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EFFECTS OF ANTIFEBRILE AND ANTICANCER DRUGS ON HUMAN CELLS AT HIGH TEMPERATUE CONDITIONS

(drug/human cells/high temperature)

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Effects of antifebrile (sulpyrine) and anticancer (5-fluorouracil) drugs on the growth of several cultured human cells at high temperature were investigated. Cultured normal human vascular endothelial (HVE) and normal human fetal lung (HAIN-55) cells at 37.2°C were sensitive to sulpyrine, and their sensitivities to the drug were markedly enhanced when they were incubated at 41.5°C. In contrast, sensitivity of malignant human cells (HeLa cells) to sulpyrine was not found at 37.2°C, however sensitivity of the cells to the drug was manifested at 41.5°C of incubation. There was no effect of 5-fluorouracil (5-FU) on the growth of HVE and HAIN-55 cells at 41.5°C, while HeLa cells showed high susceptibility to this drug at the same temperature. The results suggest the possibility that normal human cells may be sensitive to antifebrile drugs but not to anticancer drugs at high temperature, whereas malignant human cells may be susceptible to both antifebrile and anticancer drugs at high temperature.

It is necessary to establish a valid and easy method for the estimation of drug-safety. However, there is no report which estimates the suitability of drugs for patients attacked with fever at high temperature in the cell culture. In order to clarify the reaction on human cells at various ambient temperatures, which are important factors in human environment, we have been examining the effects of antifebriles on human vascular endothelial cells at high temperature (manuscript in preparation).

The purpose of this present study was to establish a method for the estimation of drug-safety by using cultured cells. This paper deals with the effects of antifebrile and anticancer drugs on the growth of normal and malignant cultured human cells at high temperature.

MATERIALS AND METHODS

1) Cells ; Cells used in this experiment are as follows : As normal human diploid cells, endothelial cells derived from human vascular endothelial (HVE) cells, and HAIN-55 strain cells (originating from human lung tissues and fibroblasts); as malignant human cells HeLa cells originating from human uterus cervical tumor, an established cell line (Fig. 1).

Procedures for preparation of HVE cells were described previously (1), and the bulk of the cells obtained were characterized as HVE cells by an enzyme-labelled antibody method for human Factor VIII antigen, and by a chromosome pattern for their normality. HAIN-55 cells were assayed for their normality by a chromosome pattern.

2) Media for cell cultures ; RPMI 1640 medium, basal Eagle's medium and Eagle's minimal essential medium were used for HVE cells, HAIN-55 cells and HeLa cells, respectively. These media were supplemented with 10% precolostrum new born calf serum (Nakashibetsu Serum Center, Hokkaido, Japan), 30 μg/ml L-glutamine and 0.2% $NaHCO_3$ solution.

3) Drugs ; Sulpyrine (one of the pyrazolone derivatives) and 5-fluorouracil (5-FU) were used in this experiment.

4) Experimental procedures ; 0.4 ml of cell suspension at a density of 0.8-1.2 x 10^5/ml in each fresh medium was plated in each well (15.5 mm) of the Multidish (Nunc Co. , Denmark) and cultured for 24 h at 37°C in a CO_2 incubator (5% CO_2). Twenty-four hours later, the medium was exchanged for 0.4 ml of fresh medium containing each of the drugs tested, and further cultured at 37.2°C, 39.4°C or 41.5°C for 3 days.

5) Assay for cytotoxicity ; The cytotoxicity of each drug to the cells was assayed as follows : Cultured cells grown on 10 x 10 mm glass cover slips were washed once with phosphate buffered saline, fixed by ethanol, stained with 3% Giemsa solution and then covered with a Sheet Mesh (Maxtaform, HF-33, England). Adherent cells in 54 areas (165 x 165 μm per area) were counted

