

Correlation of *in Vitro* and *in Vivo* Methods by Means of Mass Dose and Fiber Distribution for Amosite and Fibrous Ferroactinolite

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Oncogenesis and *in vitro* data (reported elsewhere in detail) are compared on the basis of relative activity by mass and by dimensional fiber parameters. When tumor induction is compared to the number of fibers of various lengths and aspect ratios in the dose in rats to the degree of tumor induction, a degree of difference with the long thin fiber concept of tumorigenesis by mineral fibers is noted. Consistency is re-established, however, when cognizance is taken of the change in the length and aspect ratio that took place during residence in the lung. This change resulted in a severalfold excess for ferroactinolite of all fiber lengths with high aspect ratios, produced as a result of longitudinal splitting of the introduced fibers.

The response by mass in the *in vitro* procedures did not mimic oncogenesis. When mass was so adjusted that there were an equal number of mineral fibers, aspect ratio > 3, for dose for the two minerals, agreement was closer in both the rabbit alveolar macrophage toxicity test and the clonal cytotoxicity assay in Chinese hamster ovary cells. When activity was related to the number of mineral fibers, the same aspect ratio computed to have been contained in the mass dose, agreement with the relative induction of lung tumors was closer. In all cases, erythrocyte lysis was more active in reflecting the number of mineral fibers.

Introduction

While the health hazards of exposure of workers in commercial operations involving asbestos are now well known, the risks to workers or the general population coming in contact with various mineral fiber-bearing rocks during mining, quarrying, ore beneficiation, and roads paved with crushed stone are unclear. Additionally, the natural, modified or man-made fibers that are now being proposed as asbestos substitutes require toxicological evaluation before their acceptance for specific uses. It would thus appear that the need for the toxicity testing of the above types of minerals will far exceed the available time and existing laboratory facilities, if reliance must be placed on the inhalation exposure of laboratory animals. Furthermore, although much

information is available concerning the various cytologic membrane activities of mineral fibers and the effects of intrapleural or intraperitoneal inoculations, a systematic, solid confirmation of these data as parameters for predicting health hazards to man is needed.

Hence, it would appear useful to examine a number of factors for the possibility of simplifying the health hazard testing of mineral fibers from non-commercial asbestos sources. One approach is to explore systematically, by using identical, highly characterized natural and artificial mineral fibers, the comparison of data from inhalation, intratracheal, intrapleural and/or intraperitoneal evaluations with various *in vitro* models, as well as the correlation with mineralogical characteristics such as fiber dose, size, etc. Also needed are experiments designed to search for new biological models involving animals that may be predictors of oncogenic effect, with more modest requirements in terms of the quantity of the sample, the number of animals used, and the elapsed time required. This paper explores

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the possibility of correlating various systems with pleural and pulmonary oncogenesis for two natural minerals of widely differing characteristics.

Materials and Methods

Two natural minerals, UICC amosite and fibrous ferroactinolite, were studied by means of intrapleural and intratracheal administration to Fischer 344 pathogen-free rats. The biological and mineralogical data from these studies have been reported elsewhere (1, 2). The dosing schedule and mineralogical characteristics are shown in Table 1. The accompanying three *in vitro* methods, which consisted of toxicity to rabbit alveolar macrophages (RAM), clonal cytotoxicity assays in Chinese hamster ovary (CHO) cells and lysis in sheep erythrocytes (RBC), are described in another presentation at this symposium (3). In this paper, comparisons are made between the dose required by mass and number of mineral fibers in the induction of neoplasia and the production of 50% activity in three *in vitro* systems.

Results

The *in vivo* findings summarized in Table 2 have been reported previously (1). In the intrapleural treatment, mesotheliomas noted for both amosite

(33.6%) and ferroactinolite (23%) were significantly different from the controls but not from each other. Pooled primary malignant lung tumors showed a low but statistically significant ($p = 0.05$) excess for ferroactinolite in the intratracheally treated group. Studies on the fate of the mineral fibers, made by electron microscopic examination of ashed whole lung specimens, were reported previously (2). The data showed that a longitudinal splitting of the mineral fibers occurred as a function of residence time in the lung. Since the phenomenon was much more pronounced for ferroactinolite than for amosite, there was a large increase in the number of these fibers, which also tended to be shorter and thinner than amosite fibers.

The results of the *in vitro* experiments are reported elsewhere in this symposium (3).

Discussion

In a series of experiments using various sizes of inorganic fibers, Stanton and Wrench (4) and Stanton and Layard (5) demonstrated that tumorigenesis in the pleura appears to be related to the presence of long thin fibers, especially those fibers with dimensions of $>8 \mu\text{m}$ by $< 0.25 \mu\text{m}$. Because there were nearly two orders of magnitude difference in the number of fibers of this category in our min-

Table 1. Dose schedule by mass units and number of fibers.

