The Effects of the Antifebrile Agent Sulpyrine on the Synthesis of DNA, RNA and Protein in HeLa S3 Cells at High Temperature

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Abstract: The effects of sulpyrine (56.2, 100, 178, 320, 562 μg/ml) on DNA, RNA, and protein synthesis in HeLa S3 cells at 36.7°C and at high temperature (38.8, 41.1°C) were investigated. The cells at various cell densities (2.0×10⁴, 4.0×10⁴ and 8.0×10⁴ cells/ml) were inoculated into each well of 24-well plates, and after 24-h cultivation at 37°C, adherent cells were further cultured for 48 h at high temperature with or without sulpyrine. The synthesis of DNA, RNA and protein was inhibited toward high temperature in cases of cultivations without sulpyrine. The synthesis of DNA, RNA, and protein was inhibited in a concentration-dependent manner by the addition of sulpyrine at each temperature. DNA, RNA and protein-IC₅₀ values(sulpyrine concentration inhibiting synthesis of DNA, RNA or protein by 50% relative to untreated cells) at each temperature showed a tendency to increase with the increase in cell density. DNA and protein-IC₅₀ values at each cell density at 38.8°C were not significantly lower than those at 36.7°C. However, RNA-IC₅₀ values at each cell density were significantly (p<0.005) decreased, depending on the temperature. RNA-IC₅₀ values at a cell density of 4.0×10⁴ per ml at 36.7°C and 38.8°C were decreased from 411.7±15.0 μg/ml to 221.0±23.3 μg/ml, respectively. The RNA-IC₅₀ value at 41.1°C was lower than that at 38.8°C. However, the RNA-IC₅₀ value at 41.1°C was excluded from the discussion, because DNA, RNA, and protein synthesis was markedly depressed at that temperature. These results suggest that the inhibitory effect of sulpyrine on RNA synthesis in HeLa S3 cells is enhanced at high temperature.

Key words: Sulpyrine, High temperature, Hela S3 cells

高温環境下におけるスルビリン（解熱剤）によるHeLa S₃細胞のDNA, RNA, 蛋白質合成に与える影響

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高温培養環境下（38.8°C, 41.1°C）におけるスルビリン（56.2, 100, 178, 320, 562 μg/ml）によるHeLa S₃細胞のDNA, RNA, 蛋白質合成に与える影響について, 36.7°C の場合と比較検討した。各種の細胞密度（2.0×10⁴, 4.0×10⁴, 8.0×10⁴/ml）で細胞を24穴プレートにまき, 37°C で24時間培養した。その後さらにスルビリン添加群, 無添加群に分け48時間高温環境下で培養した。スルビリン無添加の場合, DNA, RNA, 蛋白質合成ともに高温度になるにしたがい強く抑制された。細胞のDNA,
INTRODUCTION

Sulpyrine is a pyrazolone antipyretic and one of the relatively toxic drugs. Several investigations for the cytotoxicity of sulpyrine were reported. Umeda et al. (1977) studied the toxic effects of sulpyrine on the morphology of HeLa S3 and HEL cells by Panel method. Saeki et al. (1980, 1981, 1982) reported that the reaction of sulpyrine with nitrite gave rise to three N-nitroso compounds and some of which were mutagenic toward Salmonella typhimurium TA 100.

Our interest has been concerned with the temperature dependency of the cytotoxicity of sulpyrine, since the drug is administered to patients with high fever. In this paper the effects of sulpyrine on the synthesis of DNA, RNA, and protein in HeLa S3 cells at high temperature (38.8°C and 41.1°C) were reported.

MATERIALS AND METHODS

Cell line: HeLa S3 cells (Scherer et al., 1953; Puck et al., 1956), which are human malignant cells from a carcinoma of the cervix, were used in this experiment. HeLa S3 cells were graciously provided by Dr. H. Okumura of the National Institute of Health, Tokyo.

Growth medium: Eagle’s minimal essential medium (05900, Nissei Pharmaceutical Co., Tokyo) supplemented with 10% fetal bovine serum (M.A. Bioproducts, Walkersville, Md., USA), 30µg of L-glutamine per ml and 2% Meylon (NaHCO3, Otsuka Pharmaceutical Co., Tokyo) was used for culturing the cells.

Drug: Sulpyrine, which is one of the pyrazolone derivatives, was used in this experiment. Sulpyrine was dissolved in Hanks’ balanced salt solution at the concentration of 1,000 µg/ml as a stock solution. The drug was synthesized and graciously provided by Dr. T. Saeki of Shimane Medical University at Izumo.

