Carcinogenesis vol.8 no.11 pp.1711–1714, 1987

PENGAD-Bayonne, N. J.
PLAINTIFF'S EXHIBIT
1250

Chromosome aberrations in mouse bone marrow cells following *in vivo* exposure to 1,3-butadiene

Richard D.Irons[1,3], Mitsuo Oshimura[2] and J.Carl Barrett[2]

[1]Departments of Cellular and Molecular Toxicology and Experimental Pathology, Chemical Industry Institute of Toxicology, 6 Davis Drive. Research Triangle Park. NC 27709 and [2]Environmental Carcinogenesis Group. Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

[3]To whom reprint requests should be sent

Chronic exposure to 1,3-butadiene (BD) results in a marked increase in the incidence of thymic lymphoma in male B6C3F1 relative to NIH Swiss mice whereas no demonstrable differences in bone marrow (target organ) toxicity exist. Repeated exposure to BD is known to produce a macrocytic anemia and an increase in the frequency of micronuclei in circulating erythrocytes in both strains. The present study was undertaken to determine if chromosomal breakage, aneuploidy or both reflect differences in BD leukernogenicity observed between B6C3F1 and NIH Swiss mice. Mice were exposed to a single concentration of BD (1250 p.p.m.) for 6 h. Bone marrow cell preparations were made at 24, 48, 72 and 96 h after cessation of exposure. In both strains comparable increases in the frequency of chromosomal aberrations (of the chromatid type) were observed following exposure to BD. Significant differences in the number of chromosomes were not observed, although a pattern of chromosomal loss in cells from treated animals was observed. These results indicate that BD-treatment *in* vivo produces significant increases in chromatid aberrations but not aneuploidy in both strains. Therefore it is concluded that bone marrow toxicity, including cytogenetic abnormalities, is not predictive of leukemogenicity in these mice.

Introduction

1.3-Butadiene (BD*) is a colorless gas used as a monomer in the copolymerization of styrene-butadiene or 'synthetic' rubber. Chronic exposure of B6C3F1 mice to 625 or 1250 p.p.m. BD for 1 year produces a high incidence of leukemia/lymphoma as well as a variety of additional tumors (1,2). The role of endogenous retroviruses (MuLV) in murine leukemogenesis is incompletely understood, in part because increased or altered MuLV expression is frequently associated with leukemia/lymphomas irrespective of etiology, and because murine leukemogenesis models in which a role for MuLV appears likely appear to be restricted to certain strains. A comparative leukemia incidence study in two strains of mice has now been completed (R.D.Irons, unpublished results). Strains chosen for comparison were the B6C3F1 hybrid and the NIH Swiss. The B6C3F1 hybrid was chosen because it has previously been shown to develop a high incidence of lymphoma following BD exposure. The NIH Swiss mouse was chosen for comparison because it does not possess intact endogenous ecotropic retrovirus sequences, does not express ecotropic retroviruses, and rarely expresses any type of endogenous retrovirus (3,4). Results of the comparative incidence study confirm thymic lymphoma/leukemia to be the primary cause of death in B6C3F1 mice chronically exposed to BD with the incidence of thymic lymphoma/leukemia approaching 60% after 1 year. In contrast, the incidence of thymic lymphoma/leukemia in identically treated NIH Swiss mice was approximately 10% (R.D.Irons, unpublished results). Since one of the principal differences between these two strains relates to their endogenous retroviruses, a presumptive role for endogenous retrovirus in BD-leukemogenesis is suggested.

Repeated exposure of B6C3F1 or NIH Swiss mice to 1250 p.p.m. BD for 3–24 weeks results in a macrocytic anemia, a particularly striking feature of which is a large increase in the frequency of micronuclei in circulating peripheral erythrocytes (RBC) and their prominence in precursor cells in bone marrow as well (5,6). Although increases in the number of micronuclei in peripheral RBC are a reliable indicator of dyserythropoiesis in the mouse (7), it cannot be determined *a* priori whether this results from chromosomal breakage, aneuploidy or both. The present study was undertaken to ascertain (i) whether the frequency or type of chromosomal abnormalities in bone marrow target cells segregated with the incidence of leukemogenesis in these two strains and were thus predictive of leukemogenic potential, and (ii) the mechanism of induction of micronuclei following exposure to BD.