Mode of treatment	Dose, mg	No. of doses	Wt per total dose, mg	Fibers per total dose	
				Ratio > 3:1	Length $> 8 \mu\text{m}$ and diameter $< 0.25 \mu\text{m}$
Intrapleural					
Ferroactinolite	20	1	20	1.6×10^9	1.8×10^8
Amosite	20	1	20	11.8×10^9	16.6×10^7
Intratracheal					
Ferroactinolite	0.50	12	6.0	0.49×10^9	0.54×10^8
Amosite	0.25	12	3.0	1.71×10^9	25×10^8

Table 2. Pleural and lung lesions.

Treatment	Number of animals	No. of lesions (%)				
		Mesotheliomas	Lung tumors	Metastatic tumors	Atypical bronchiolar-alveolar cell hyperplasia	Granuloma
Intrapleural studies						
Ferroactinolite	135	31 (23%)	2 (1.5%)	0 (0)	4 (3%)	27 (20%)
UICC amosite	137	46 (33.6%)	1 (0.73%)	0 (0)	0 (0%)	42 (30.7%)
Sham control	134	2 (1.5%)	1 (0.75%)	0 (0)	0 (0%)	0 (0%)
Untreated control	219	0 (0%)	1 (0.43%)	1 (0.46%)	2 (1.3%)	0 (0%)
Intratracheal studies						
Ferroactinolite	561	5 (0.89%)	22 (3.9%)	12 (2.1%)	37 (6.6%)	0 (0%)
UICC amosite	139	2 (1.4%)	1 (0.71%)	0 (0%)	11 (7.9%)	0 (0%)
Sham control	212	0 (0%)	2 (0.6%)	2 (0.94%)	0 (0%)	0 (0%)
Untreated control	219	0 (0%)	1 (0.43%)	1 (0.46%)	3 (1.3%)	0 (0%)

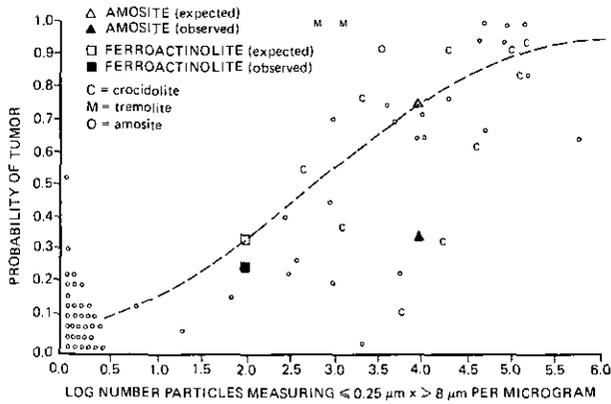


FIGURE 1. Observed and expected values for amosite and ferroactinolite plotted on logistic regression model of Stanton et al. (6) and Stanton and Layard (5), with their parameters estimated from data consisting of various fibrous inorganic minerals that are not specified here, and three asbestos fibers: tremolite (M), crocidolite (C), and amosite (O). Note that their data exhibit considerable scatter about the estimated regression curve with wider variance for asbestos. (Δ) Number of pleural tumors expected for amosite according to log of fiber number ($\geq 8 \mu\text{m} \times \leq 0.25 \mu\text{m}$). (\blacktriangle) number of tumors observed; (\square) number of pleural tumors expected for ferroactinolite on basis of log of fiber number ($\geq 8 \mu\text{m} \times \leq 0.25 \mu\text{m}$); (\blacksquare) number of pleural tumors observed for ferroactinolite.

erals (amosite $8.3 \times 10^6/\text{mg}$; ferroactinolite $0.09 \times 10^6/\text{mg}$) (Table 1) and our induction of pleural tumors being approximately equal, our data appear somewhat inconsistent with the long fibers concept mentioned above. When our data are compared to the average curve of the most recent model of Stanton et al. (6), it predicts closely for ferroactinolite but overpredicts by a probability of 0.32 amosite (Fig. 1). Since Stanton's dose was larger (40 mg versus 20 mg per rat) and since his application of the material on glass fiber discs would tend to concentrate the fibers over a smaller area of the pleural surface, one might expect a greater tumor yield on a per-fiber basis, thus bringing tumor incidence for amosite higher in regard to the predictive curve of their model. If we assume the same degree of influence for ferroactinolite, the effect of this mineral would be underpredicted by the same degree. While these differences are interesting and while it might be speculated that the *in vivo* splitting that was much greater for ferroactinolite than for amosite might have been contributory, additional dose response data would be required to verify this in light of the scatter of data points in the model and the fact that we have but a single datum for each mineral.