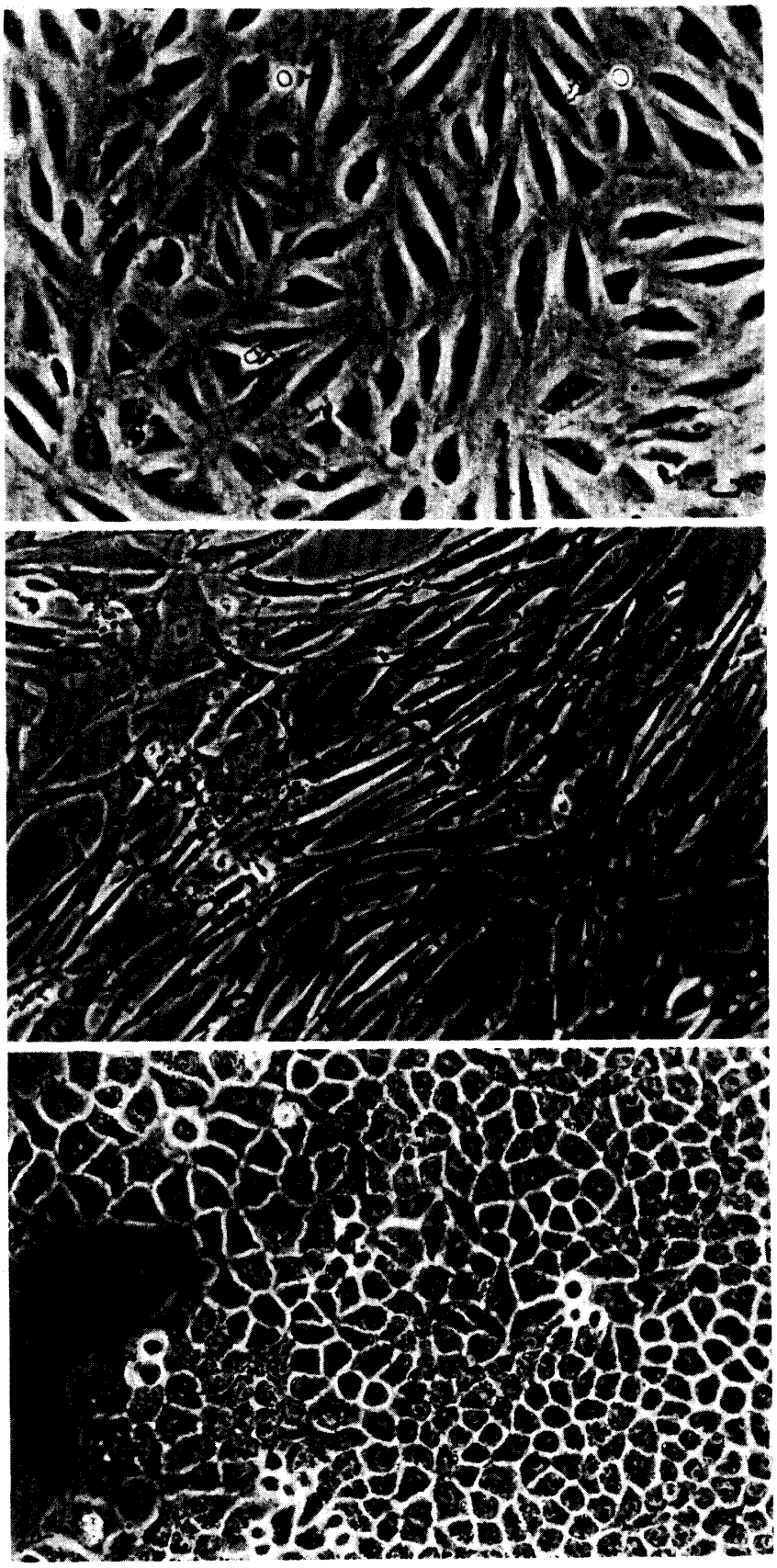

Fig. 1. Micrographs of cultured cells used in this experiment. HeLa cells(A), HAIN-55 cells(B), and HVE cells(C)

to determine the number of viable cells per well under a light microscope. A cell morphology was also observed under a phase contrast microscope (Olympus, IMT-413, Tokyo). It was confirmed in a preliminary experiment that in cell cases in the experiment, most of the viable cells and of the dead cells were observed in adherent and floating conditions, respectively.

