USE OF A MICROBIAL TEST TO DETECT THE MUTAGENIC EFFECT OF INDUSTRIAL MINERAL DUSTS

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UDC 613.033-078

KEY WORDS: asbestos; industrial mineral dusts; rats; microbial test in an in vivo system; mutagenic effect

One of the most important industrial factors which may exert a harmful action on human health is the numerous industrial mineral dusts (IMD). In particular, the carcinogenic action of various types of asbestos has been established, but other IMD have received much less study from this standpoint. Since close correlation exists between mutagenicity and carcinogenicity, data on the mutagenic effect (ME) of IMD are interesting. However, data of this kind in the literature are sporadic and contradictory [2, 7, 13, 16], probably due to the lack of research into the problem and the inadequate use of modern mutagenicity tests in relation to IMD. The aim of the present investigation was to study the possibility of using the microbial test, one of the most informative and widely used modern methods of assessment of ME, in the case of IMD.

EXPERIMENTAL METHOD

The microbial test was used in an in vivo system [8, 12]. Male rats were the intermediate hosts, and Salmonella typhimurium strains TA 1534, TA 1950, and TA 1537, with mutations in different histidine loci and enabling detection of ME of substances acting in accordance both with the reading frame shift type (TA 1534 and TA 1537) and to the base pair replacement type (TA 1950), served as the indicator organism. The IMD for testing, after preliminary sterilization for 1 h at 105°C, were injected intraperitoneally into the animals once in the form of a suspension in 0.5 ml of physiological saline and in a dose of 125 mg/kg (0.2 LD_{50}). After 24 h the animals were given an intraperitoneal injection of 5 ml of a culture of microorganisms, and 6 h later they were sacrificed. These experimental conditions were worked out in preliminary experiments. Taking the culture of microorganisms from the peritoneal cavity and seeding were carried out by the standard method [8]. The results were obtained in two or more repetitions, 4 to 8 animals in each case. Control animals were given sterile physiological saline. As a positive control we used cyclophosphamide in a dose of 45 mg/kg. ME of the dust was estimated from the number of colonies of revertants on histidine-deficiency agar with a minimal glucose concentration after culture for 48 h at 37°C. The bactericidal action (BCA) of the IMD was determined as the fraction of microorganisms surviving on nutrient agar after incubation for 24 h at 37°C.

EXPERIMENTAL RESULTS

In the experiments of series I on three strains of microorganisms, Bazhenovo chrysotile asbestos was tested in vivo. As Table 1 shows, asbestos had ME sufficient to increase the frequency of back mutations in two strains (TA 1534 and TA 1950). Spontaneous mutation rate under these circumstances was $(0.49\text{-}21.53)\cdot10^{-8}$, in agreement with data in the literature [4, 12]. During assessment of the degree of ME of asbestos, a multiplicity index was calculated, reflecting numerically the excess of the mutation rate in the experiments over the control [8]. This index was found to be maximal when strain TA 1534 was used, when it was 27. Cyclophosphamide, which was used as the positive control, had ME similar to that of asbestos in vivo, and caused a 20-fold increase in the number of histidine-dependent revertants above the control level. It must be pointed out that ME of asbestos, unlike cyclophosphamide, was exhibited against the background of a marked BCA of the dust. The percentage of surviving

Medical Research Center for Preventive Medicine and Health Care of Factory Workers, Ministry of Health of the RSFSR, Sverdlovsk. (Presented by Academician of the Academy of Medical Sciences of the USSR B. T. Velichkovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 522-525, November, 1990. Original article submitted November 16, 1989.

