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Talc - Macrophages.

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Dear Bill:

As I related to you on the telephone, we have spent more time studying effects of talc than we anticipated. Since we have found a cytotoxic effect, I felt that we should pursue it in more detail and obtain data which can be compared to other studies. This has required doing more short-term incubation experiments in the absence of serum. I believe that we are now completing the initial phase of the talc experiments and have a reasonably good picture of its effects. I will describe the key experiments, but not present all of the data at this time.

Most of the relevant data on mineral toxicity to macrophages used incubations of a few hours. We have examined enzyme leak and viability in four experiments using our finely ground quartz and the size-fractionated talc. The incubations always occured with shaking, and with cell concentrations of 2.5 X 10^6/ml and with one exception in Hank's salts without serum. In general, the talc seems to be cytotoxic at concentrations of 0.5 mg/ml and higher (Fig 1 and 2). There is a correlation between cytotoxicity as estimated by trypan blue and release of enzymes, both lysosomal hydrolases (B-glucuronidase and acid phosphatase) and lactate dehydrogenase (cytoplasmic). There are problems in both estimations which may contribute to error. The trypan blue method requires some subjective judgement when counting blue cells, particularly when the cells are covered with large pieces of talc and when clumped together with talc. An additional potential problem is that only intact dead cells will stain blue, and cells which have completely lysed will not. The enzyme assays have a problem in that the enzymes are apparently adsorbed by the talc particles. When incubated with 0.5 mg/ml talc the activity of all enzymes in a broken cell suspension decreased. Unfortunately, this inhibition will vary in suspension in which the talc is attached to or injested by macrophages thereby decreasing the available surface area. We are now analyzing the data which should provide the proper controls for the enzyme leak-talc interaction.

The effects of both talc and quartz on viability (trypan blue) are largely abolished in 1% and completely reversed in 2 - 3% rabbit serum (Fig 3). The protective effect of serum has been observed with more toxic forms of silica, this suggests that although toxicity is lower in talc it occurs by the same

mechanism.

Using the interpation of Allison for silica, the serum proteins coat the particles, shielding oxygens which react with and disrupt membranes by hydrogen bonding. Over a longer period of time, however, these proteins are either digested away or exchange with membranes for the critical sites. Although the cytotoxicity is reduced by serum during a three hour period, the culture experiments show that it merely delays using the effect.

We have cultured macrophages which had been exposed to 0.5 mg/ml talc in 10% serum for one hour in four separate experiments. The culturing occurs in M-199 medium supplemented with 10% serum. In general, talc reduced attachment to the flasks by about 10 - 20% compared to controls and after 24 hours the number of talc cells on the flasks was about one-half that of the controls. The slope of a plot of cells remaining vs. time is more negative for talc cells than controls (Fig 4). The numbers of talc cells were 56% and controls were 76% of initial numbers after 8 days. A semi-log plot of these data give a half-time of 14.5 and 22.5 days for talc and control populations respectively. No significant release of enzymes could be measured in the experiments because the rate of cell lysis was too slow to be detectable in the presence of the serum in the medium. The serum contains all of the enzyme activities we measure and thereby increases the background of the assays enough to decrease sensitivities.

We are presently conducting a culture experiment with cells exposed to 0.125, 0.25 and 1.0 mg/ml talc. The data are not in yet, but after four days it appears that the lower concentrations have no effect on attachment or survival and the higher concentration has a greater effect than talc at 0.5 mg/ml.

The two approaches, i.e., short duration-no serum and exposure in the presence of serum followed by culturing for several days, agree quite well. In both kinds of experiments the lowest dose which reproducibly resulted in an effect clearly different from control was 0.5 mg/ml. Koshi et. al., reported that 100-200 ug/ml inhibited one half of the dehydrogenase (his viability assay) in his peritoneal macrophage suspension compared to 50 ug/ml for quartz. Using his classification, I would rank your talc with his least toxic minerals, i.e., effective above 0.5 mg/ml. I should point out that the doses he describes are total doses in 2 ml and that he used serum in his medium. Based on the data we have concerning talc, I would predict that it can cause pneumoconiosis, but only if very high concentrations reach the deep lung. This seems to be the case from what I have read, i.e., talc pneumoconiosis occurs only after heavy industrial exposure for many years.

I have considered the possibility that the smaller birefrigent particles in the talc are responsible for the cytotoxicity but think it doubtful. In the experiment shown in Fig. 4, only 12% of the cells were in contact with or contained these other particles. In the experiment which is still underway, the cells exposed to 1 mg/ml talc are reduced in number to about one-third of control and yet only 7% of them contained the other particles. In the experiment shown in Figure 1, the percentage of cells which contained these particles was 17% at 0 min, 11% at 45 min, 24% at 90 min, and 24% at 180 min, respectively.

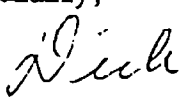
In the first two cases, not enough of the cells contained the particles to account for the loss rate. In the last case one would have to postulate that all of the cells which contained even one particle died within three hours to account for the observed cell death. In general, about 90% of the cells incubated with 0.5 mg/ml talc in the absence of serum and 70% exposed to this level in the presence of serum are attached to (or contain) talc. We have these data for every experiment and, therefore, can relate effect to percent of cells containing talc and other material. As is obvious from the data shown here, the quartz which I used was a relatively non-toxic form. I have written Allison in England for some of the more toxic forms, e.g., tridymite. Others report that these are toxic at levels of less than 50 ug/ml, more than ten times that we observed with talc. I will repeat a short-term incubation study and a culture study with some of this when it arrives. I would appreciate your help in locating some of this material in case Allison does not come through.

It would be interesting to see if the effect of poly-2-vinyl-pyridine-N-oxide (PVPNO) protects macrophages from talc as it does from silica (A.C. Allison and M.D. Harrow, Arch. Int. Med., 9:271, 1971). This would implicate hydrogen bonding with membrane groups as the likely mechanism of action. If you are concerned about possible cytotoxicity and fibrogenicity of talc, you may be interested in pursuing the possibility of coating the material with a protective layer. Several other polymers as well as aluminum have been shown to be effective in reducing cytotoxicity as well as fibrosis in animals.

The next stage of the work will be a study of the cytotoxicity of corn starch and TCP in a similar and hopefully briefer manner than with talc. The EM lab is now preparing electron micrographs of talc-containing macrophages for determination of particle size and ultrastructural effects. I will send you prints when they are ready.

I am considering the questions concerning relating in vitro to in vivo dose we discussed on the telephone and will either call you or write you when I feel I have a reasonable approach in mind. I do feel that finding what percent of the macrophages contain talc in exposed animals would provide important data.

Cordially,



R. P. Schneider, Ph.D.
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Enclosures

RPS:rs