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Short Communication

THE EFFECTS OF CADMIUM ON THE ADHESION AND GROWTH OF NORMAL AND MALIGNANT HUMAN CELLS AT HIGH TEMPERATURES

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The effects of heat and cadmium (Cd) treatment on the adhesion and subsequent growth of normal (HAIN-55) and malignant (HeLa S3 and HGC-27) human cultured cells were investigated. When malignant human cells were preincubated with 2.0 ppm Cd, damage of the so-treated cells was temperature-dependent. In contrast, the subsequent growth of HAIN-55 cells was not affected by Cd treatment at high temperatures. There was no effect of 2.0 ppm Cd on the adhesiveness of normal and malignant human cells at high temperatures. Compared with findings in malignant cells, the adhesive function and growth of normal human cells were markedly less sensitive to a high temperature or Cd.

The expression of the toxic effects of certain pollutants is temperature-dependent and differs markedly from species to species. In the mouse, the toxicity of cadmium (Cd) is enhanced by acclimating the host animals to a low temperature(1). In rats, the degree of Cd-induced testicular and epididymal hemorrhage depends on the environmental temperature(2). On the other hand, many studies of Cd cytotoxicity in cell culture have been made (3-5). However, there is no report that estimates the effects of Cd on cultured human cells at various ambient temperatures, which are important factors in the human environment. It is valid to study the toxicity of Cd by using human cells. We investigated the effects of Cd on the adhesion to the culture dish and growth

of normal and malignant human cells at high temperatures, since the capacities are most important properties when attempting to elucidate the temperature-dependency of Cd toxicity of human cells.

1) Cell lines: As normal human diploid cells, HAIN-55 of a fibroblastic cell line was used in this experiment. The normality of the cell line was assessed on the basis of the chromosomal pattern. HAIN-55 cells with a fibroblastic nature originated from fetal lung tissues(6). As human malignant cells, HeLa S3(7,8) (human malignant cells from carcinoma of the cervix) and HGC-27(9) cells (human malignant cells from metastatic lymph nodes in case of gastric cancer) were used in this experiment. The malignancy was confirmed by a heterotransplantation test in hamsters and nude mice. HAIN-55 and HeLa S3 cells were donated by Dr. H. Okumura, of the National Institute of Health, Tokyo and HGC-27 cells from Professor H. Saito of Shimane Medical University, Izumo.

2) Media: Eagle's basal medium was used for HAIN-55 cells and Eagle's minimal essential medium for HeLa S3 and HGC-27 cells. When these media were used for cell growth, 10% fetal bovine serum (M. A. Bioproducts, USA), 30 μg/ml L-glutamine and 2% Meylon ($NaHCO_3$, Otsuka Pharmaceutical Co., Tokyo) were supplemented, respectively.

3) Chemicals: Cadmium chloride was dissolved in distilled water at the concentration of 1,000 ppm as a stock solution. The concentration of the Cd solution (2.0 ppm) for experiments that inhibited the cell growth of HeLa S3 cells by 50% relative to untreated cells was measured by an atomic absorption spectrophotometer (180-80, Hitachi, Tokyo).

4) Cell adhesion test: To examine the cell adhesion to the 35-mm culture dish surface (Falcon, 301, USA), in all cases of confluent monolayer cultures, the cells were released by treatment with 0.25% trypsin in Ca^{2+} - Mg^{2+}-free phosphate buffered saline (PBS(-)). The cell suspension was immediately diluted with the above mentioned medium corresponding to the particular kind of cell, and cells were collected by centrifugation (250 g for 6 min) and washed once with the Hanks' balanced salt solution (HBSS) (pH 7.4). The pellets were resuspended in HBSS, with or without 2.0 ppm Cd at the concentration of 1.0×10^5/ml. Then, 2.0 ml aliquots of the cell

suspension were inoculated into 35-mm culture dishes and cultured at 37°, 39°, 41°, 43° or 45°C in 5% CO_2 in 95% humidified air (CO_2 incubator). After three h of incubation, the medium was aspirated from the dish, and the dish was rinsed three times with 1.0 ml of PBS(-). Attached cells were released by treatment with 0.25% trypsin - 0.05% EDTA in PBS(-), and suspended in the corresponding medium; the number of cells was counted in an electronic particle counter (Coulter Electronic, Hialeah, Florida, USA). The results were expressed using the parameter "Adhesion index" calculated as follows: Adhesion index = (the number of attached cells/the number of cells plated) x 100 (%).

