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Activity of human carcinogens in the Salmonella and rodent bone marrow cytogenetic tests *

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Recently Shelby and Zeiger (1990) have concluded that organic chemicals that are carcinogenic for rodents, active in the Salmonella and rodent bone marrow cytogenetic tests are highly probably also human carcinogens.

The aim of this paper is to add some data to the results presented in two papers by Shelby (1988, 1990) for they did not include some data obtained by Soviet investigators and data obtained recently. In 1990 two more drugs were added to the list of agents of group 1 – cyclosporin (cyclosporin A) and thiotepa (Tomatis and Bartsch, 1990) – and the results of the investigations of these agents in the Ames assay and cytogenetic tests are absent in Shelby's papers (1988, 1990). I also add some other human carcinogens – ethanol, ionizing radiation – and one industrial process – aluminum production (Tomatis and Bartsch, 1990).

Asbestos is inactive in the classical Ames assay (IARC, 1987) but Soviet investigators (Frash et al., 1990) using the modified Ames assay (host-mediated assay) have shown that chrysotile asbestos (125 mg/kg in saline, 1/5 of LD_{50}) is mutagenic in Salmonella. The hosts were male Wistar rats which were intraperitoneally treated with asbestos. 24 h later the rats were treated intraperitoneally with Salmonella and animals were killed 6 h after that. The results show that asbestos caused a 27-fold increase in strain TA1534, a 4.2-fold increase in TA1950, but was inactive in strain TA1537 of Salmonella. Various asbestoses 24 h after intraperitoneal injection (500 mg/kg b.w. in saline) caused statistically significant increases in the number of polychromatic erythrocytes with micronuclei (MN) in bone marrow of CBA mice (Vanchougova et al., 1985). Chrysotile asbestos (from Bazenovsk) induced 2.63%, chrysotile A 1.0%, chrysotile B 1.04%, krokidolite 1.47%, antophylite 1.38% and amosite 1.13% cells with MN vs. 0.51% in intact controls. Under the same conditions chrysotile asbestos caused a 3.7-fold increase in the number of bone marrow cells with chromosomal aberrations (CA) of C57Bl/6 mice but ceolite asbestos caused a 3-fold increase only 28 days after treatment (Dournev et al., 1990). Soviet investigators have considered that the mutagenic and clastogenic effects of asbestos are due not to direct interaction of fibers with target cells, but to increased

* Addition to the paper of M.D. Shelby and E. Zeiger, Mutation Research, 1990, 224, 257–261.

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TABLE I

MUTAGENICITY IN SALMONELLA AND CLASTOGENICITY IN RODENT BONE MARROW AND CARCINOGENICITY IN ANIMALS OF IARC GROUP 1 CARCINOGENS

Carcinogen	Salmonella mutagenicity	Rodent bone marrow clastogenicity	Carcinogenicity in animals
Organic compounds, combinations or groups			
1 aflatoxins	+	+	S
2 4-aminobiphenyl	+	+	S
3 analgesics containing phenacetin	+	+	L
4 azathioprine	+	+	L
5 benzene	–	+	S
6 benzidine	+	+	S
7 betel quid and tobacco	+	+	L
8 bis(chloromethyl)ether	+	±	S
9 chlorambucil	+	+	S
10 chlornaphazine	+	+	L
11 cyclophosphamide	+	+	S
12 cyclosporin *	–	–	L
13 ethanol *	±	±	I
14 melphalan	+	+	S
15 MOPP	+	+	ND
16 mustard gas	+	+ *	L
17 myleran	+	+	L
18 2-naphthylamine	+	+	S
19 thiotepa *	+	+	S
20 tobacco, smokeless	+	+	I
21 tobacco smoke	+	+ *	S
22 treosulphan	+	+	ND
23 vinyl chloride	+	+	S
Tars and oils			
24 coal-tar pitches	+	+ *	S
25 shale oil	+ *	+ *	S
Hormones			
26 diethylstilbestrol	–	+	S
Metals			
27 arsenic compounds	–	+	L
28 chromium compounds	+	+	S
29 nickel and nickel compounds	–	+ *	S
Fibers			
30 asbestos	± *	+ *	S
31 talc containing asbestiform fibers	–	–	I
Industrial process			
32 aluminum production *	+	+	ND
Other			
33 ionizing radiation *	+	+	S

* Added to the list of Shelby and Zeiger (1990). +, positive, –, negative and ±, inconclusive results in the short-term tests; S, sufficient, L, limited, I, inadequate evidence of carcinogenicity; ND, no data in long-term experiments using animals.

