ANALYSES OF CHROMOSOME ABERRATIONS IN LYMPHOCYTES AND BONE MARROW CELLS INDUCED BY RADIATION OR BENZENE

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ABSTRACT

The chromosome and chromatid type aberration can be induced by benzene and the dicentric and ring ones were not observed in vitro experiment but observed in vivo one. In vitro experiment a good linear regression can be given between benzene concentrations and total aberration cells while power regression for radiation dose. The chromosome aberrations induced by benzene combined with radiation in rabbit blood lymphocytes are higher than in bone marrow cells.

Keywords Gamma-radiation, Benzene, Combined-effect, Chromosome aberration

1 INTRODUCTION

The curves of dose response for the frequency of induced chromosome aberrations obtained in vitro irradiated lymphocytes are used to give reliable estimates of the radiation dose in cases where individuals have been accidentally exposed to radiation[1~3]. However, in some special environment chromosome aberrations can be also induced by other factors, especially chemical ones except radiation. Therefore, combined effects must be considered in order to estimate accurately radiation dose. The studies on chromosome aberration induced by benzene and its metabolic products combined with radiation have been reported[4~7], but the studies on combined cytogenetic effects of blood lymphocytes and bone marrow cells in vivo are yet rare. Therefore, the chromosome aberrations induced by ^{60}Co γ-rays radiation combined with benzene in vitro human peripheral lymphocytes and in vivo rabbit peripheral lymphocytes and bone marrow cells were examined in the present investigation.

2 MATERIALS AND METHODS

2.1 In vitro experiments

Two donors are healthy male and female aged 18 and 19, neither therapeutic or diagnostic irradiation, nor history of occupational irradiation, viral vaccination or drug intake. Blood was drawn from the cubital veins of each donor into a sterile heparinized plastic syringe, 0.5 ml blood was added into culture solution consisting of 5 ml RPMI 1640 medium, 1.5 ml calf serum, 0.5 ml BudR (60 μg/ml) in a tin-walled glass tube.

Samples of control group are neither treated by benzene nor by ^{60}Co γ-rays. Samples of group B are treated by benzene. The final benzene concentrations of B1~4 samples

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are 0.0007, 0.0014, 0.0028, 0.0056 mol/L, respectively. R1~4 samples of group R are irradiated by ^{60}Co γ-rays at 3.33 mGy/min and kept at constant temperature of 37°C. Their doses are 0.5, 1.0, 2.0, 3.0 Gy, respectively. Samples of group B+R were obtained through B1~4 samples irradiated.

At the same time 0.5 ml PHA was added to each human blood sample, then incubated for 48 h at 37°C in dark before fixation. After 43 h colchicine was added to each culture to give a final concentration of 0.1 μg/ml and cells were harvested 5 h later. Thereafter, the cells were treated with 0.075 mol/L kcl for 15 min at 37°C, fixed with absolute mathyl alcohol acetic acid (3:1) and spread on the slides. Air dried slides were stained by the UPG (ultraviolet plus giemsa) technique to provide scoring of exclusively M1 cells identified by uniformly stained sister chromatid.

2.2 In vivo experiments

Weighing 3.26~2.76 kg, 6~7 months old, 30 healthy male rabbits are divided into 6 groups. Every group was 5 ones. Animals of control, radiation; low benzene, low benzene + radiation; high benzene, high benzene + radiation groups are injected subcutaneously 0.6 ml peanut oil, 0.6 ml peanut oil + 0.06 ml (corresponding 17.7 mg/kg body weight) benzene, 0.6 ml peanut oil + 0.6 ml (corresponding 177 mg/kg body wight) benzene/(d·rabbit); respectively, 5 d per week for 14 weeks. After injection, radiation, low benzene + radiation, high benzene + radiation groups are irradiated by ^{60}Co γ-rays at 0.899 mGy/min×60 d.

The blood drawn from the ear vein at different times of experiment are cultured for 48 h at 38°C in the dark before fixation. Other procedures of chromosome preparation are the same as those described above.

After experiment end, the bone marrow cells from rabbit femurs are flashed out using 6 ml RPMI 1640 and transplanted into culture bottle, then 0.1 μg colchicine is added. Usually, 2 cultures are set up for each rabbit. The cultures are incubated at 38°C for 5 h. Thereafter, chromosome preparations are finished according to common method.

2.3 Chromosome analysis

Chromosome analysis is carried out in metaphase containing 46 centromeres in man and 44 ones in rabbit. All detectable aberrations are recorded except chromosomal gaps. The dose-effect relationships are determined for the dicentric plus rings (including dicentric, centric rings and polycentric chromosomes), the acentric (including terminal deletions, minutes and acentric rings), aberrational cells (including chromosome type aberration cells), total aberrations, total aberration cells (including chromosome type and chromatid type aberration cells) and chromatid aberrations (including chiefly chromatic deletions and breakages).