5) Cell growth test: The effects of Cd (2.0 ppm) on the growth of human cells at high temperatures were examined as follows. About 2 x 10^5 of the cells in 2.0 ml of growth medium were seeded onto 35-mm culture dishes and cultured at 37°C for 24 h in a CO_2 incubator. After the cultivation, adherent cells were washed twice with 2.0 ml of PBS(-) and exposed to 2.0 ml of HBSS, with or without 2.0 ppm Cd at 37°, 39°, 41°, 43° or 45°C for three h. The cell sheets were then washed twice with PBS (-) and cultured in growth medium at 37°C in a CO_2 incubator for various intervals. The medium was replaced with a fresh one every 72 h. After various intervals, the floating cells were washed out twice with PBS(-) and the attached cells were treated with 0.25% trypsin - 0.05% EDTA in PBS(-) to disperse the cells. The number of viable cells was counted with a hemocytometer by the dye exclusion test using 0.2% nigrosin solution in PBS(-).

The effects of heat or Cd treatment on the adhesion of human cells are shown in Table I. In the case of HeLa S3 cells without Cd, the adhesion indices at temperatures of less than 41°C were more than 80%, while marked suppression of the indices was seen at temperatures over 43°C. Comparing these adhesion indices with and without Cd, no significant difference was indicated. When HGC-27 cells were cultured under the same conditions, results similar to those with HeLa S3 cells were obtained. The adhesion indices of HAIN-55 cells cultured without Cd at high temperatures were more than 50% and did not show temperature-dependency. There was no significant difference between the index with and without Cd. As compared with the malignant human cells (HeLa S3 and HGC-27 cells), the adhesive ability of the normal human cells

Table I. EFFECT OF HEAT AND Cd ON THE ADHESION OF HUMAN CELLS

Temperature	HeLa S3		HGC-27		HAIN-55	
	Cd(-)	Cd(+)	Cd(-)	Cd(+)	Cd(-)	Cd(+)
37°C	76.5 *	81.5	85.5	97.5	57.5	55.0
39°C	93.5	85.0	82.0	85.5	55.0	59.5
41°C	83.0	83.5	81.5	89.0	61.0	63.5
43°C	46.9	50.0	26.1	30.0	60.5	51.5
45°C	16.7	20.7	19.0	18.0	52.0	55.0

* ; Adhesion index (%)

