EFFECT OF TEMPERATURE ON THE FREQUENCY OF CHROMOSOME ABERRATIONS AND MICRONUCLEI IN CULTURED CHINESE HAMSTER CELLS

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ABSTRACT We previously reported that both hyperthermia and hypothermia induced micronuclei in mouse bone marrow cells (Asanami and Shimono, 1997a, 1997b, 1999). To investigate the effects of temperature on chromosome aberration *in vitro*, we conducted chromosome aberration and micronucleus tests under hyper- and hypothermic conditions using Chinese hamster lung (CHL) cells. In the chromosome aberration test, we observed positive responses at 40 °C and 41 °C for 24 hr, and at 42 °C for 6 hr and over. In the micronucleus test, we observed positive responses at 31 °C, 33 °C, and 40 °C for 24 hr, and at 42 °C for 2 hr. The results suggest that in CHL cells, hypothermic conditions can induce micronuclei while hyperthermic conditions can induce both chromosome aberrations and micronuclei.

KEY WORDS: *In vitro* chromosome aberration test, *In vitro* micronucleus test, Temperature, Chinese hamster lung cells

INTRODUCTION

Chromosome aberration (CA) tests using cultured mammalian cells have been widely used in evaluating the clastogenicity of chemicals. Micronuclei (MN) are caused by chromosome breakage and disturbance of the mitotic apparatus (Heddle, 1973; Schmid, 1973; Krishna and Hayashi, 2000). The MN assay, therefore, detects both clastogenicity (chromosome breakage) and aneugenicity (alteration of chromosome number due to chromosome lagging that results from dysfunction of the mitotic apparatus) when performed appropriately (Krishna and Hayashi, 2000). Recently, the *in vitro* MN test could be an alternative to the CA test for screening the genotoxicity of chemicals.

Heat treatment exerts numerous effects on mammalian cells, including chromosome aberrations (Dewey *et al.*, 1971), alterations in chromosomal proteins (Tomasovic *et al.*, 1978), inhibition of DNA repair (Jorritsma and Konings, 1983), damage to membranes (Yatvin, 1977) and the mitotic apparatus (Dewey *et al.*, 1971), and MN induction (Komae *et al.*, 1999). We found no reports, however, regarding the effects of

hypothermic conditions on CA or MN induction in mammalian cells, although we previously reported that both elevated and depressed body temperature induced MN in mouse bone marrow cells (Asanami and Shimono, 1997a, 1997b, 1999). We therefore confirmed the effects of hyper- and hypothermic conditions on induction of CA and MN *in vitro*.

MATERIALS AND METHODS

Cells

CHL/IU cells were obtained from the National Institute of Health Sciences. They were maintained in Eagle's minimum essential medium supplemented with 10% heat-inactivated (56 °C for 30 min) calf serum.

Chromosome aberration test

2×10^4 cells were seeded in 60 mm plastic plates, treated 3 days later at 31, 33, 40, 41, or 42 °C for 1, 2, 3, 6, or 24 hr, and then cultured in fresh medium at 37 °C for another 23, 22, 21, 18, or 0 hr, respectively. Slides were prepared as described by Sofuni *et al.* (1990), with minor modifications. Briefly, colcemid (final con-

centration 0.2 mg/mL) was added to the culture for 2 hr before cell harvesting. The harvested cells were trypsinized and incubated in 0.075 M KCl hypotonic solution for 15 min at 37 °C and fixed 3 times with ice-cold 1:3 acetic alcohol. A drop of cell suspension was placed on a clean glass slide, air-dried, and stained with Giemsa solution. All slides were coded, and 100 or 200 well-spread mitotic metaphases were analyzed. The frequencies of cells with chromosome aberrations were recorded together with types of aberrations (chromatid or chromosome gap, break, exchange, polyploidy, etc).

Micronucleus test

5×10^4 cells were seeded in 60 mm plastic plates, treated 2 days later at 31, 33, 40, or 42 °C for 1, 2, 3, 6, or 24 hr, and then cultured with fresh medium at 37 °C for another 47, 46, 45, 42, or 24 hr, respectively. We conducted the *in vitro* MN test as described by Matsuoka *et al.* (1993). Briefly, after heat treatment, the cells were treated with trypsin supplemented with EDTA to make a single cell suspension and incubated in hypotonic (0.075 M) KCl solution for 10 min at room temperature. The cells were fixed in three or more changes of 1:3 acetic alcohol, then suspended in methanol containing 1% acetic acid. A drop of cell suspension was placed on a clean glass slide and air-dried. The preparation was stained with 40 mg/mL acridine orange solution and immediately observed by fluorescence microscopy with blue excitation. All slides were coded, and the number of cells with MN was recorded based on the observation of 1000 intact interphase cells. The number of mitotic cells appearing in the same microscopic field was also recorded.

Statistical analysis

Statistical analysis was performed by comparison with the concurrent negative control (37 °C treatment group) using Fisher's exact test (Yoshimura, 1987).

RESULTS

For CA test, hyperthermic conditions (40 or 41 °C for 24 hr, 42 °C for 6 or 24 hr) induced a significant increase in structural CA, including chromatid or chromosome gap, chromatid break, chromatid exchange, and fragmentation. Six hr at 42 °C significantly induced polyploidy. No CA was observed under hypothermic conditions (Table 1).

For MN test, both hyperthermic (40 °C for 24 hr, 42 °C for 2–24 hr) and hypothermic (31 and 32 °C for 24 hr) conditions induced a significant increase in MN

frequencies (Table 2).