3 RESULTS

3.1 In vitro experiments

Table 1 shows that for dose effect of benzene, a good fit was given only between total aberration cell rate and benzene concentration, i.e. $Y(\%) = 1.40 + 108.70\ D(\text{mol/L})$; for

Table 1
Yield and SEF values of chromosome aberrations in human blood lymphocytes

%

Groups	Doses		Cells scored	Chromosome aberrations					
	Ben./ mmol·L^{-1}	γ-rays/ Gy		d.+r.(SEF)	ace.(SEF)	t.ab.	ab.c.	Chromat. ab.	t.ab.c.
Control	0	0	400	0	0.25	0.25	0.25	0	0.25
	0.7	0	400	0	0.75	0.75	0.75	0.75	1.50
	1.4	0	400	0	0.75	0.75	0.75	0.75	1.50
B	2.8	0	400	0	1.00	1.00	1.00	0.75	1.75
	5.6	0	400	0	1.25	1.25	1.25	0.75	2.00
	0	0.5	200	3.00	7.50	10.50	10.50	0	10.50
	0	1.0	200	8.50	12.00	20.50	19.50	1.00	19.50
R	0	2.0	200	15.50	25.50	41.00	39.00	0.50	39.00
	0	3.0	200	22.00	47.00	69.00	57.50	1.50	58.50
	1.4	0.5	200	2.00(0.4953)	6.50(0.8676)	8.50	8.50	0.50	9.00
	1.4	1.0	200	4.00(0.7571)	12.50(0.9510)	16.50	16.50	1.00	17.50
B_2+R	1.4	2.0	200	17.50(1.1575)	31.50(1.0244)	49.00	43.00	0.50	43.00
	1.4	3.0	200	40.00(1.4869)	43.50(1.0651)	83.50	69.50	5.00	69.50
	2.8	0.5	200	4.00(0.9201)	7.00(0.9207)	11.00	9.00	1.00	10.00
	2.8	1.0	200	4.50(1.0346)	14.00(1.0414)	19.00	18.50	1.00	19.50
B_3+R	2.8	2.0	200	21.50(1.1630)	32.50(1.1716)	54.00	44.50	3.50	45.50
	2.8	3.0	200	31.00(1.2459)	55.00(1.2470)	86.00	67.00	5.00	67.50
	5.6	0.5	200	3.50(1.0034)	9.00(1.1687)	12.50	12.50	2.00	14.00
	5.6	1.0	200	8.00(1.1908)	17.00(1.1644)	25.00	23.00	1.50	24.00
B_4+R	5.6	2.0	200	16.00(1.2520)	34.50(1.1234)	50.50	45.00	3.00	46.00
	5.6	3.0	200	35.50(1.3131)	45.00(1.0892)	81.00	63.00	1.50	63.00

Notes: ben. = Benzene, d.+r. = Dicentric plus rings, ace. = Acentric, t.ab. = Total aberrations, ab.c. = Aberration cells, chromat.ab.=Chromatid aberrations, t.ab.c.=Total aberration cells

Table 2
Values of k and n estimated by fitting the various aberration types to $Y = kD^n$

Groups		d.+r.	ace.	t.ab.	ab.c.	t.ab.c.
R	k	7.1490	13.7113	21.0036	20.0309	20.0754
	n	1.0947	1.0156	1.0383	0.9540	0.9616
	P	<0.05	<0.05	<0.01	<0.01	<0.01
B_2+R	k	5.4128	13.5146	19.2375	18.3317	19.0707
	n	1.7071	1.0970	1.3040	1.1937	1.1554
	P	<0.05	<0.01	<0.01	<0.01	<0.01
B_3+R	k	7.3969	15.0600	22.6718	19.4148	20.6521
	n	1.2639	1.1473	1.1844	1.1387	1.0846
	P	<0.05	<0.01	<0.01	<0.01	<0.01
B_4+R	k	7.9840	17.1078	25.2963	23.3913	24.8873
	n	1.2421	0.9151	1.0362	0.9117	0.8850
	P	<0.01	<0.01	<0.01	<0.01	<0.01

R or B+R, a good fit is given between aberration yield and dose i.e. $Y(\%) = kD^n$ (Gy), where k, n both are coefficients (see Table 2).