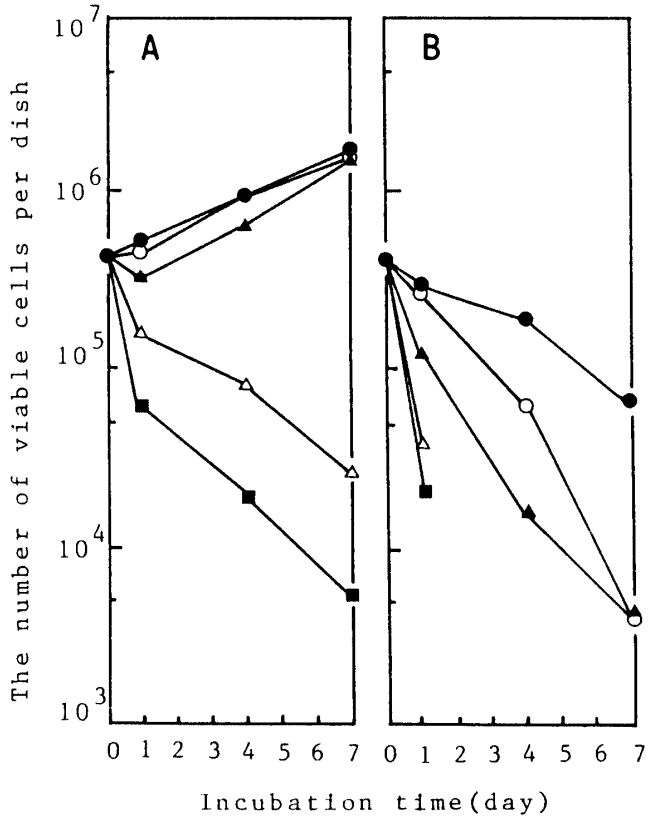

Fig.1. Effects of heat and Cd treatment on the subsequent growth of HeLa S3 cells. HeLa S3 cells were precultivated in HBSS in the absence (A) or presence (B) of 2.0 ppm of Cd at various temperatures for 3 h followed by subsequent cultivation in growth medium for up to 7 days (see "MATERIALS AND METHODS" for details). (●): 37°C, (○): 39°C, (▲): 41°C, (△): 43°C , and (■): 45°C. Each point represents the mean of three samples. Standard error was less than 5% of the mean for all points.

(HAIN-55 cells) was markedly less sensitive to high temperatures.

Fig. 1 shows the effects of Cd treatment of HeLa S3 cells at various temperatures for 3 h. As indicated in Fig. 1-A, precultivation of HeLa S3 cells in HBSS without Cd and at a temperature of less than 41°C did not affect the subsequent

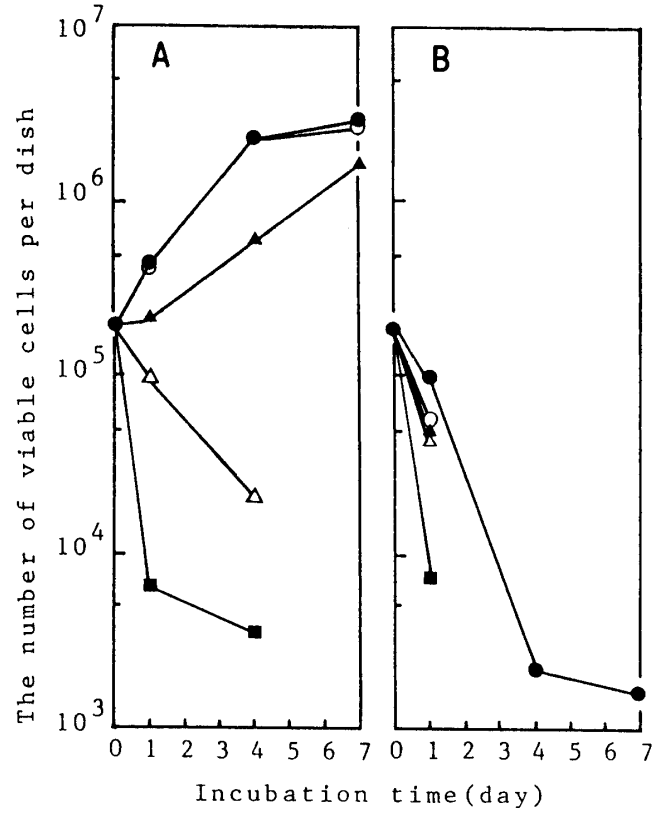

Fig.2. Effects of heat and Cd treatment on the subsequent growth of HGC-27 cells. Details are the same as in Fig.1.

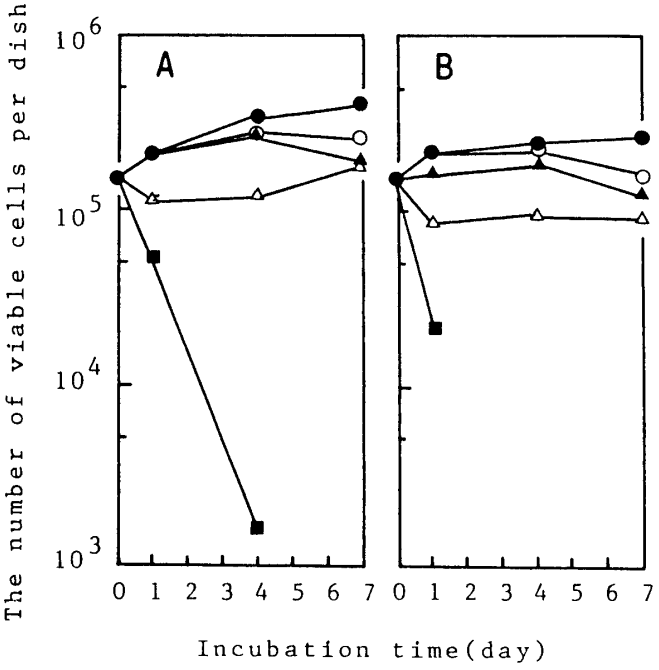

Fig.3. Effects of heat and Cd treatment on the subsequent growth of HAIN-55 cells. Details are the same as in Fig.1.

