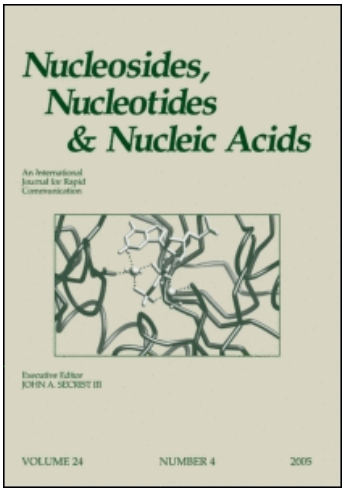

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Cytotoxic and Apoptotic Effects of Novel Heterodinucleoside Phosphates Consisting of 5-Fluorodeoxyuridine and Ara-C in Human Cancer Cell Lines

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ABSTRACT

In search for possible alternatives in the treatment of human malignancies we investigated several new heterodinucleoside phosphates consisting of 5-Fluorodeoxyuridine (5-FdUrd) and Arabinofuranosylcytosine (Ara-C). We show that all dimers tested inhibited the number of colonies of CCL228, CCL227, 5-FU resistant CCL227 and HT-29 human colon tumor cells with IC_{50} values ranging from 0.65 to 1 nM. Dimer # 2 inhibited the number of sensitive and Ara-C resistant H9 human lymphoma cells with IC_{50} values ranging from 200 to 230 nM. Since no significant difference in the cytotoxicity of the dimers could be observed between sensitive and

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resistant cells, these compounds might be used in the treatment of 5-FU and Ara-C resistant tumors.

Key Words: Ara-C; 5-Fluorodeoxyuridine; Apoptosis; Colon carcinoma; Lymphoma.

INTRODUCTION

Various heterodinucleoside phosphates consisting of 5-Fluorodeoxyuridine (5-FdUrd) and Arabinofuranosylcytosine (Ara-C) have recently been synthesized as alternative chemotherapeutic agents. 5-Fluorodeoxyuridine is being used against human colon tumors, whereas Ara-C is one of the most effective agents in the treatment of hematological malignancies. These dimers enter the tumor cell due to their high affinity to lipid membranes. After cellular uptake, the 5′-phosphate derivative of 5-FdUrd and Ara-C is supposed to be released via enzymatic cleavage. Due to the release of the 5′-phosphate derivative, 5-FdUrd needs not to be phosphorylated in the tumor cell. In addition, as being a part of the dimer, Ara-C can be transported to the tumor site without degradation. In our present study, we tested three of these amphiphilic dimers which had already exhibited antitumor activity in leukemia and prostate cancer cell lines.[1–5] We investigated the cytotoxic effects in various human colon tumor cell lines as well as in Ara-C sensitive and resistant H9 human lymphoma cells.[6] Besides, we analyzed the induction of apoptosis in HT-29 cells.

MATERIALS AND METHODS

Chemicals and Supplies

Dimers # 2, # 2A and # 3 were provided by Prof. Herbert Schott, University of Tuebingen, Germany. All other chemicals were commercially available and of highest purity.

Cell Culture

CCL228, CCL227 and HT-29 cells were purchased from ATCC (American Type Culture Collection, Rockville, MD, USA). The 5-FU resistant CCL227 cells were provided by Prof. Robert M. Mader, University Hospital, Vienna, Austria. The sensitive and Ara-C resistant H9 cells were provided by Prof. Ram P. Agarwal, University of Miami, Florida. Cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS) (GIBCO, Grand Island Biological Co., Grand Island, NY, USA), and with 1% penicillin-streptomycin in humidified atmosphere containing 5% CO_2. Cell counts were determined using a microcellcounter CC-108 (SYSMEX, Kobe, Japan).

Clonogenic Assay

Cells growing in the logarithmic phase of growth were plated in 24 well Costar plates (10^3/well) and incubated with various concentrations of dimers for 7 days at

37°C. After trypan blue staining, colonies (> 40 viable cells) were counted using an inverted microscope (Olympus) at 40 × magnification.

Growth Inhibition Assay

Logarithmically growing H9 cells were seeded at a density of 0.1×10^6 cells/ml in 25 cm^2 Nunc tissue culture flasks and exposed to various concentrations of 5-FdUrd, Ara-C and dimer # 2. Cells were incubated for 72 hours, then counted. Viability of cells was determined by staining with trypan blue. Results were calculated as number of viable cells.

Hoechst Dye 33258 and Propidium Iodide Double Staining

Cells were incubated with various concentrations of dimers for 24 hours. Hoechst 33258 (HO, Sigma, St. Louis, MO) and propidium iodide (PI, Sigma, St. Louis, MO) were added directly to the cells to final concentrations of 5 μg/ml and 2 μg/ml, respectively. After incubation for 60 minutes at 37°C, cells were examined by flourescence microscopy (Zeiss Axiovert 35) with Dapi filters and photographed on Kodak Ektachrome P1600 films. Cells were differentiated into four groups: viable, early apoptotic, late apoptotic and necrotic cells.