RESULTS

1) Effects of sulpyrine on the growth and morphology of cultured human cells at high temperature ;

The effects of sulpyrine on the growth of HeLa cells at high temperature are shown in Fig.2. In the case of 37.2°C (Fig. 2-A), the number of adherent cells was 7.8 x 10^4/well after 24 h incubation (at zero time). After 3 days, the number of adherent cells increased to approximately 2.7 x 10^5/well. In contrast, after the same period the number of cells treated with 130 or 250 μg/ml concentration of sulpyrine was 2.2 x 10^5 and 1.4 x 10^5/well, respectively. That is, the growth of cells at 37.2°C was slightly suppressed by the drug. When the cells were cultured at 39.4°C (Fig. 2-B), the number of cells decreased with an increase of sulpyrine concentration as well as in the case of incubation at 37.2°C (Fig. 2-A), and the inhibitory effects of sulpyrine in cell adhesion and growth at 41.5°C against HeLa cells was more conspicuous than at 37.2°C or 39.4°C and the control cases at 41.5°C (Fig. 2-C).

As shown in Fig. 3, when HAIN-55 cells were incubated at 37.2°C (Fig. 3-A), the number of control cells gradually increased, and was 1.4 x 10^5/well after 3 days incubation, however, cells treated with sulpyrine (130 or 250 μg/ml) were not able to proliferate. When the cells were incubated at 39.4°C (Fig. 3-B), results obtained from cells incubated at 39.4°C were almost as well as in the case of results at 37.2°C, and the decrease in the number of adherent cells was more effective with an increase in the concentration of sulpyrine. At 41.5°C (Fig. 3-C), the growth inhibitory effect was more conspicuous than at 37.2°C or 39.4°C. The growth inhibitory effect of sulpyrine against HAIN-55 cells incubated at 41.5°C was enhanced as well as against HeLa cells.

The effects of sulpyrine on the growth of HVE cells at high temperature are shown in Fig. 4. At 37.2°C, the growth of HVE

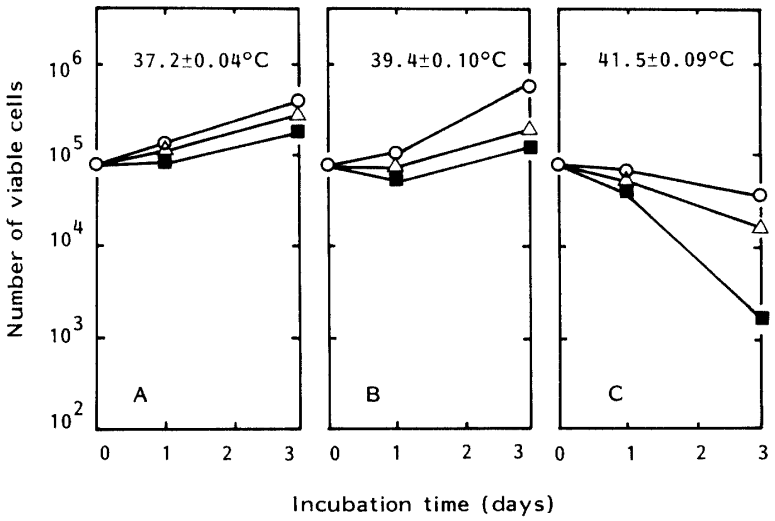

Fig. 2. Effects of sulpyrine on the growth of HeLa cells. 0.4 ml of cell suspension at a density of 1.0 x 10^5/ml in minimal essential medium supplemented with 10% precolostrum new born calf serum, 2% Meylon and 30 mg/ml L-glutamine (growth medium) was plated on to each well of a Multidish and cultured for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. After that the medium was exchanged with 0.4 ml of fresh medium containing sulpyrine, and further cultured at 37.2°C, 39.4°C or 41.5°C for 3 days under 5% CO_2. At indicated intervals, the residual adherent cells were counted as described under Materials and Methods. Each points represents the mean of three samples. Standard error was less than 10% of the mean for all points. In the following figures, the experimental methods are the same as mentioned above. Control(○), Cells + 130 μg/ml(△), Cells + 250 μg/ml(■)