0007-4888/90/0011-1547$12.50

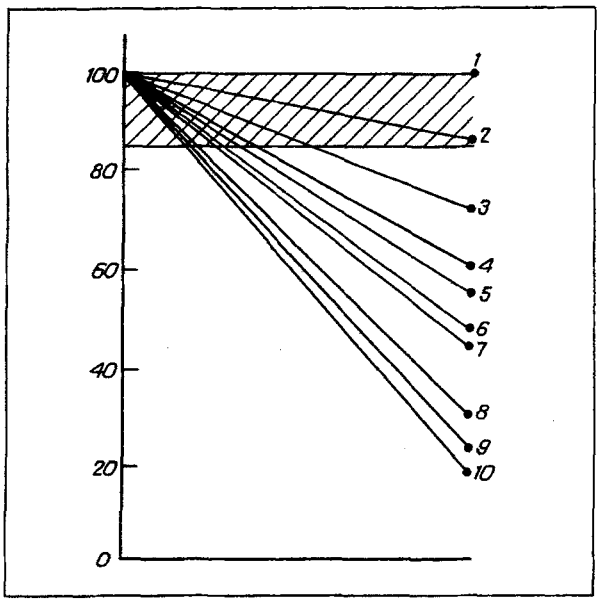

Fig. 1

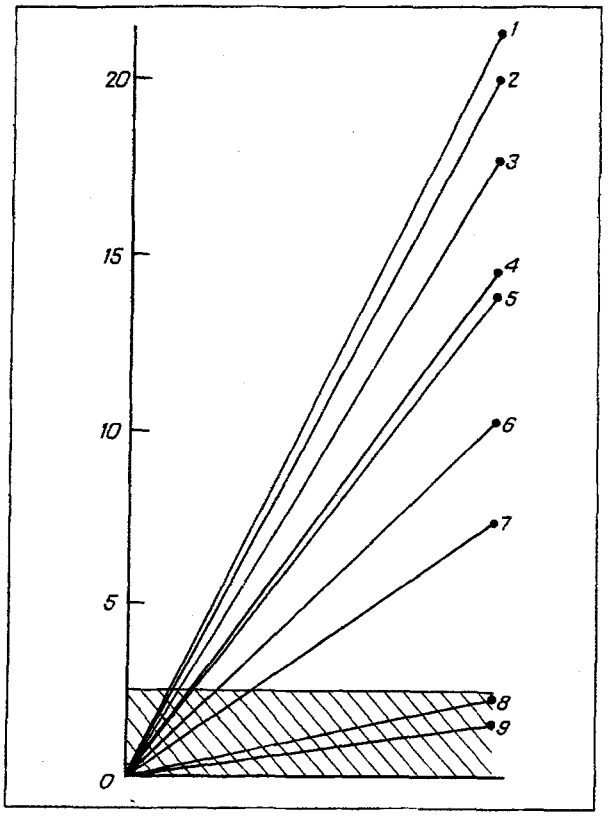

Fig. 2

Fig. 1. Bactericidal action of industrial mineral dusts (according to survival rate of microorganisms). Ordinate, percentage of surviving cells. 1) Control; 2) sivol; 3) cement; 4) forsterite from asbestos; 5) asbestos cement; 6) STBF; 7) UTBF; 8) nemalite; 9) antigorite; 10) Bazhenovo chrysotile – asbestos.

Fig. 2. Mutagenic action of industrial mineral dusts in microbial test in vivo. Ordinate, index of multiplicity. 1) Bazhenovo chrysotile – asbestos; 2) nemalite; 3) STBF; 4) asbestos cement; 5) cement; 6) UTBF; 7) sivol; 8) forsterite from asbestos; 9) antigorite.

cells averaged 60. This value of BCA is considered acceptable for detection of ME of substances in the in vivo microbial test [8]. Data on ME of asbestos relative to strain TA 1534 indicates that the mutagenic activity of asbestos is connected with the histidine locus HIS D 3053 and takes place in accordance with a reading frame shift mechanism. Since maximal ME of asbestos was exhibited against strain TA 1534, it also was used in subsequent experiments to test a series of fibrous and nonfibrous IMD, forsterite, sivol, nemalite, antigorite, cement, asbestos cement, superthin basaltic fibers (STBF), and ultrathin basaltic fibers (UTBF), were tested.

The experiments showed that some of these IMD possessed marked BCA (Fig. 1). Evidence on BCA of a number of IMD (different types of asbestos, nemalite, etc.) is given in [15]. No clear dependence could be discovered between BCA and the fibrous structure of the dusts. On the one hand, with the example of asbestos and forsterite (a product of thermal processing of asbestos at 850°C) it could be observed that a disturbance of the fibrous structure during heat treatment considerably (by about half, $p < 0.025$) reduced BCA. Sivol, on the other hand, which does not differ from asbestos in its fibrous structure but contains no magnesium or iron ions, nevertheless differed sharply from asbestos in its BCA. Other fibrous dusts, namely nemalite, STBF, UTBF, and asbestos cement – differing considerably in their dispersemorphological composition, had a similar level of BCA. The two nonfibrous dusts which were studied, namely antigorite and cement, possessed BCA, and lowered the survival rate of the bactorial cells by 5 and 2 times, respectively compared with the control ($p < 0.025$). It will be evident that the chemical composition of the IMD was more important than their fibrous or nonfibrous nature for survival of the cells. In fact, comparison of the bactericidal properties of asbestos and its nonfibrous chemical analog antigorite shows them to be virtually equal in BCA, and superior in this respect to the other dusts.

TABLE 1. Frequency of Back Mutations (10^{-8}) of Salmonella typhimurium Strains TA 1534, TA 1537, and TA 1950, under the Influence of Chrysotile-Asbestos in the in vivo Microbial Test

Experimental conditions	Mutation rate		
	TA 1534	TA 1537	TA 1950
Physiological saline (control)	10,56	5,08	2,67
Chrysotile-asbestos	286,56	9,19	11,22
p	<0,025	—	<0,025
Degree of ME	27,14	1,81	4,20

Note. Mean values of frequency of reverse mutations are given.

