Short Communication

COMBINED CYTOGENETIC EFFECTS OF BENZENE AND RADIATION ON CULTURED HUMAN LYMPHOCYTES

ベンゼン投与および放射線照射の複合による
培養ヒトリンパ球染色体の変化

It is frequently reported that occupational exposure to ambient benzene can induce chromosome aberrations^{1,2)} in peripheral blood lymphocytes as well as in the bone-marrow cells. Experimental studies have been accumulated on induced chromosome changes³⁻⁵⁾ in bone-marrow cells or in peripheral lymphocytes. Similar chromosome changes are also found in the workers exposed to radiation accidentally^{6,7)} and A-bomb survivors in Hiroshima and Nagasaki.^{8,9)}

There is a increasing possibility of synergistic effect of mutagenic or clastogenic agents in the environmental circumstances.^{10,11)} Then, in the present study the author observed *in vitro* combined effects of benzene and radiation on chromosome aberrations in human peripheral lymphocytes after 53 hours incubation.

Materials and Methods

About 0.3 ml of peripheral blood freshly drawn from a normal male adult was suspended in approximately 5 ml of culture medium which consisted of medium TC-199 (80%) and fetal calf serum (20%) with 500 units of penicillin and 0.5 mg of streptomycin. In the first study, benzene was added to the culture media with final concentrations of 3.0×10^{-3} M, 1.0×10^{-3} M, 2.0×10^{-4} M and 4.0×10^{-5} M. Immediately after this treatment, caps of the vessels were tightened. In the second, benzene was added at levels of 1.0×10^{-3} M, 2.0×10^{-4} M, and 4.0×10^{-5} M and the caps were tightened. The blood samples in the benzene-added media and controls were exposed to gamma-rays emitted from 4000 Ci ¹³⁷Cs source at room temperature. Total dose was 100 rad at a dose-rate of 33-35 R/min calibrated in advance.

All samples were initiated with phytohemagglutinin-P and incubated at 37°C for 53 hours including the last 6-hr treatment with deacetylmethyl colchicine (Colcemid-CIBA) at the final concentration of 0.5 µg/ml. Chromosome preparations were made according to the standard air-drying method¹²⁾ by a slight modification and were stained with Giemsa.

Microphotographs were made for randomly selected cells with sufficiently separated chromosomes.

On these pictures structural chromosome aberrations were recorded as dicentrics, rings, acentric fragments, breaks and gaps (of both chromosome-type and chromatid-type) with reference to numerical analysis of the chromosome-set, consulting the original slide when necessary.

Results

Structural chromosomal aberrations per cell and percentage of the cells with structural chromosome change induced by benzene exposure are tabulated in Table 1. None of dicentrics and rings was observed. At relatively higher concentrations of benzene, all types of deletions except chromosome-type breaks, and percentage of the aberrant cells increased significantly as statistically indicated, though chromosome-type deletions were tested by chi-square method with Yates' correction.

Results indicated that leukocytes in their late S-G2 stage may have higher chromosomal sensitivity to benzene than those in the G0-G1 stage, as the frequency of chromatid-type deletions was very higher than that of chromosome-type ones.

Structural chromosome changes induced by 100 rad irradiation soon after the addition of benzene are described in Table 2 as dicentrics & rings per cell, chromosome-type deletions per cell and chromatid-type deletions per cell. There are also shown percentages of the cells with chromosomal aberrations whose increase had a good dose-response relationship with benzene concentration.

Discussion

On chromosome effect of benzene, Koizumi *et al.*⁵⁾ reported previously that there was 21.8 % of cells with breaks and gaps at benzene concentration of 1.1×10^{-3} M but there was only 15.3% of the aberrant cells at a higher concentration of 2.2×10^{-3} M, though in controls it was 8.5%, in 72-hr cultured human leukocytes. This is because some aberrant cells may not survive to the next cell division.¹³⁾ Most of the metaphase leukocytes harvested after 72 hours of incubation were in their second division in culture from the initiation with PHA. The present study showed a good relationship between benzene concentration and percentage of the cells with structural chromosome aberrations (Table 1), as leukocytes were harvested in their first division after 53 hours of culture.¹³⁾

Gaps per cell, obtained by subtracting breaks per cell from breaks and gaps per cell, was increased remarkably according to the benzene concentration in both tables. The data were suggestive of a mechanism of benzene on chromosomal aberrations if gaps observed were caused mainly by indirect effect on histone or other connector proteins which consti-

Table 1. Effects of benzene on chromosome aberrations of 53-hr cultured human lymphocytes (Mean $\pm$ S.D.).