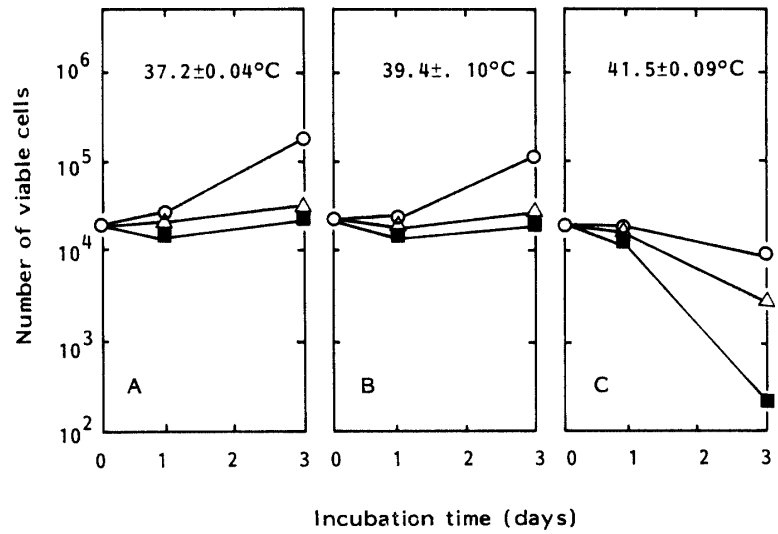

Fig. 3. Effects of sulpyrine on the growth of HAIN-55 cells. 0.4 ml of cell suspension at a density of 0.9 x 10^5/ml in growth medium was plated on to each well of a Multidish. Control(○), Cells + 130 μg/ml(△), Cells + 250 μg/ml(■)

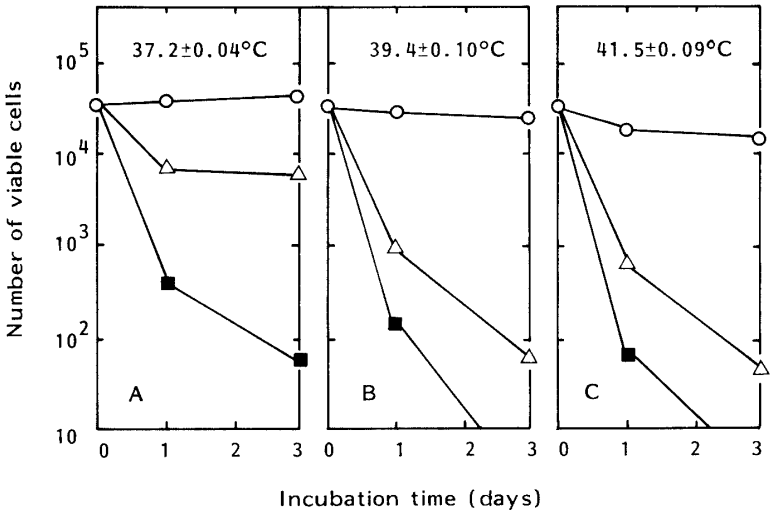

Fig. 4. Effects of sulpyrine on the growth of HVE cells. 0.4 ml of cell suspension at a density of 1.2 x 10^5/ml in growth medium was plated on to each well of a Multidish. Control(○), Cells + 130 μg/ml(△), Cells + 250 μg/ml(■)

cells exhibited a concentration-dependent pattern by the addition of sulpyrine (Fig. 4-A). The inhibitory effect of sulpyrine on the growth of HVE cells at 39.4°C (Fig. 4-B) or 41.5°C (Fig. 4-C) was more conspicuous than that at 37.2°C, and the growth inhibitory effect of the drug against HVE cells was more marked than that against HeLa or HAIN-55 cells.

The effects of sulpyrine on the morphology of cultured human cells at high temperature are shown in Fig. 5. When HeLa cells were cultured at 41.5°C without sulpyrine, granular degenerated cytoplasm and spindle-shaped cells were seen under microscopical observation (Fig. 5-A-1). Almost all HeLa cells treated with sulpyrine (250 μg/ml) at 41.5°C were dispatched from the surface of the dish (Fig. 5-A-2). In the case of HAIN-55 cells incubated at 41.5°C, there is no remarkable degeneration in cell morphology (Fig. 5-B-1), whereas, cells treated with sulpyrine at 41.5°C were necrotic (Fig. 5-B-2). When HVE cells were cultured at 41.5°C (Fig. 5-C-1), cells were fully spread, however, when the cells were treated with sulpyrine (250 μg/ml) at 41.5°C, the cytoplasm of the cells was shrunk and many granules were found (Fig. 5-C-2).