According to the effect equation and the data in Table 1, "synergetic effect factor (SEF)" defined by Morimoto[6] was calculated as follows: SEF= $N(n,D)/N(n,0) + N(0,D) - N(0,0)$, where $N(n,D)$, $N(n,0)$, $N(0,D)$ and $N(0,0)$ were chromosome aberration yields of B+R, B, R and control groups, respectively. When SEF=1, the combined effect can be thought completely additive, as SEF>1, the more synergetic effect can be regarded as (also see Table 1).

3.2 In vivo experiments

3.2.1 Chromosome aberrations of peripheral blood lymphocytes

Table 3 shows that the dicentric plus rings in low and high benzene groups were observed, this finding was different from those in vitro experiment; and linear relationship of dose effect was not found except accentric aberration yield in high benzene group ($Y(\%) = 0.155 + 0.021\ (\pm 0.003)\ D$ (g)). For SEF values, see Table 4.

Table 3

Yield of chromosome aberrations in rabbit blood lymphocytes %

Groups	Time of exp/ d	Dose ben/ g	Dose γ/ Gy	Cells scored	dic. + r	ace	Total aber	aber cells	chromat aber	Total aber cells
	0	0	0	1000	0	0.20	0.20	0.20	0	0.20
	53	0	0	1000	0	0.20	0.20	0.20	0	0.20
Control	76	0	0	1000	0	0.40	0.40	0.40	0	0.40
	95	0	0	1000	0	0.30	0.30	0.30	0.10	0.30
	0	0	0	1000	0	0.20	0.20	0.20	0.20	0.40
	53	2.112	0	1000	0	0.30	0.30	0.30	0.10	0.40
Low ben.	76	2.904	0	1000	0.10	0.40	0.40	0.40	0.30	0.80
	95	3.696	0	1000	0	0.70	0.70	0.70	0.40	1.10*
	0	0	0	1000	0	0.20	0.20	0.20	0.10	0.30
High	53	21.12	0	1000	0	0.50	0.50	0.50	0.20	0.70
ben.	76	29.04	0	800	0.13	0.75	0.88	0.88	0.38	1.25*
	95	36.96	0	800	0.13	1.00	1.13	1.13	0.63	1.75**
	0	0	0	1000	0	0.20	0.20	0.20	0	0.20
radiat.	53	0	2.1576	1000	0.20	1.30**	1.50**	1.40**	0	1.40**
	76	0	2.9667	800	0.30	1.50**	1.80**	1.80**	0.20	2.00**
	95	0	3.7758	800	1.00*	3.38**	4.38**	4.13**	0.20	4.38**
	0	0	0	1000	0	0.10	0.10	0.10	0.20	0.30
Low ben.	53	2.112	2.1576	1000	0.30	1.50**	1.80**	1.60**	0	1.60**
+	76	2.904	2.9667	1000	0.70*	1.50**	2.20**	2.00**	0.70*	2.70**
radiat	95	3.696	3.7758	1000	0.80*	4.40**	5.20**	4.70**	1.00	5.70**
High	0	0	0	1000	0	0.10	0.10	0.10	0	0.10
ben.	53	21.12	2.1576	1000	0.20	1.60**	1.80**	1.70**	0.10	1.70**
+	76	29.04	2.9667	1000	0.50	1.70**	2.20**	2.00**	0.40	2.30**
radiat.	95	36.96	3.7758	700	2.20**	4.80**	7.00**	6.57**	1.00	7.60**

Notes: exp=experiment, ben=benzene, dic+r=dicentric + rings, ace=acentric, aber=aberration, radiat=radiation, chromat=chromatid, γ = γ-rays,

*=Compared with the control $P < 0.05$, **=Compared with the control $P < 0.01$

3.2.2 Chromosome aberration yield in bone marrow cells

The dicentric plus rings in low benzene, radiation, high benzene + radiation groups were observed, but no statistical difference among various groups (see Table 5). Table 5 shows that chromosome aberrations in bone marrow cells can be induced by chronic benzene injection and γ-rays, and being mainly the acentric. The acentric, total aberrations, aberration cells and total aberration cells in the high benzene + radiation and low benzene + radiation groups were all higher than those of the radiation, high benzene and low benzene groups; and there were statistical differences except the acentric, total aberrations and aberration cells for high benzene + radiation group and low benzene + radiation group were not statistically different from the radiation group. It was illustrated

that chromosome aberrations induced by low dose γ-rays can be significantly enhanced by benzene treatment. Table 5 also shows that induced chromosome aberrations in the blood lymphocytes were higher than in the bone marrow cells on the 95th d.