Another point to consider is the possibility that fibers shorter than $8 \mu\text{m}$ might have been contributory in our material. It was noted that a large number of shorter, extremely thin fibers occurred with

ferroactinolite after residence in the body of the rat. There appears to be a growing trend to ascribe importance to fibers of shorter length. Pott (7) has constructed a hypothetical, three-dimensional model from which he deduces that tumorigenesis may be a function of cumulative potency of fibers of various dimensions, with activity grading upward to long thin fibers.

From a reanalysis of the data of Stanton and Layard (5), Bertrand and Pezerat (8) have postulated that tumorigenesis may be a continuous function of aspect ratio. Stanton et al. (6), in their most recent paper in which additional dimensional categories were considered, have stated, "What is perhaps most likely than the existence of a narrow range of sizes within which particles are carcinogenic and outside of which they are not is that the probability of tumor falls as particle diameter increases and length decreases." They mention also in this paper

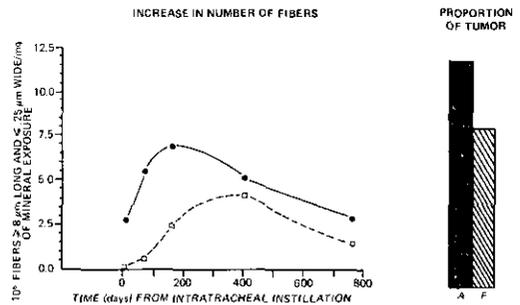


FIGURE 2. Change in number of fibers (category $\geq 8 \mu\text{m} \times \leq 0.25 \mu\text{m}$) with residence time in lung compared to proportion of pleural tumors (A = amosite; F = ferroactinolite). Note that the increase with time is proportionately greater for ferroactinolite than for amosite.

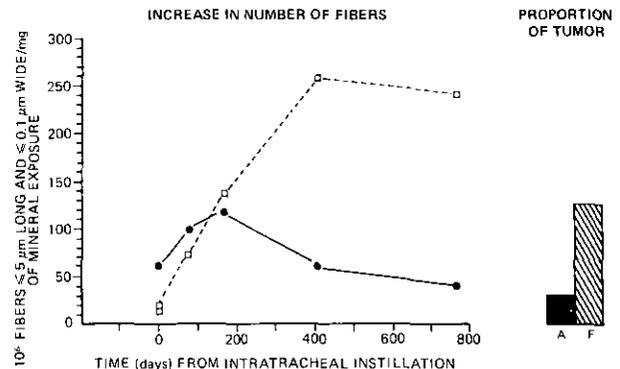


FIGURE 3. Change in number of fibers (category $\leq 5 \mu\text{m} \times \leq 0.1 \mu\text{m}$) with residence time in the lung compared to proportion of pulmonary tumors (A = amosite; F = ferroactinolite). Note that the increase of relatively short, extremely thin fibers is greater for ferroactinolite than for the longer fibers (Fig. 2).

that the asbestos fibers tended to deviate from the expected values of the estimated regression line, with amosite and tremolite giving higher values and crocidolite giving lower values than those predicted from the regression curve (Fig. 1). While they suggest that the variance may have been due to problems in assessing particle size for the asbestos fibers, we offer that another possibility might be in the difference in the rate of *in vivo* longitudinal splitting of the respective asbestos fibers or that the many shorter fibers that might be expected to have been present in the asbestos may have been contributory.

In view of the foregoing, it seems tenable to suggest that the differential in the rate of *in vivo* splitting between amosite and ferroactinolite may have been a factor in the relatively high incidence of pleural tumors for the latter mineral by intratracheal inoculations.

Dose-response relationships and the possible role of fiber size characteristics are less understood for the induction of tumors of the lung by entrance to

the pulmonary tissue via the airway, because problems of aerodynamics, clearance, and lower incidence of tumors make such data difficult to obtain. However, while the parameters of fiber size are not well understood, it is fairly certain that induction of pulmonary tumors is in some way connected with number and size characteristics of asbestos fibers.