2) Effects of 5-FU on the growth and morphology of cultured human cells at high temperature ;

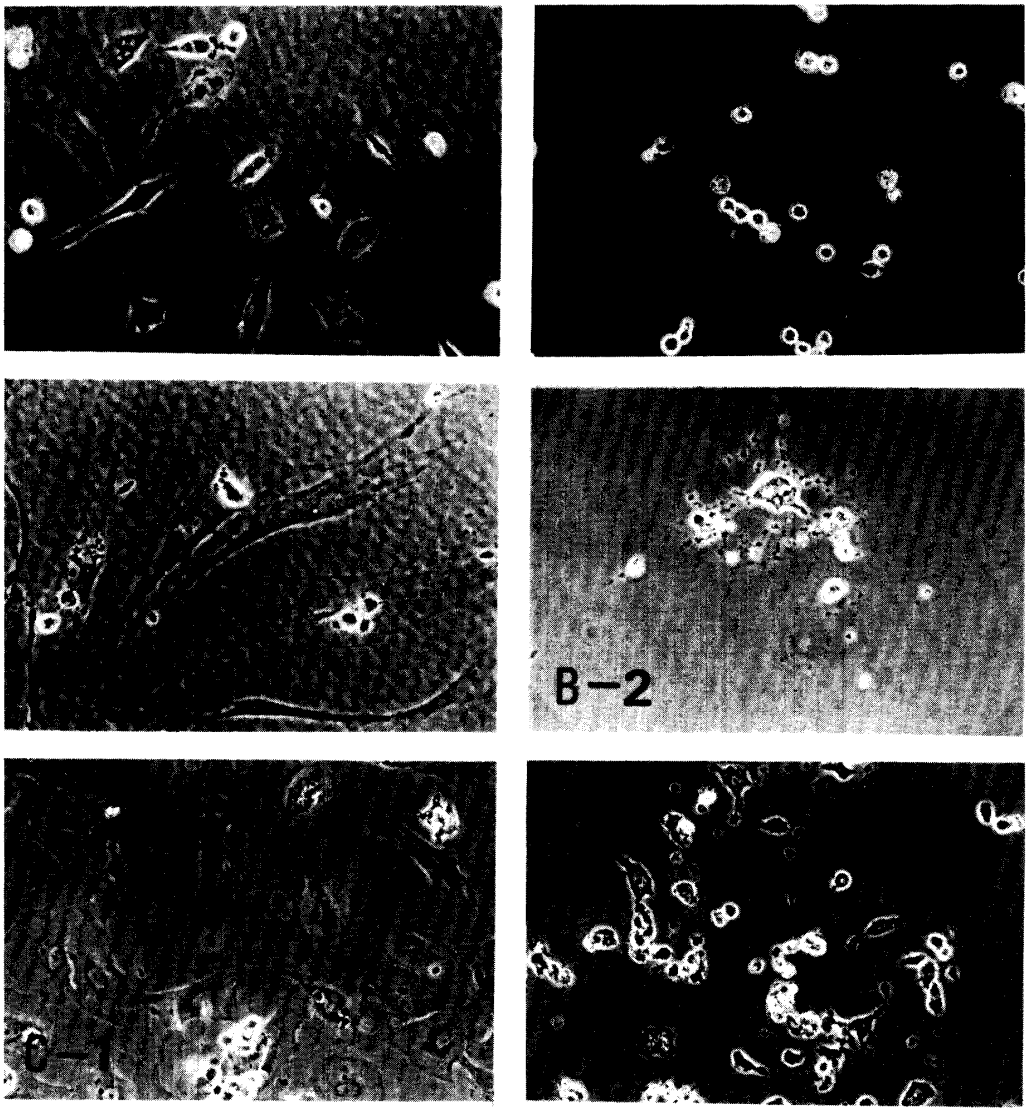

Fig. 5. Effects of sulpyrine on the morphology of cultured human cells. HeLa cells cultured without sulpyrine at 41.5°C(A-1), HeLa cells cultured with 250 μg/ml sulpyrine at 41.5°C(A-2), HAIN-55 cells cultured without 5-FU at 41.5°C(B-1), HAIN-55 cells cultured with 250 μg/ml sulpyrine at 41.5°C(B-2), HVE cells cultured without sulpyrine at 41.5°C(C-1), HVE cells cultured with 250 μg/ml sulpyrine at 41.5°C(C-2)

The effects of 5-FU on the growth of HeLa cells are illustrated in Fig. 6. The growth of the inhibitory effect of 5-FU against HeLa cells at various temperatures (37.2°, 39.4° and 41.5°C) was dependent on the drug concentration, and the inhibitory effect of 5-FU at 41.5°C against the cells was enhanced more markedly than that at 37.2°C or 39.4°C (Fig. 6-C).