RESULTS

Effect of Dimers # 2 and # 2A on the Colony Formation of HT-29 Human Colon Cancer Cells

HT-29 cells were plated in 24 well Costar plates as described in the methods section and then incubated with various concentrations of dimers # 2 and # 2A. After 7 days, the number of colonies was counted. Dimer # 2 inhibited the formation of tumor cell colonies with an IC_{50} of 15 nM and dimer # 2A with an IC_{50} of 8 nM, respectively.

Effect of Dimers # 2 and # 3 on the Colony Formation of Sensitive and 5-FU Resistant CCL227 Human Colon Cancer Cells

CCL227 cells were plated in 24 well Costar plates as described above and then exposed to various concentrations of dimers # 2 and # 3. Dimer # 2 yielded an IC_{50} value of 0.7 nM after incubating the sensitive CCL227 cells for 7 days, and 1.0 nM after incubating the 5-FU resistant CCL227 cells, respectively. Dimer # 3 inhibited the formation of sensitive CCL227 cell colonies with an IC_{50} of 0.6 nM and the formation of 5-FU resistant CCL227 cell colonies with an IC_{50} of 0.7 nM.

Effect of Dimers # 2 and # 3 on the Colony Formation of CCL228 Human Colon Cancer Cells

CCL228 cells were plated in 24 well Costar plates and then incubated with various concentrations of dimers # 2 and # 3. After 7 days of exposure, colonies were counted.

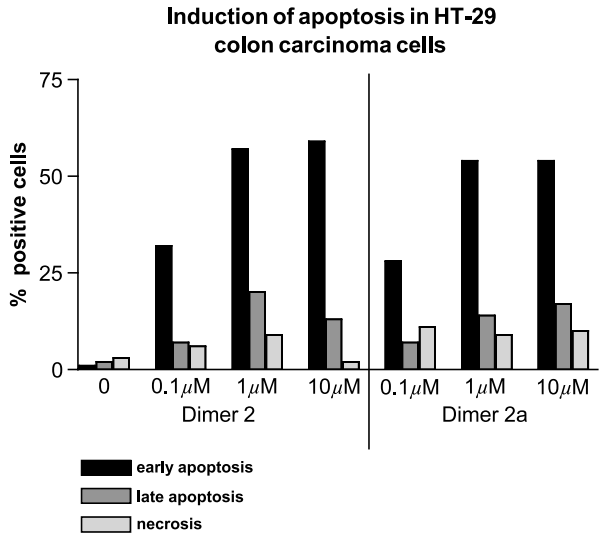

Figure 1. Induction of apoptosis by dimers # 2 and # 2A.

Dimer # 2 yielded an IC_{50} of 0.9 nM, and dimer # 3 inhibited the formation of tumor cell colonies with an IC_{50} of 0.7 nM, respectively.

Effect of Dimer # 2 on the Growth of Sensitive and Ara-C Resistant H9 Human Lymphoma Cells

Sensitive and Ara-C resistant H9 cells were seeded at a cell concentration of 0.1×10^6 cells per ml and exposed to various concentrations of dimer # 2. After 3 days of incubation, cell number was counted using a microcellcounter. Dimer # 2 inhibited the growth of native lymphoma cells with an IC_{50} of 230 nM and the growth of Ara-C resistant cells with an IC_{50} of 200 nM.

Induction of Apoptosis in HT-29 Human Colon Carcinoma Cells by Dimers # 2 and # 2A

HT-29 cells were incubated with 0.1, 1 and 10 μM of dimers # 2 and # 2A. After 24 hours of exposure, cells were double stained with hoechst/propidium iodide. We could observe a dose dependent increase of apoptotic cells. Up to 77% of cells showed signs of early or late apoptosis after incubation with 1 μM of dimer # 2 for 24 hours. Results are shown in Fig. 1.

DISCUSSION

We conclude that the examined novel heterodinucleoside phosphates exhibit in vitro cytotoxic activity against human colorectal cancer cells and human lymphoma cells. Furthermore, they are able to induce programmed cell death in HT-29 cells and are capable of circumventing 5-FU resistance in CCL227 cells as well as Ara-C

resistance in H9 cells. Therefore, these dimers (consisting of 5-FdUrd and Ara-C) might be an additional option for the treatment of sensitive and resistant colon tumors and hematological malignancies.

ABBREVIATIONS

5-FdUrd	5-fluorodeoxyuridine
Ara-C	arabinofuranosylcytosine
Dimer # 2	arabinocytidylyl-(5′ → 1)-2-O-octadecyl-*rac*-glycerylyl-(3 → 5′)-5-fluoro-2′-desoxyuridin
Dimer # 2a	5-Fluoro-2′-desoxyuridylyl-(5′ → 2)-1-O-octadecyl-*rac*-glycerylyl-(3 → 5′)-arabinocytidin
Dimer # 3	5-Fluoro-2′-desoxyuridylyl-(3′ → 5′)-N^4-oleoyl-2′,3′-di-O-acetylarabinocytidin

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