growth of the cells, while marked killing and/or damaging were seen in the cases where the cells were treated at temperatures higher than 43°C. When HeLa S3 cells were precultivated in HBSS with Cd under the same conditions (Fig. 1-B), damage of the treated cells was observed. Thus, damage of the cells by Cd treatment seemed to be temperature-dependent.

Precultivation of HGC-27 cells in HBSS without Cd and at a temperature of less than 39°C did not affect the subsequent growth of the cells (Fig. 2-A). At 41°C, compared with HeLa S3 cells, slight damage was seen. Marked killing and/or damaging were seen at temperatures higher than 43°C, as with HeLa S3 cells. When HGC-27 cells were precultivated in HBSS with Cd under the same conditions, damage of the cells was shown in a temperature-dependent manner (Fig. 2-B).

Data from the same experiments on the subsequent growth of HAIN-55 cells are illustrated in Fig. 3. Precultivation of the cells in HBSS without Cd and at a temperature of less than 41°C did not affect the subsequent growth of the cells (Fig. 3-A). At 43°C, in contrast with HeLa S3 cells, the subsequent growth of HAIN-55 cells was slightly suppressed, but reached confluence at day 7 of cultivation. However, marked damage of the cells was observed at 45°C. With HAIN-55 cells precultivated in HBSS with Cd under the same conditions (Fig. 3-B), the subsequent growth of the cells was not affected by Cd. As compared with HeLa S3 cells, the subsequent growth of HAIN-55 cells was remarkably less sensitive to heat or Cd.

We studied the effects of Cd on the adhesive function and growth of normal and malignant human cultured cells at high temperatures. As shown in Table I, there was no effect of Cd on the adhesion of normal and malignant human cultured cells, even at a high temperature. Hildebrand *et al*(10) observed that, when Cd-resistant Chinese hamster ovary cells were maintained in suspension culture rather than in monolayer, an even greater resistance to Cd was seen. It is suggested that Cd-mediated cytotoxicity against cultured cells can be altered depending on the stages of the adhesion and growth cycle of the cells. This may explain the failure of Cd to inhibit adhesion of either the normal or malignant cultured cells. The adhesiveness of malignant cells, compared to normal cells, was more severely suppressed by heat treatment. Normal human cells seem to be more resistant to

high temperature in both phases, i.e., the adhesion and the growth of cells, than are the malignant human cells.

Precultivation of HeLa S3 and HGC-27 cells (malignant human cells) in HBSS without Cd and at a temperature of less than 41°C did not affect the subsequent growth of the cells, which, however, was inhibited by Cd treatment in a temperature-dependent manner. In contrast with malignant human cells, the growth of HAIN-55 cells (normal human cells) was not affected by heat treatment at temperatures of less than 43°C or by Cd treatment at high temperatures. HAIN-55 cells are more resistant to heat or Cd than are HeLa S3 and HGC-27 cells. These findings are in agreement with those of Bender and Shram(11) and Mondovi *et al.*, (12) who reported that tumor cells tended to be more sensitive to heat on the basis of their growth compared to normal cells. Further studies to determine whether normal human cells are less sensitive to Cd than malignant human cells on the basis of their growth using other human cells originating from various tissues are needed.

The growth inhibitory effects of Cd on malignant human cells were enhanced at high temperatures. At temperatures higher than physiological or normal ones, suppression of synthesis of nucleic acids occurs(13,14). Moreover, disturbance in the nucleic acid synthesis of rat liver and endothelial cells of uterine vessels is also caused by Cd(3-5). Thus, it may be that the toxic effects of Cd on malignant human cells are synergistically elevated at high temperatures. In this connection, it is noteworthy that Nahid and Kurt(10) demonstrated that the heat-mediated potentiation of radiation lethality and direct damaging effect of heat are based on different cellular mechanisms.

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