Experimental procedure: A large number of HeLa S3 cells were harvested by treatment with 0.25% trypsin in Ca2+-Mg2+-free phosphate buffered saline (PBS (−)). The cell suspension was immediately diluted with the medium, and the cells were collected by centrifugation (250 × g for 6 min). The pellets were resuspended in the medium containing 15% dimethyl sulfoxide (045-07215, Wako Chemical Industries, Osaka) at the density of 1.0 × 10⁶ cells/ml and dispensed into glass ampules and stored at −80°C until needed (Paul, 1975).

After the frozen cells were thawed by immersion in a water bath at 37°C for 3 min, they were inoculated into culture flasks and cultured at 37°C for 48 h. The medium was aspirated from the culture flasks, and the cells were released by treatment with 0.25% trypsin in PBS (−) and suspended in growth medium. The cells at various densities (2.0 × 10⁴, 4.0 × 10⁴, 8.0 × 10⁴ cells/ml) in 0.5 ml of growth medium were seeded into each well (15.5 mm) of 24-well plates (25820, Corning Glass Works, N.Y., USA) and cultured at 37°C in 5% CO₂ in 95% humidified air (CO₂ incubator). Twenty-four h later, the medium was replaced with 0.5 ml of growth medium containing various concentrations of sulpyrine, and then to each well was added 1.11 pCi of methyl-3H-thymidine (NET-027X, New England Nuclear, Boston Mass., USA), (5-3H)-uridine (NET-174, New England Nuclear), or DL-(4, 5-3H)-leucine (TRK-75, New England Nuclear) to measure DNA, RNA, and protein synthesis, respectively (Gierthy and Frenkel, 1984). Further cultivation was carried out at 36.7±0.01°C, 38.8±0.04°C, or 41.1±0.06
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0.5 ml of cell suspension in minimal essential medium supplemented with 10% fetal bovine serum, 2% Mello and 30μg of L-glutamine per ml was plated in each well of a 24-well plate and cultured for 24 h at 37°C in a humidified atmosphere containing 5% CO2. After that the medium was replaced with 0.5 ml of the medium containing sulpyrine (0.0, 56.2, 100, 178, 320, 562 μg/ml) and radioisotope, and further cultured at 36.7°C, 38.8°C or 41.1°C for 48 h under 5% CO2 (see "Materials and Methods" for details). (A): Counts per minute (CPM) of radioisotope found per well, (B): CPM as percent of untreated culture (without sulpyrine) at each temperature. (○): 36.7°C, (■): 38.8°C, (▲): 41.1°C. Each points represents the mean of three samples. Standard error was less than 5% of the mean for all points.

The effects of high temperature and sulpyrine treatment on DNA synthesis in HeLa S3 cells at a density of 4.0 x 10^4 cells/ml are shown in Fig. 1. When the cells were cultured without sulpyrine at each temperature, the synthesis of DNA being presented by the CPM (counts per minute) value / well was depressed toward high temperature (Fig. 1-A). The inhibition of DNA synthesis, which is indicated by the decrease in the CPM values / well, increased with an increase in sulpyrine concentration at each temperature (Fig. 1-A). When CPM values

RESULTS

The effects of high temperature and sulpyrine treatment on DNA synthesis in HeLa S3 cells at a density of 4.0 x 10^4 cells/ml are shown in Fig. 1. When the cells were cultured without sulpyrine at each temperature, the synthesis of DNA being presented by the CPM (counts per minute) value / well was depressed toward high temperature (Fig. 1-A). The inhibition of DNA synthesis, which is indicated by the decrease in the CPM values / well, increased with an increase in sulpyrine concentration at each temperature (Fig. 1-A). When CPM values
as percentage of the values in untreated cultures at each temperature were analyzed (Fig. 1-B), there were no significant differences in the inhibitory effects on DNA synthesis among the different temperatures. DNA-IC$_{50}$ values (sulpyrine concentration inhibiting synthesis of DNA by 50% relative to untreated cells) at the various temperatures also did not show significant differences from each other.

As illustrated in Fig. 2-A, in the case without sulpyrine, the synthesis of RNA was depressed with an increase in temperature. RNA synthesis at 36.7°C and 38.8°C was...
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Table 1 Comparison of IC$_{50}$ values (sulpyrine concentration inhibiting synthesis of DNA, RNA or protein by 50% relative to untreated cells) at high temperature$^1$.

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<th>Cell density</th>
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<tr>
<td></td>
<td>2.0 $\times$ 10$^4$/ml</td>
<td>4.0 $\times$ 10$^4$/ml</td>
<td>8.0 $\times$ 10$^4$/ml</td>
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<tr>
<td>DNA</td>
<td>36.7°C</td>
<td>38.8°C</td>
<td>36.7°C</td>
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|            | 106.7 ± 2.7  | 91.0 ± 4.1 | 195.0 ± 17.8 | 103.0 ± 8.1*
|            |              |            | 411.7 ± 15.0 | 221.0 ± 23.3** |
| RNA        | 36.7°C       | 38.8°C     | 36.7°C     | 38.8°C     |
|            | 53.3 ± 0.5   | 52.7 ± 2.0 | 91.3 ± 4.8  | 93.0 ± 4.9 |
|            |              |            | 149.3 ± 2.3 | 149.5 ± 7.0 |

$^1$ Shown is the mean IC$_{50}$ values ± standard error of the mean for at least nine samples.

