2′-Deoxycytidine Decreases the Anti-tumor Effects of 5-Fluorouracil on Mouse Myeloma Cells

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2′-Deoxycytidine (dCyd), a pyrimidine nucleoside found at high concentrations in the plasma of cancer patients with a poor prognosis after chemotherapy, is considered to be a biomarker for breast cancer. 5-Fluorouracil (5FU) is a nucleoside analog and is used as an anti-tumor agent in patients whose plasma dCyd concentrations are increased. Because both dCyd and 5FU are pyrimidine analogues, it is possible that they have pharmacokinetic/pharmacodynamic interaction, by which the anti-cancer efficacy of 5FU would be reduced. Here, we examined the effects of dCyd on the cytotoxicity of 5FU on mouse myeloma SP2/0-Ag14 (SP2/0) cells lacking hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) and RH4 hybridomas with HGPRT under asynchronous conditions. The reduced cell viability by 5FU was restored by co-, but not pre-, treatment of dCyd in both SP2/0 and RH4 cells, but this effect in the former tended to be greater than that in the latter, suggesting a possible involvement of HGPRT in the interaction, although this might not be a major mechanism. Moreover, dCyd administration to SP2/0 myeloma-bearing mice tended to shorten their 5FU-induced prolonged survival in vivo. Collectively, these results indicate that dCyd decreases the anti-tumor efficacy of 5FU and that a metabolic pathway via HGPRT is involved partially in this interaction. The evaluation of dCyd as a biomarker is believed to provide valuable information for effective and safe chemotherapy with 5FU.

Key words 2′-deoxycytidine; 5-fluorouracil; biomarker; hypoxanthine-guanine-phosphoribosyl transferase; cell viability; survival

Recently, various kinds of biomarkers have been found to be useful for the diagnosis and therapy of diseases. The NIH definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.” Specifically, tumor markers have been essential in the early diagnosis and follow-up of therapy on special malignancies. For example, carcinoembryonic antigen (CEA), prostate specific antigen (PSA) and CA 19-9 are tumor-associated antigens or tumor-specific antigens that are increased in the sera of patients with colorectal cancer, prostatic cancer or pancreatic carcinoma, respectively, and Bence-Jones protein is specific to multiple myeloma. Of note, cytosine arabinoside (ara-C) and cytarabine (ara-A), which are cytosine analogs, are used in the treatment of acute myelogenous leukemia. We examined the effects of dCyd on the cytotoxicity of 5FU on mouse myeloma SP2/0-Ag14 (SP2/0) cells lacking hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) and RH4 hybridomas with HGPRT under asynchronous conditions. The reduced cell viability by 5FU was restored by co-, but not pre-, treatment of dCyd in both SP2/0 and RH4 cells, but this effect in the former tended to be greater than that in the latter, suggesting a possible involvement of HGPRT in the interaction, although this might not be a major mechanism. Moreover, dCyd administration to SP2/0 myeloma-bearing mice tended to shorten their 5FU-induced prolonged survival in vivo. Collectively, these results indicate that dCyd decreases the anti-tumor efficacy of 5FU and that a metabolic pathway via HGPRT is involved partially in this interaction. The evaluation of dCyd as a biomarker is believed to provide valuable information for effective and safe chemotherapy with 5FU.

Key words 2′-deoxycytidine; 5-fluorouracil; biomarker; hypoxanthine-guanine-phosphoribosyl transferase; cell viability; survival
biomarker not only for diagnosis/prognosis but also for the prediction of 5FU sensitivity in breast cancer patients.

MATERIALS AND METHODS

Chemicals  dCyd and 5FU were obtained from Sigma (St. Louis, MO, U.S.A.). RPMI 1640 medium (RPMI) was obtained from Nikken Biomedical Laboratory (Kyoto). Fetal bovine serum (FBS) was from Sanko Junyaku (Tokyo). Plastic tissue culture dishes of 100-mm diameter were from IWAKI Co. (Tokyo). Penicillin and streptomycin were obtained from Meiji Seika Co. (Tokyo). Phosphate buffered saline (PBS) was from Takara Shuzo Co., Ltd. (Shiga). Trypan blue was from Wako Pure Chemical Ind., Ltd. (Osaka). A 0.22-μm Filter (MilllexTM–GV) was purchased from Millipore Corporation (Bedford, MA, U.S.A.).

Cell Cultures of SP2/0-Ag14 Myeloma Cells and RH4 Hybridomas  SP2/0-Ag14 mouse myeloma cells (SP2/0) were obtained from the Collection of Cancer Cell Lines (National Institute of Hygienic Science, Tokyo). RH4 hybridoma was produced in our laboratory as reported previously. These cells were maintained in culture in RPMI containing 10% heat-inactivated FBS, penicillin at 100 U/ml and streptomycin at 100 μg/ml. The cells were grown in 100-mm plastic tissue culture dishes under a humidified atmosphere of 5% CO2 at 37 °C.