As shown in Fig. 7, a slight growth inhibitory effect of 5-FU against HAIN-55 cells was observed, depending on the

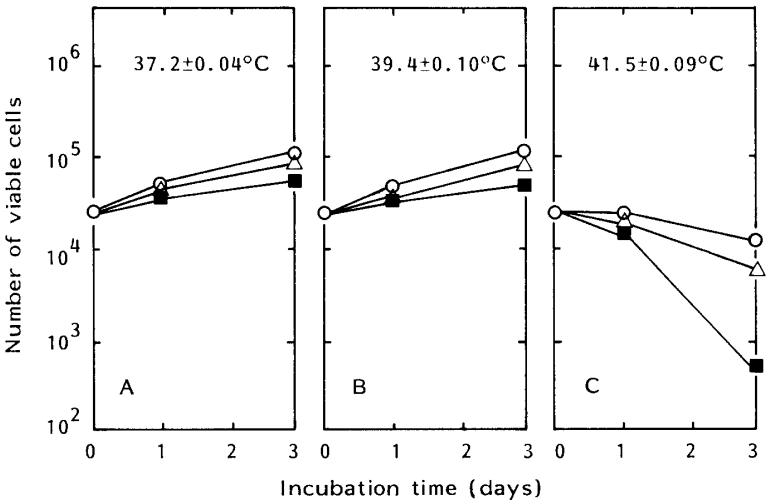

Fig. 6. Effects of 5-FU on the growth of HeLa cells. 0.4 ml of cell suspension at a density of 0.8 x 10^5/ml in growth medium was plated on to each well of a Multidish. Control(○), Cells + 43 ng/ml(△), Cells + 85 ng/ml(■)

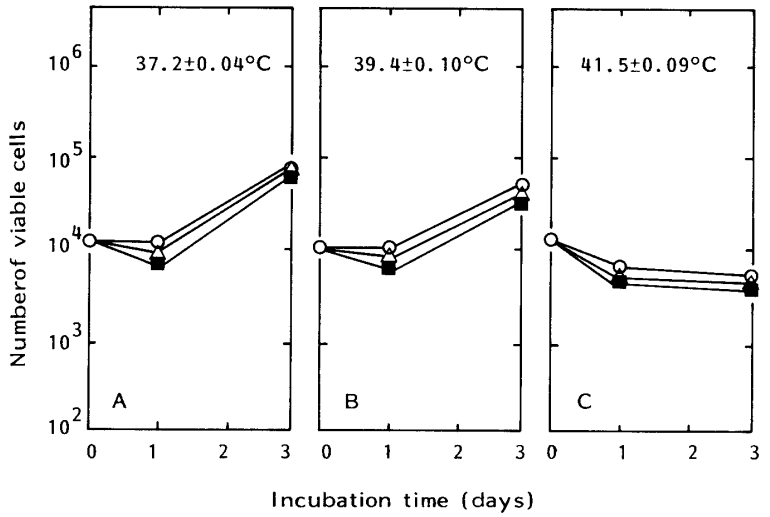

Fig. 7. Effects of 5-FU on the growth of HAIN-55 cells. 0.4 ml of cell suspension at a density of 0.8 x 10^5/ml in growth medium was plated onto each well of a Multidish. Control(○), Cells + 43 ng/ml(△), Cells + 85 ng/ml(■)

concentration of 5-FU, however, the growth inhibition of HAIN-55 cells at 41.5°C was not enhanced by the addition of 5-FU (Fig. 7-C).