Materials and methods

Male B6C3F1 mice (4–6 weeks old) were obtained from Charles River Breeding Laboratories (Wilmington, MA), quarantined for a 2-week period. and randomly assigned to control and treatment groups. Male NIH Swiss mice were random bred from breeding stock obtained from the National Cancer Institute (Frederick, MD). Serologic examination of sentinal animals during the breeding and quarantine period confirmed the absence of antibody titers io pathologic murine viruses in either strain. Mice were individully housed in hanging stainless steel wire cages in 8 m^3 chambers and exposed io 1250 p.p.m. BD or ambient air for 6 h. 1,3-Butadiene, >99.5% purity, was supplied by Exxon Corporation (Baton Rouge, LA) in 49 lb cylinders. A detailed description of analytical procedures and chamber exposure conditions has been published elsewhere (5).

Three animals from *each* group (treated and control. NIH Swiss and B6C3F1) were killed 24, 48, 72 *or* 96 h after a single 6 h exposure io BD. Three hours prior to each time point mice were injected i.p. with 0.4 ml Colcemid (100 μg/ml). Animals were killed with CO_2, and bone marrow cells harvested by flushing femurs with minimum essential medium (MEM) containing 10% fetal calf serum. Chromosomal preparations were made from cells treated with hypotonic solution (0.075 M KCl) for 20 min. fixed with ethanol/acetic acid (3:1), air dried, and stained with 5% Giemsa's solution. For *each* animal 50 well spread metaphases were analyzed for chromatid and chromosome aberrations. Data was *analyzed* first by the Ansari-Bradley test *for* equal dispersions and then tested for significant differences using the Mann–Whitney *U* test (8,9).

Results

Table I summarizes cytogenetic abnormalities in bone marrow cells from B6C3F1 and MH Swiss mice exposed to 1250 p.p.m. BD for 6 h. Exposure to BD resulted in a high frequency of chromatid breaks as well as increases in chromatid and isochromatid gaps in both strains. Moderate increases in fragments

*Abbreviations: BD, 1,3-butadiene; RBC, erythrocytes; MEM, minimum wential medium.

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Table I. Frequencies of chromatid aberrations in bone marrow cells from control and BD-treated B6C3F1 and NIH Swiss mice 24, 48, 72 and 96 h after exposure

Strain	Treatment/ animal	Time	Chromosome No.				Chromatid aberrations[a]							Total aberrations		% Aneuploid cells
			38	39	40	41	ctg[b]	ictg	ctb	ictb	frag	exch.	marker	with gaps	without gaps	
NIH Swiss	Control	24														
	1				50		1		1		1					
	2			2	48		1									
	3			2	48											
														4	2	2.7
	Treated	24														
	1			1	49		4	1	6							
	2			3	47		3	2	5							
	3			3	47		4	1	5		1	1				
														33[c]	18[c]	4.0
B6C3F1	Control	24														
	1			1	49		1									
	2				50											
	3			1	49			1								
														2	0	1.3
	Treated	24														
	1		1	3	46		6	5				1				
	2				50		10	2	6		1					
	3			2	48		5	2	8		1					
														47[c]	17[c]	4.0
NM Swiss	Control	48														
	1			1	49		1									
	2			2	48				1							
	3				50		1									
														3	1	2.0
	Treated	48														
	1			2	48		1									
	2			2	48		1	3			3		2			
	3		1	3	46		2	3			2					
														17	7	5.3
B6C3F1	Control	48														
	1			2	48		1									
	2			1	49											
	3			1	49			1								
														2	0	2.7
	Treated	48														
	1			1	49		3		1							
	2			2	48		4									
	3			2	48		2	2	3		1	1				
														17[c]	6	3.3
NM Swiss	Control	72														
	1			2	48		1		1							
	2				50		1									
	3			1	49											
														3	1	2.0
	Treated	72														
	1			5	45		1		1							
	2			2	48											
	3			3	47				2							
														4	3	6.7
B6C3F1	Control	72														
	1			1	49		1									
	2				50											
	3				50											
														1	0	0.6
	Treated	72														
	1				50											
	2			2	48		1		3							
	3			3	47											
														4	3	3.3