Measurement of Cell Viability in Vitro  dCyd and 5FU were dissolved in sterilized PBS at a given concentration and filtrated through a 0.22-μm filter. Cell suspensions at a density of 1×105 cells/ml were plated in 24-well (1.0 ml per well) plates containing a given dCyd- or 5FU-containing test solution. After 72h incubation, the cells were removed from each well by pipetting, and then the cell viability was evaluated by a trypan blue exclusion test.

As for pre-treatment with dCyd, a SP2/0 cell suspension at a density of 1×104 cells/well (200 μl/well) was plated in a 96-well plastic tissue culture plate and incubated at 37 °C for 24h. The medium was replaced with a fresh medium containing dCyd (pre-treatment) or PBS (co-treatment) at a given concentration. Four days later, the medium was replaced with a designated medium containing 5FU (pre-treatment) or dCyd+5FU (co-treatment), and the cells were cultured for two days. Thereafter, the cell viability was evaluated (Fig. 3).

Survival of Mice Bearing SP2/0 Myeloma Tumors  BALB/c mice (male, five weeks old, weighing 20—25 g) were obtained from Japan SLC (Hamamatsu). The drug administration schedule is shown in Fig. 4. SP2/0 myeloma cell suspensions were inoculated into the mice (intraperitoneally (i.p.), 1×106 cells/mouse). From the next day through the 8th day, the test solution (control: PBS, dCyd: 0.1 mmol/kg, 5FU: 0.15 mmol/kg, dCyd+5FU: dCyd at 0.1 mmol/kg/5FU at 0.15 mmol/kg) was administered intraperitoneally on a daily basis, and the survival was evaluated.

The protocol used here was performed according to the guidelines of the Japanese Society for Pharmacology and was approved by the Committee for Ethical Use of Experimental Animals at Setsunan University.

Statistical Analysis of Cell Growth  Data were analyzed by the F-test for variance and the Student’s t-test using the DA-stat program for significance. Survival curves were calculated using the Kaplan–Meier method, and differences in survival periods were analyzed with the log-rank test.

RESULTS

Effect of dCyd Co-treatment with 5FU on SP2/0 Cell Viability  The effects of 5FU, both alone and in combination with dCyd (dCyd+5FU), on the viability of SP2/0 cells are shown in Fig. 1. 5FU dose-dependently decreased the cell viability. 1 mM dCyd had no effect on the cell viability, and its combination with 5FU significantly increased the effects.
compared with the case of 5FU alone (Fig. 1a). At 1 μM of 5FU, dCyd in the concentration range from 0.1 to 10 mM increased the 5FU-induced decrease of cell viability by up to approximately 30%, and the increment of cell viability with dCyd did not show dose-dependency (Fig. 1b).

Effect of dCyd Co-treatment with 5FU on RH4 Cell Viability
As shown in Fig. 2a, there was a dose-dependent decrease of the viability of RH4 hybridoma cells by the 5FU treatment, and this cytotoxic effect in RH4 cells was observed at lower concentrations of 5FU than that in SP2/0 cells. As with the case of SP2/0 cells, dCyd showed the dose-independent increase on the 5FU (0.3 μM)-treated cell viability at 0.1, 1 and 10 mM in RH4 cells, with increased effects on the viability of approximately 20% (Fig. 2b).

Effect of dCyd Pre-treatment with 5FU on SP2/0 Cell Viability
Co-treatment of dCyd with 5FU significantly increased the cell viability of SP2/0 cells compared to the case of 5FU alone. In contrast, the pre-treatment with dCyd for four days did not show any effect on the 5FU-induced decrease of SP2/0 cell viability (Fig. 3).

Effect of dCyd Treatment with 5FU on Survival of SP2/0-Bearing Mice
With the transplantation of SP2/0 cells to mice, their survival was time-dependently decreased in the control and dCyd groups, with the mean survival times calculated as 25 and 24 d, respectively. This survival time of mice was significantly increased to 48 d by the 5FU administration, and this prolonged survival tended to be decreased to 40 d by the co-administration of dCyd (Fig. 4).

DISCUSSION
In this study, we examined whether dCyd had any effect on 5FU’s anti-tumor activity in vitro and in vivo. Treatment with dCyd diminished the cytotoxic effect of 5FU in SP2/0 and RH4 cells in vitro, and the prolonged survival of SP2/0-bear-
transport system for dCyd should lead us to a better understanding of the interaction between dCyd and 5FU, and this project is now underway in our laboratory.

In conclusion, we showed that dCyd decreased the anti-tumor effects of 5FU in in vitro and in vivo experiments. These findings suggest that the evaluation of dCyd in plasma should be a useful biomarker not only for diagnosis/prognosis but also for the prediction of 5FU sensitivity in breast cancer patients.

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