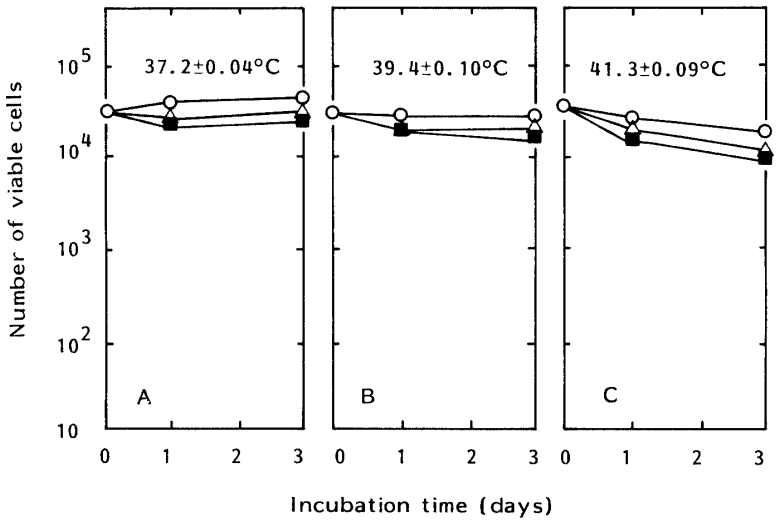

Fig. 8. Effects of 5-FU on the growth of HVE cells. 0.4 ml of cell suspension at a density of 1.2 x 10^5/ml in growth medium was plated on to each well of a Multidish. Control (○), Cells + 43 ng/ml(△), Cells + 85 ng/ml(■)

Fig. 8 shows the effects of 5-FU on the growth of HVE cells. When HVE cells were cultured at various temperatures, the growth of cells was slightly inhibited by the addition of 5-FU. However, the growth inhibition was not so enhanced by the drug at 41.5°C (Fig. 8-C).

Fig. 9 demonstrates the effects of 5-FU on the morphology of cultured human cells at high temperature. In the case of HeLa cells incubated at 41.5°C, granular degeneration in cytoplasm, spindle-shaped and dispatched cells are shown in this photograph (Fig. 9-A-1). Morphological exchanges of HeLa cells treated with 5-FU (85 ng/ml) were also observed as well as control cells, however the number of attached cells was slightly reduced as compared with that of cells untreated with 5-FU at 41.5°C (Fig. 9-A-2). There is no remarkable degeneration in morphology of HAIN-55 cells in the presence (Fig. 9-B-2) or absence (Fig. 9-B-1) of 5-FU at 41.5°C. When HVE cells were treated with 5-FU at 41.5°C, remarkable degeneration on cell morphology was not observed (Fig. 9-C-2) as compared with control cells at 41.5°C (Fig. 9-C-1).

