliferation. Cell death was directly associated with the phagocytosis of fibers. SEM preparation indicated that asbestos fibers were readily contained in the cells and sometimes penetrated the cell membrane. The ingestion of long fibers by cells is a slow process and it may be incomplete, thus the increased permeability may be sustained for many hours (14). The cytotoxicity by asbestos fibers could be explained at least partially by the disruption of integrity of cell membrane. Fibers with a length that exceeds the cell diameter may remain partly extracellular or several cells may become attached to one fiber, forming a structure like a 'string of beads'. Interestingly, asbestos body formed in human lung tissue after exposure to asbestos fibers is a similar structure resembling the string of beads.

In our study chrysotile asbestos was more potent in the inhibition of cell proliferation at higher doses of fibers. The cytotoxicity results are in agreement with the data of other investigators which showed that chrysotile was more effective in the inhibition of the growth of cell populations than crocidolite using macrophage-like cell lines (15), epithelial cells (16, 17), mesothelial cells (18), and fibroblasts (9, 19).

As already pointed out in the work of many researchers (3, 9), asbestos fibers may exert their carcinogenic effect with the physical properties of the fibers rather than the chemical properties of the mineral. Mitotic interference induced by asbestos fibers physically may be closely related with the carcinogenicity (20). The multinucleate giant cell formation, an indicator of mitotic interference, is not a specific change caused by asbestos fibers but a kind of chronic inflammatory reaction caused by a foreign body in the lung (21). Other mineral fibers of similar dimensions may disturb mitosis in a similar way and induce multinuceate giant cells. These cells are formed when cells attempting to undergo mitosis fail to complete cytoplasmic division. They grow in size and undergo DNA replication and nuclear divisions before they die. These cells are unlikely to give rise to tumor cells directly because they cannot continue multiplication (11). However, they may be useful in evaluating the effects on animal and human cells since multinucleate giant cells can easily be counted and the ability to induce formation of multinucleate giant cells reflects the ability to interfere with mitosis.

In the present study, crocidolite and chrysotile increased the frequency of multinucleate giant cell over 100-fold after 72 hours' treatment with 20 μg/ml. Chrysotile was more potent in inducing the multinucleate giant cells at the dose.

In summary, crocidolite and chrysotile fibers induced cytotoxicity in vitro, as indicated by the inhibition of V79 cell proliferation. The cytotoxic effect of these asbestos fibers was closely associated with the phagocytic activity. The effect of the asbestos fibers on the development of multinucleate giant cells was also evaluated. The physical characteristics of the fibers may play a role in the development of these cells. This index of mitotic disturbance seems to be a useful marker in the carcinogenesis assay of asbestos fibers.

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