lipid peroxidation induced by asbestos, Ionizing radiation (Dubinin, 1986) and thiotepa (IARC, 1987) were active in the Ames assay and cytogenetic tests. Tobacco smoke (Balansky et al., 1988) and sulfur mustard (Ashby et al., 1991) induced MN in bone marrow of mice. Ethanol had inconclusive activity in the Ames assay (De Flora et al., 1984) and it induced a very low level of CA (2.6% vs. 0 in control), polyploidy (7.8% vs. 1% in control) and aneuploidy (36.6% vs. 15.6% in control) in bone marrow cells of male Wistar rats 24 h after oral administration (3000 mg/kg b.w., 1/5 of LD_{50}) (Barilyak and Kozachouk, 1985). At best, ethanol should be considered equivocal in the rodent bone marrow test for CA. Crude shale oil was directly mutagenic in strain TA98 (indicated at least twice the background level) and elicited a mutagenic response in four Salmonella strains (TA98, TA100, TA102 and TA104) with exogenous metabolic activation (indicated activity at least 1.5 times the background level) (Bogovski et al., 1990). Shale oil also increased the CA level in bone marrow cells of mice (Mayne and Deaven, 1982). Cyclosporin is inactive in the Ames assay and cytogenetic tests (IARC, 1990). Extracts of coal tar pitches induced significant increases in MN in bone marrow of CBA mice 24 h and 48 h after intraperitoneal injection (data were not presented in the paper, Frash and Vanchougova, 1987). Nickel dusts (1/2 of LD_{50} in saline) statistically significantly (2–3 times, depending on the nickel contents in the dusts) increased the frequency of MN in bone marrow of mice 24 h after injection (Kisseliova et al., 1989) and MN and CA in bone marrow cells of rats (data were not presented in the paper, Zhong et al., 1989). Emission dusts from aluminum plants were strongly mutagenic with exogenous metabolic activation in Salmonella (Pinter et al., 1990). Soviet investigators have shown that wild mice trapped in the region and in the territory of an aluminum plant had increased CA levels in the bone marrow cells (8.2% vs. 1.67% in control, Gileva et al., 1990).

In Table 1 are presented only agents which are investigated in the Ames assay and cytogenetic tests. Among 33 agents only talc containing asbestiform fibers and cyclosporin are inactive in two tests, and ethanol gives inconclusive results. Consequently, the activity of 33 human carcinogens in the battery of two short-term tests (activity at least in one test system) is 90.9–93.9% (it depends on one inconclusive result, ethanol). Benzene, cyclosporin, diethylstilbestrol, arsenic compounds, nickel and talc containing asbestiform fibers are inactive in the Ames assay, asbestos and ethanol give inconclusive results. So, the sensitivity of this assay is 75.8–81.8% (it depends on two inconclusive results). In the cytogenetic tests only talc containing asbestiform fibers and cyclosporin are inactive, bis(chloromethyl)-ether and ethanol give inconclusive results. So, the sensitivity of cytogenetic tests in identification of 33 human carcinogens is 87.9–93.9% (it depends on two inconclusive results). Cytogenetic tests are more sensitive in the identification of carcinogens than the Ames assay at least for this group of agents. It is interesting that some carcinogens inactive in the Ames assay or cytogenetic tests or giving inconclusive results increased the CA level in the lymphocytes of humans exposed to these agents. Such agents are benzene, nickel, arsenic compounds, bis(chloromethyl)ether (IARC, 1987), cyclosporin (IARC, 1990), ethanol (Obe et al., 1986), asbestos (Ressner et al., 1990). So I think that if an agent is inactive in the Ames assay or cytogenetic tests in rodents, the reaction of human somatic cells to this agent is very important.

I suppose that cyclosporin induces CA in human lymphocytes not via genotoxic mechanisms but via its immunosuppressive property. It is known that genome instability is connected with immune system abnormalities (Ilyinshikh, 1990) and cyclosporin-induced immunosuppression leads to the elevation of the number of mutant cells. Increased numbers of lymphocytes with CA were observed only after 1 year of cyclosporin therapy. But investigations concerning clastogenicity in rodents were performed 24–48 h after cyclosporin treatment. Voogt (1989) also concluded that another immunosuppressive agent, azathioprine, induces tumors in humans via its immunosuppressive property but not via genotoxicity. So the Ames assay and cytogenetic tests in rodents cannot identify the genotoxicity of any hormonal and some inorganic agents as described by Shelby and Zeiger (1990), nor immunosuppressive agents with mechanisms of action other

than genotoxicity. I suppose that the genotoxicity of some such agents is identified in short-term tests only by chance.

It would be of interest to compare the results of long-term studies of carcinogenicity in anim..s (Tomatis and Bartsch, 1990) and the activity of carcinogens in two short-term tests. Only 30 of the 33 carcinogens were studied in long-term studies. Three agents showed inadequate evidence of carcinogenicity in animals (ethanol, smokeless tobacco and talc containing asbestiform fibers) and eight agents had limited evidence of carcinogenicity (analgesics containing phenacetin, azathioprine, betel quid with tobacco, chlornaphazine, cyclosporin, mustard gas, myleran and arsenic compounds). Only 19 agents (63.3%) had sufficient evidence of carcinogenicity in animals and altogether 27 agents (90%) had sufficient or limited evidence of carcinogenicity. Consequently the sensitivity of long-term experiments using animals is almost the same as for batteries of short-term test including the Ames assay and cytogenetic tests (at least for this group of agents).

Conclusion

The additional data have confirmed the conclusion of Shelby and Zeiger (1990) than the greatest concern should center on agents that are carcinogenic for animals, mutagenic in Salmonella and rodent clastogens. In the case of inconclusive or negative results in rodent clastogenicity or in the Ames assay the reaction of human somatic cells to this agent is very important. I agree with Ashby and Tennant (1991) that 'non-genotoxic rodent carcinogens cannot be automatically neglected as possible human carcinogens' and I suppose that all hormonal or immunosuppressive agents that are carcinogenic for animals (sufficient or limited evidence) but inactive in short-term tests may be carcinogenic for humans, because they induce tumors not via genotoxic mechanisms.

The battery consisting of two short-term tests (the Ames assay and rodent clastogenicity) is very sensitive in identification of genotoxic carcinogens. It is very important for genotoxic agents are prevalent among human carcinogens and also among agents probably and possibly carcinogenic for humans (Tomatis and Bartsch, 1990).

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