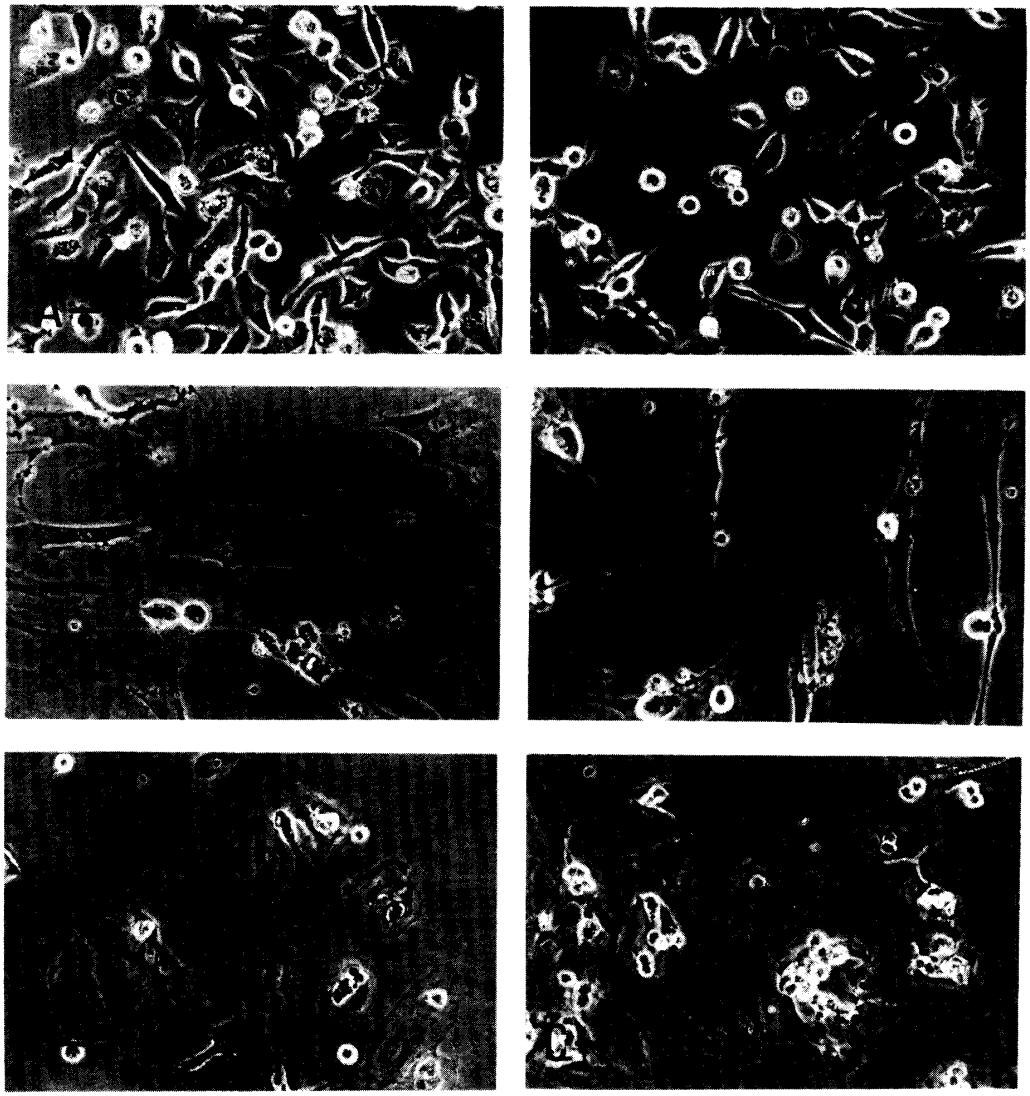

Fig. 9. Effects of 5-FU on the morphology of cultured human cells. HeLa cells cultured without 5-FU at 41.5°C(A-1), HeLa cells cultured with 85 ng/ml 5-FU at 41.5°C(A-2), HAIN-55 cells cultured without 5-FU at 41.5°C(B-1), HAIN-55 cells cultured with 85 ng/ml 5-FU at 41.5°C(B-2), HVE cells cultured without 5-FU at 41.5°C(C-1), HVE cells cultured with 85 ng/ml 5-FU at 41.5°C(C-2)

DISCUSSION

It was revealed that heat alters various cell functions such as oxygen uptake, synthesis of nuclear acids and protein and membrane stabilization (2-5). In the present experiment, the cytotoxicity of sulpyrine against cultured human cells tested was enhanced by incubation at high temperature (41.5°C), which may be due to a combination of temperature and sulpyrine, because sulpyrine cytotoxically acted on cultured cells at 37.2°C and

the growth of cells in the absence of sulpyrine was slightly inhibited at 41.5°C. Nahid et al. (5) demonstrated that heat potentiation of radiation lethality and direct heat death are two distinct phenomena and the two types of damage must be mediated by different cellular mechanisms, therefore, further studies are needed to determine whether or not the same cellular mechanisms are responsible for heat potentiation of sulpyrine lethality and direct heat death.

Bender and Shramm (6) , and Mondovi et al. (3) reported that there was a tendency for the tumor cells to be more sensitive to heat than the normal cells. It is well known that 5-FU which is absorbed by pinocytosis inhibits a cellular DNA synthesis. In the present study, it was found that the sensitivity of tumor cells to 5-FU at 41.5°C was higher than that of normal cells at the same temperature. The difference in sensitivity of normal and tumor cells to 5-FU may be due to the difference in the rate of cell-division and/or mitotic indices between them, and the study further suggests that DNA synthesis in tumor cells which were considerably damaged or altered by cultivation at 41.5°C may be blocked more and more by the presence of 5-FU. From the results, it seems possible that a combination of anticancer drugs with heat is useful for cancer treatment.

HVE cells have been used by many investigators (7-12). We also applied HVE cells in this study as normal human diploid cells. Remarkable cytotoxicity of 5-FU to HVE cells was not observed. The results suggest that cancer treatment with 5-FU by an intravenous injection is possible without injury to vascular cells. This experimental model in cell culture is valid and it is easy to estimate drugs for patients attacked with fever.

The results of these experiments indicate that : 1) it is essential to estimate drug-safety at high temperature, when drugs are administered to patients attacked with fever. 2) The cytotoxicity of drugs to cultured human cells differed between normal and malignant human cells, so it is necessary to choose suitable human cultured cells for an estimation of drug-safety, and if possible, normal and malignant cells originating from the same tissues should be used.

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