* $p<0.025$, ** $p<0.005$ for the difference between values at 36.7°C and 38.8°C.

inhibited in a concentration-dependent manner by the addition of sulpyrine. When the cells were cultured at 41.1°C, RNA synthesis in the cells was markedly depressed. RNA-IC$_{50}$ values decreased with an increase in temperature (Fig. 2-B). There were significant ($p<0.005$) differences among RNA-IC$_{50}$ values at each temperature, which indicates that the cells were less tolerant to sulpyrine at high temperature (38.8°C, 41.1°C) than at 36.7°C.

Fig. 3 shows the effect of sulpyrine on protein synthesis in the cells at each temperature. When the cells were cultured without sulpyrine at each temperature, the inhibition of protein synthesis increased toward high temperature as well as in the cases of DNA and RNA (Fig. 3-A). There was a dose-response relationship between sulpyrine and protein synthesis at each temperature. There were no significant differences among protein-IC$_{50}$ values among the temperatures (Fig. 3-B).

Table 1 gives the DNA, RNA, and protein-IC$_{50}$ values at various cell densities at 36.7°C and 38.8°C. IC$_{50}$ values at 41.1°C were excluded from Table 1, because DNA, RNA, and protein synthesis was markedly depressed at that temperature. DNA, RNA, and protein-IC$_{50}$ values at 36.7°C and 38.8°C increased according to the cell density, which indicates that the cells at high cell density were more tolerant to sulpyrine than those at low cell density. RNA-IC$_{50}$ values at each cell density at 38.8°C were significantly lower than those at 36.7°C, and the inhibitory effect of sulpyrine on RNA synthesis was enhanced at high temperature.

**DISCUSSION**

The present study has examined the effects of sulpyrine on the synthesis of DNA, RNA and protein in HeLa S3 cells at high temperature, as a preliminary study for the temperature dependency of the cytotoxicity of sulpyrine.

These experiments were performed with HeLa S3 cells of the same generation by using frozen cells. Preliminary experiments (data not shown) showed that there were large deviations among the results when cells of different generations were used. It is important to conduct experiments with cells of the same generation in order to make the experimental conditions in as similar as possible.

With respect to the synthesis of DNA, RNA and protein in HeLa S3 cells, the tolerance of the cells to sulpyrine at each temperature (36.7°C, 38.8°C and 41.1°C) was increased according to the cell density ($2.0 \times 10^4, 4.0 \times 10^4, 8.0 \times 10^4$ cells/ml). There seem to be two possible reasons for this phenomenon; at higher cell density some cells were not affected by sulpyrine molecules; or the metabolic cooperation through
gap junctions among cells was increased by higher cell density (Farguhar and Palade, 1963; Goodenough and Revel, 1970; Hooper and Subah-Shape, 1981).

It is unknown why DNA and protein-inhibitory effects of sulpyrine were not enhanced at high temperature. Further studies to investigate the reasons are needed. However, the inhibitory effect of sulpyrine on RNA synthesis in HeLa S3 cells was significantly enhanced at high temperature. Synthesis of nucleic acids and protein was depressed toward high temperature in the cases of cultivations without sulpyrine. Then, it may be that RNA-inhibitory effect of sulpyrine is synergistically elevated at high temperature. It has been shown that high temperature alters various cell functions such as oxygen uptake, synthesis of nucleic acids and protein, and membrane stabilization (Cavaliere et al., 1967; Mondovi et al., 1969; Nahid and Kurt, 1983). The enhancement of the RNA-inhibitory effect of sulpyrine at high temperature may be involved by some alterations in the various cell functions.

**CONCLUSION**

The present study has examined how the effect of sulpyrine on the synthesis of DNA, RNA and protein in HeLa S3 cells is influenced by high temperature. These experiments showed that 1) the synthesis of DNA, RNA and protein was inhibited toward high temperature in cases of cultivations without sulpyrine; 2) the synthesis of DNA, RNA and protein was inhibited in a concentration-dependent manner by the addition of sulpyrine; 3) the IC50 values (sulpyrine concentration inhibiting synthesis of DNA, RNA or protein by 50% relative to untreated cells) at each temperature showed a tendency to increase with an increase in cell density; 4) RNA-IC50 values at each cell density were significantly decreased according to the temperature; the inhibitory effect of sulpyrine on RNA synthesis in HeLa S3 cells was enhanced at high temperature.

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