Concentration of benzene treated	Number of cells examined	Cells with aberration [†] (%)	Chromosome-type aberration		Chromatid-type aberration	
			Breaks per cell	Breaks & gaps per cell	Breaks per cell	Breaks & gaps per cell
Control	236	10.2 $\pm$ 2.1	0	0.004 $\pm$ 0.004	0.025 $\pm$ 0.010	0.106 $\pm$ 0.021
4.0 $\times$ 10 ⁻⁵ M	146	15.1 $\pm$ 3.2	0	0.027 $\pm$ 0.014	0.048 $\pm$ 0.018	0.144 $\pm$ 0.031
2.0 $\times$ 10 ⁻⁴ M	135	18.5 $\pm$ 3.7*	0.007 $\pm$ 0.007	0.030 $\pm$ 0.015	0.052 $\pm$ 0.020	0.170 $\pm$ 0.035
1.0 $\times$ 10 ⁻³ M	159	22.0 $\pm$ 3.7**	0	0.031 $\pm$ 0.014	0.050 $\pm$ 0.020	0.208 $\pm$ 0.036**
3.0 $\times$ 10 ⁻³ M	145	29.0 $\pm$ 4.5**	0.014 $\pm$ 0.010	0.048 $\pm$ 0.018*	0.076 $\pm$ 0.023*	0.303 $\pm$ 0.046**

Cells with aberration[†]; cells with chromosome- and/or chromatid-type breaks & gaps.* significant at $P < 0.05$, ** significant at $P < 0.01$ (chi-square test).Table 2. Combined effects of benzene and radiation of chromosome aberrations of 53-hr cultured human lymphocytes (Mean $\pm$ S.D.).

Concentration of benzene treated	Number of cells examined	Cells with aberration (X)	Dicentric and rings per cell	Chromosome-type deletion		Chromatid-type deletion	
				Breaks per cell	Breaks & gaps per cell	Breaks per cell	Breaks & gaps per cell
Control (100rad only)	211	29.4 $\pm$ 3.7	0.100 $\pm$ 0.022	0.085 $\pm$ 0.020	0.118 $\pm$ 0.024	0.052 $\pm$ 0.016	0.104 $\pm$ 0.022
4.0 $\times$ 10 ⁻⁵ M (+ 100rad)	128	34.4 $\pm$ 5.2	0.117 $\pm$ 0.030	0.109 $\pm$ 0.029	0.133 $\pm$ 0.032	0.063 $\pm$ 0.022	0.172 $\pm$ 0.037
2.0 $\times$ 10 ⁻⁴ M (+ 100rad)	142	40.9 $\pm$ 5.4*	0.162 $\pm$ 0.034	0.099 $\pm$ 0.026	0.134 $\pm$ 0.031	0.085 $\pm$ 0.024	0.211 $\pm$ 0.039**
1.0 $\times$ 10 ⁻³ M (+ 100rad)	144	50.5 $\pm$ 5.9**	0.215 $\pm$ 0.039**	0.118 $\pm$ 0.029	0.174 $\pm$ 0.035	0.104 $\pm$ 0.027	0.257 $\pm$ 0.042**

Cells with aberration; cells with dicentric and rings and chromosome- and/or chromatid-type breaks & gaps.

* significant at $P < 0.05$, ** significant at $P < 0.01$ (chi-square test).

tute chromosomes in combination with DNA strands. The combined effect of benzene and radiation on chromosomes seems to be synergistic only in dicentric and rings, and to be additive in the other aberrations. To make the mechanism of the synergistic effect clear, further study is being prepared.

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