DISCUSSION

We demonstrated here that hyperthermic conditions significantly induced both CA and MN in cultured CHL cells, while hypothermic conditions significantly induced only MN.

Dewey (1989) reported that the major cellular effects of hyperthermia result primarily from the denaturation and aggregation of proteins in the cell, and there are few DNA single-strand or double-strand breaks induced by heat. We suggested that the mechanism of the MN induction by hyperthermia is a disturbance of the mitotic apparatus (Asanami and Shimono, 1997a) and inhibition of DNA repair (Asanami and Shimono, 1999). Therefore, hyperthermic conditions can induce both structural CA and numerical CA *in vitro* due to the disruption of protein structures leading to disturbance of the mitotic apparatus and inhibition of enzyme activity associated with DNA repair systems. This would also be responsible for the observed MN induction in this test.

Hypothermia, on the other hand, has not previously been reported to induce CA or MN *in vitro*. In *in vivo* studies, Asanami and Shimono (1997b) showed that hypothermia induced MN in mouse bone marrow cells, and suggested that the mechanism was a disturbance of the mitotic apparatus. In the present study, hypothermic conditions induced MN, but not structural CA. The results suggest that hypothermic conditions (31 and 32 °C for 24 hr) may also disrupt the mitotic apparatus, but we speculate that hypothermia may not be strong enough to induce polyploidy as hyperthermia.

In conclusion, we confirmed that both hyper- and hypothermic conditions directly affect CA or MN frequencies *in vitro*. Hyperthermia and hypothermia, therefore, could directly affect MN frequencies *in vivo*. It seems that hyperthermic conditions can induce both structural CA and numerical CA, but a hypothermic condition can produce only aneuploidy in CHL cells.

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Temperature effect on chromosome aberrations and micronucleus induction *in vitro*.**Table 1.** Effects of temperature on the frequency of chromosome aberrations in CHL cells.

Temperature (°C)	Treatment -recovery (hr)	No. of cells analyzed	MI (%)	Number of aberrations							TAG	Poly
				gap	ctb	cte	csb	cse	other	mult		
31	0-24	100	4.5	1	1	0	0	0	0	0	2	0
	24-0	200	2.3	0	0	0	0	0	0	0	1	0
33	0-24	100	4.7	0	0	0	0	0	0	0	0	0
	24-0	200	3.6	1	0	0	0	0	0	0	1	0
40	0-24	200	6.1	1	0	0	0	0	0	0	1	1
	1-23	200	5.4	2	0	0	0	0	0	0	2	3
	2-22	200	6.7	2	1	0	0	0	0	0	3	1
	3-21	200	6.4	3	0	0	0	0	0	0	3	0
	6-18	200	6.6	3	2	0	0	0	0	0	5	1
	24-0	200	4.6	3	4	2	0	0	0	0	9**	3
41	0-24	200	7.0	2	0	0	0	0	0	0	2	1
	1-23	200	7.1	1	0	0	0	0	0	0	1	0
	2-22	200	7.2	1	0	0	0	0	0	0	1	1
	3-21	200	6.2	2	1	0	0	0	0	0	3	1
	6-18	200	7.3	2	0	0	0	0	0	0	2	0
	24-0	200	5.2	7	4	5	0	0	0	0	16**	0
42	0-24	200	6.6	1	0	0	0	0	0	0	1	2
	1-23	200	5.5	3	0	0	0	0	0	0	3	2
	2-22	200	5.5	1	1	0	0	0	0	0	2	4
	3-21	200	5.9	3	0	1	0	0	0	0	4	8
	6-18	200	5.2	2	5	4	0	0	0	0	9**	10*
	24-0	200	1.2	18	49	14	0	0	45	73	173**	1

gap, chromatid or chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; other, fragmentation; mult, more than 9 aberrations; TAG total number of aberrant cells including gaps; Poly, polyploidy; MI, Mitotic index (%); **p<0.01 vs control (Fisher's exact test).

Table 2. Effects of temperature on the micronucleus frequency in CHL cells.

Temperature (°C)	Treatment -recovery (hr)	No. of cells analyzed	MI (%)	Micronucleated cells / 1000 cells	
				Individual data	Mean $\pm$ SD (%)
31	0-48	4000	1.9	13, 19, 16, 18	16.5 $\pm$ 2.6
	24-24	4000	1.7	30, 43, 35, 43	37.8 $\pm$ 6.4**
33	0-48	4000	1.7	10, 11, 10, 11	10.5 $\pm$ 0.6
	24-24	4000	2.3	49, 56, 39, 54	49.5 $\pm$ 7.6**
40	0-48	2000	2.1	12, 10	11.0 $\pm$ 1.4
	1-47	2000	2.0	14, 10	12.0 $\pm$ 2.8
	2-46	2000	2.2	15, 11	13.0 $\pm$ 2.8
	3-45	2000	1.5	12, 10	11.0 $\pm$ 1.4
	6-42	2000	2.3	15, 10	12.5 $\pm$ 3.5
	24-24	2000	2.1	34, 47	40.5 $\pm$ 9.2**
42	0-48	2000	2.2	19, 8	13.5 $\pm$ 7.8
	1-47	2000	1.8	10, 12	11.0 $\pm$ 1.4
	2-46	2000	2.4	17, 38	27.5 $\pm$ 14.8**
	3-45	2000	2.7	38, 41	39.5 $\pm$ 2.1**
	6-42	2000	2.8	41, 37	39.0 $\pm$ 2.8**
	24-24	2000	0.8	251, 272	261.5 $\pm$ 14.8**

MI, Mitotic index (%); **p<0.01 vs control (Fisher's exact test).

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