In our intratracheal experiments, tumor induction in the lung was significant only for ferroactinolite, whereas on the basis of fiber numbers, the opposite effect would have been expected. While the lung tumor data are incompatible with the number of fibers administered, *in vivo* splitting, which was much greater for ferroactinolite than for amosite, resulted in a very large increase of very thin fibers. Since the mode of fiber length was shorter for ferroactinolite, splitting resulted in a disproportionate increase in shorter fibers of high aspect ratio (compare Fig. 2 and 3). Thus, it might be speculated that a continuum of fiber lengths of very thin fibers may have accounted for the stronger pulmonary tumorigenesis of the ferroac-

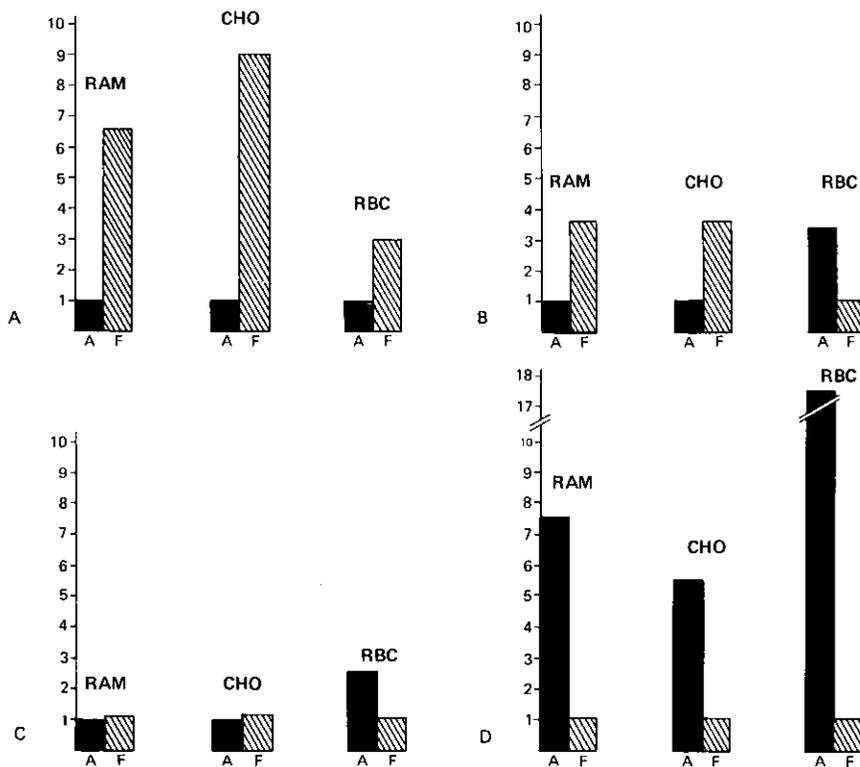


FIGURE 4. Summary of results of *in vitro* and *in vivo* experiments with amosite and ferroactinolite: Effects are shown as ratio of units required to yield 50% activity (LC/50). In each instance, the greatest effect is represented by the number one. (A) Response by unit mass; (B) response when mass was adjusted to achieve unity in number of mineral fibers (aspect ratio ≥ 3); (C) response when expressed by number of fibers, aspect ratio ≥ 3 ; (D) response when expressed as number of fibers, $\geq 8 \mu\text{m} \times \leq 0.25 \mu\text{m}$.

tinolite, as well as the smaller than expected differences in the induction of pleural tumors.

It is clear that the *in vitro* dose response computed on a unit-mass basis does not compare with our results for tumorigenesis (Fig. 4a). RAM and CHO appear to equate best with our results for pleural when the dose response is expressed in units of fibers, aspect ratio > 3 (Fig. 4b and 4c). RBC lysis, which reflected fiber numbers more sensitively, mimics tumorigenesis in the lung, which as we have pointed out above may be related to the relatively short, very thin fibers that were predominant in ferroactinolite after considerable residence time in the lung. Such fibers did not occur in the *in vitro* system as the result of splitting; nonetheless, a greater degree of lysis was noted for ferroactinolite when the two minerals were compared on the basis of dose response for fibers with aspect ratio > 3. When *in vitro* response was computed on the basis of fiber size > 8 μm by < 0.25 μm , the dose response was strongly slanted toward ferroactinolite (Fig. 4d). We presume that this apparent response is artifactual, because the paucity of this length category, coupled with the high reactivity of the more numerous shorter fibers, would naturally lead to such results in a calculated dose response.

It appears from a comparison of our *in vitro* and *in vivo* data that correlation is best when the *in vitro* response is expressed in unit fibers, aspect ratio > 3. The strong shift in favor of the RBC lysis at this point suggests that the red cell is more sensitive to these fibers or that some other constituent of the ferroactinolite may be especially reactive. The content of quartz was negligible in this sample. The remainder of the nonfibrous material of the sample consisted of magnetite, ferroactinolite, hornblend fragments, and nonfibrous silicate particles

(1). From information available to us, there is no reason to believe that this material would be either tumorigenic or reactive in the *in vitro* systems employed. Such material is not known to be lytic; therefore, the data suggest that both the tumorigenesis and *in vitro* response are the result of the mineral fibers and that the results equated best when the *in vitro* dose was expressed in terms of mineral fiber, aspect ratio > 3.

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