Table 4
Calculated "SEF" in combined effect of radiation with benzene on chromosome aberrations of rabbit blood lymphocytes

Groups	Times of exp/d	Dose ben/g	Dose γ/Gy	dic+r	ace	Total aber	aber cells	Total aber cells
Low ben.+radiat.	53	2.112	2.1576	1.50	1.07	1.13	1.07	1.00
	76	2.904	2.9667	1.70	1.00	1.16	1.05	1.13
	95	3.696	3.7758	0.80	1.16	1.09	1.04	1.12
High ben.+radiat.	53	21.12	2.5176	1.00	1.00	1.00	1.00	0.89
	76	29.04	2.9667	1.16	0.92	0.96	0.88	0.81
	95	36.96	3.7758	1.95	1.12	1.34	1.32	1.33

Table 5
Comparison of chromosome aberrations between lymphocytes and marrow cells

Groups	Dose ben/g	Dose γ/Gy	Cell type	Cells scored	dic +r	ace	Total aber	aber cells	Chromat aber	Total aber cells
Control	0	0	L	1000	0	0.30	0.30	0.30	0.10	0.30
	0	0	M	1000	0	0.20	0.20	0.20	0	0.20
Low ben	3.696	0	L	1000	0	0.70	0.70	0.70	0.4	1.10
	3.696	0	M	1000	0.10	0.70	0.70	0.70	0	0.70
High ben	36.96	0	L	800	0.13	1.00	1.13	1.13	0.63	1.75
	36.96	0	M	800	0	0.88	0.88	0.88	0.36	1.25
Radiat	0	3.7758	L	800	1.0	3.38	4.38	4.13	0.20	4.38
	0	3.7758	M	800	0.13	1.25	1.38	1.38	0	1.38
Low ben	3.696	3.7758	L	1000	0.80	4.40	5.20	4.70	1.00	5.70
+radiat	3.696	3.7758	M	1000	0	1.80	1.80	1.80	0.20	2.00
High ben	36.96	3.7758	L	700	2.20	4.80	7.00	6.57	1.00	7.60
+radiat	36.96	3.7758	M	1000	0.20	1.70	1.90	1.90	0.20	2.10

Notes: L=Lymphocytes, M=Marrow cells

4 DISCUSSION

Chromosome aberrations of the Human in vitro peripheral blood lymphocytes as well as rabbit's peripheral blood lymphocytes and bone marrow cells could be induced by benzene treatment . Induced chromosome aberration types are chromosome and chromatid types, being mainly the former. In vitro experiment results differ from those of Morimoto[6], where benzene induced chromosome aberrations were mainly chromatid-type, especially gaps. The results of in vivo experiment are in agreement with those of previous studies[6,9], where benzene-induced aberrations are mainly chromosome type. The reason for this could be explicated as follows. a. The gaps were not recorded in our data because chromatid gaps (where biological significance is not clear yet) were most partly affected by factors of human techniques, thus, not used as quantity indicators of

radiation dose. Of course, gaps can be induced by benzene. Tompa *et al.*[10] demonstrated that among structural chromosome aberrations, increase in chromatid-type (gaps) is more significant than in chromosome-type aberrations, recorded gaps can be used as indicator of genetoxic effect of benzene. b. Benzene and its metabolites, such as phenol, have been thought to be a potent chromosome breaking agent. They might directly induce the chromosome-type aberration in the body[6,7], benzene concentrations in present experiment were higher than those of Morimoto, thus chromosome type aberrations induced by benzene were relatively higher. c. The cells with primary chromatid-type lesions could be observed as derivant chromosome type aberrations after in vivo one or more replications.

For 1.4 mmol/L benzene combined with 0.5 and 1.0 Gy γ-rays, the SEFs<1 in the dicentric plus ring and the acentric, this could be the problems of scoring at low aberration yields with the statistical errors associated with the small number of cells observed or of lower abilities of breaking chromosome and inhibiting repair of chromosome broken.

Ding Zhicheng *et al*[11] have reported that a good linear regression equation can be given between the accumulated doses and chromosome aberration rates in each irradiation groups, where rabbits were chronically irradiated by ^{60}Co γ-rays at 0.01 and 0.025 Gy/8h. Our observations indicate that chromosome aberration yields in radiation group or radiation combined with benzene groups increased with accumulated doses of γ-rays, however, no significant linear correlations between the doses and effects were found. This variation may be a consequence of less dose points before accumulated dose 2 Gy and chromosome aberration yields remained a constant after 2 Gy.

Chromosome aberration yields increased with increase in benzene concentration under 2.0 Gy γ-rays but not entirely at 3.0 Gy, such as the yields of the dicentric plus ring at 1.4 mmol/L benzene combined with 3.0 Gy γ-rays were higher than at 2.8 and 5.6 mmol/L benzene combined with the same dose. Whether these changes reflect induced interval phase death or delay division of cells at the higher benzene concentration combined with higher γ-rays is subjected to further study.

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