Complete agreement likewise was not found between BCA of the dusts and their ME (Fig. 2). For instance, antigorite and forsterite, substances with quite strong BCA, possessed minimal ME, which was not statistically significant compared with the spontaneous mutation rate. It could accordingly be concluded that these dusts are inert in vivo. Conversely, sivol, a dust with marked ME, virtually did not reduce the survival rate of the microorganisms. For most IMD, including asbestos, nemalite, UTBF, STBF, asbestos cement, cement, and sivol, definite correlation was observed between the BCA and ME. This will be clear from a comparison of orders of activity of the above-mentioned IMD, in which these dusts lay in virtually the same order: with respect to BCA – asbestos > nemalite > UTBF > STBF > asbestos cement > cement > sivol, and with respect to ME – asbestos > nemalite > STBF > asbestos cement > cement > UTBF > sivol. The index of multiplicity under these circumstances varied from 7.4 for sivol to 22 for asbestos. Unlike BCA, ME of the dusts correlated strongly with their fibrous composition. All fibrous dusts possessed ME, and absence of fibers in the specimen of IMD (antigorite) or a change in their length and diameter during each treatment (forsterite) led to abolition of the ME of the dust. The exception was the nonfibrous cement dust, which exhibited mutagenic properties similar to those of asbestos cement dust. Its activity in this case can be attributed to possible contamination of the cement by traces of hexavalent chromium which, as we know, possesses both mutagenic and carcinogenic action.

The ability of asbestos and several other IMD to index gene mutations in the histidine locus of Salmonella typhimurium TA 1534 which we found in the in vivo microbiral test is in agreement with data in the literature, according to which different types of asbestos can induce gene mutation in the hypoxanthine-guanine-phosphoribosyl transferase locus in cultures of Chinese hamster lung and ovarian cells [2, 10]. It is interesting to note that the ability of asbestos to induce gene mutations is independent of activation of the microsomal fraction S_9 of rat liver, and was found, just as in our experiments, only when the decrease in survival rate of the cells was taken into account [10]. In the literature, the microbial test for asbestos was used in its classical variant, namely Ames' test, with negative results [13, 16]. The authors cited attributed this result either to inability of liver fraction S_9 to activate asbestos in vitro, or to the fact that bacterial cells, unlike mammalian cells, do not phagocytose fibers [13, 16]. The use of a mammal as the intermediary, however, enabled ME of several IMD to be detected, for in that case activation of the dusts (if it does take place) or their biotransformation occur actually in the intact organism, with involvement of all enzyme systems necessary for this purpose. In this case it is interesting to note that some other chemical mutagens, whose ME could not be found by Ames' test, gave a positive result in the in vivo test: dimethylnitrosamine, cycasin, etc, [12]. As regards the possible mechanisms of the ME of a number of IMD, discovered in this test system, they are evidently linked with biochemical changes arising in the organism exposed to the action of dust. For instance, in studies of the molecular mechanisms of the biological action of asbestos conducted in recent years, activation of free-radical reactions [3], intensification of lipid peroxidation [17], etc., hve been established. Highly active metabolites arising in this way are known to possess ME.

Results of mutagenicity tests are often used to predict the potential carcinogenicity of substances [1, 2, 9]. There are sporadic references in the literature to the carcinogenic action of the dusts which we tested, and obtained by traditional long-term animal experiments. The carcinogenic action of chrysotile-asbestos [1], nemalite [14], UTBF and STBF

[1, 5], and asbestos cement [6] and the absence of such in antigorite [1], sivol [11], and cement [1] have been demonstrated. If these data are compared with the results of the microbial test in vivo, almost complete coincidence of mutagenic and carcinogenic effects will be noted for the IMD studied. This coincidence was not found only for sivol and cement, and accordingly, the results for these dusts with respect to ME in the in vivo test, must be regarded as falsely positive for the prediction of carcinogenicity. We know that the in vivo microbial test sometimes gives false positive predictions in relation to certain chemical substances also [12].

The use of the in vivo microbial test can thus reveal ME and can predict, with greater probability, the potential carcinogenicity of IMD, as a new object for applied genetics.

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TEST OF GENOMIC DNA OF BACTERIOPHAGE FD 103 REVEALS HYPERVARIABLE REGIONS OF HUMAN GENOME

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UDC 616-079.3:575

KEY WORDS: genome, bacteriophage

The bacteriophage with diameter FD 103 of E. coli is related to the bacteriophage with diameter M13, which contains a cluster of tandem repeating sequences of DNA hybridized with hypervariable regions of the human genome [1]. It was shown in the present investigation that blot-hybridization of the DNA with diameter FD 103 with human chromosomal DNA also reveals multiple interindividual hypervariability of DNA restriction fragments, which are inherited in accordance with Mendel's laws. The genetic replica of the DNA of an individual, detectable by hybridization with DNA with diameter FD 103, is similar, but not identical, to the genetic replica revealed by means of DNA M13mp19 (Fig. 1). The differences are characteristic of high-molecular weight restriction fragments of DNA (5000 base pairs) and may be due to mutational noncoincidences in clusters of tandem repeating sequences of DNA with diameter M13

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(Presented by Academician of the Academy of Medical Sciences of the USSR M. E. Vartanyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, p. 525, November, 1990. Original article submitted December 28, 1989.

0007-4888/90/0011-1550$12.50