Table I. Continued

Strain	Treatment/ animal	Time	Chromosome No.				Chromatid aberrations[a]							Total aberrations		% Aneuploid cells
			38	39	40	41	ctg[b]	ictg	ctb	ictb	frag	exch.	marker	with gaps	without gaps	
NIH Swiss	Control	96			50											
	1			1	49		1									
	2			2	48											
	3															
														1	0	2.0
	Treated	96														
	1			1	49		1		1		1					
	2			2	48		1									
	3			3	47				1				1			
														6	4	4.0
B6C3F1	Control	96														
	1				50											
	2			1	49		1									
	3			2	48											
														1	0	2.0
	Treated	96														
	1			1	49		1		1		1					
	2			2	48		1						1			
	3			3	47				1							
														6	4	5.3

[a]50 metaphases were examined per animal ($n = 3$).
[b]ctg: chromatid gap, ictg: isochromatid gap. ctb: chromatid break, ictb: isochromatid break. frag: fragment, exch.: chromatid exchange, marker: marker chromosome.
Treated significantly different from control ($P < 0.05$).

and chromatid exchanges were noted; however, chromosome-type abnormalities were not observed. The maximum frequency of abnormalities in both strains was observed at 24 h and diminished with time, presumably as a result of cytotoxicity. No significant numerical chromosome changes were observed in cells from control or treated animals in either experiment, although a consistent pattern of chromosome loss was observed in treated animals. No extranumerary chromosomes were observed in all the cells examined.

Discussion

Chronic exposure to 1250 p.p.m. BD has previously been shown to produce a high tumor incidence in B6C3F1 mice, thymic lymphoma being the principal cause of death. Previous studies of radiation- or chemical-induced murine thymic lymphoma have indicated that bone marrow damage and/or involvement plays an obligatory or enabling role in the development of the disease. Consistent with these previous observations, bone marrow toxicity was also observed in B6C3F1 or NIH Swiss mice following exposure to BD (5,6). A prominent feature of toxicity was the increased frequency of micronuclei in bone marrow cells and peripheral erythrocytes. The frequency of circulating micronuclei was found to be increased more than 5-fold in B6C3F1 and 7-fold in NIH Swiss mice after 6 weeks of BD exposure (5,6).

Cytogenetic analysis of bone marrow cells indicates that exposure to 1250 p.p.m. BD results in a marked increase in chromatid aberrations in both strains. Although a pattern of chromosome loss, consistent with loss of chromosome segments (i.e. segmental aneuploidy), accompanied BD treatment, extranumerary chromosomes were not observed. These results suggest that the mechanism of micronuclei formation following BD exposure is more likely associated with chromatid breaks than mitotic spindle events. Therefore, the treatment-related increase in micronuclei previously observed following exposure to this compound, appears to correlate with cytogenetic damage in bone marrow cells.

Molecular mechanisms of murine leukemogenesis have been the subject of intensive study, although the respective roles of endogenous MuLV and bone marrow damage are not yet completely understood. In virtually all experimental models of leukemogenesis the bone marrow is an obligate target organ. In strain-specific radiation-induced leukemogenesis both bone marrow damage and MuLV are implicit requirements (10–12). Chemically induced lymphomas frequently demonstrate altered MuLV expression, yet it is not clear whether host DNA damage is a direct or indirect requirement for leukemogenesis in these cells. The results reported here indicate that BD induces cytogenetic abnormalities in murine bone marrow cells, and that these occur independent of both endogenous retroviral background and leukemogenesis in these animals.

Acknowledgements

The authors thank Mr Mark Higuchi and Ms Come N.Smith for expert technical assistance in the conduct of these studies.

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Received on May 14, 1987; accepted on August 6. 1987