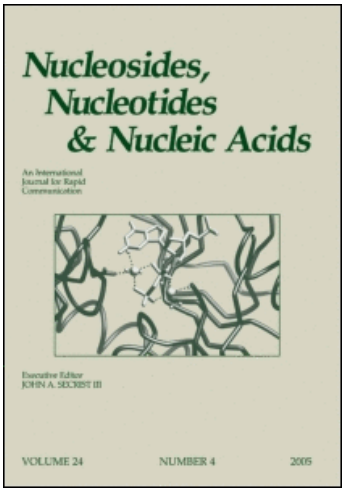

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I. Hubeek[a]; G. J. Peters[b]; A. J. F. Broekhuizen[a]; G. J. L. Kaspers[a]
[a] Department of Pediatric Hematology/Oncology, VU University Medical Center, Amsterdam, The Netherlands [b] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

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Modulation of Cytarabine Induced Cytotoxicity Using Novel Deoxynucleoside Analogs in the HL60 Cell Line

I. Hubeek,[1,*] G. J. Peters,[2] A. J. F. Broekhuizen,[1] and G. J. L. Kaspers[1]

[1]Department of Pediatric Hematology/Oncology and [2]Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

ABSTRACT

In order to enhance the cytotoxicity of ara-C in the HL60 cell line the following deoxynucleoside analogs were used: cladribine, fludarabine and gemcitabine. HL60 cells were co-incubated with ara-C and each of the modulators at the ratios of their respective IC50s. Cytotoxicity was determined with the MTT-assay and drug interactions were evaluated with the combination index (CI) method (Calcusyn; Chou & Talalay). CI < 1, CI ± 1 and >1 indicate synergism, additive effect and antagonism, respectively. We observed moderate synergism between ara-C/cladribine and ara-C/gemcitabine, with CIs of 0.76 ± 0.14 and 0.82 ± 0.04, respectively. The interaction between ara-C/fludarabine resulted in moderate antagonism (CI = 1.29 ± 0.11). In conclusion, in this in vitro study we showed that the cytotoxicity of ara-C can be succesfully modulated in the HL60 cell line by cladribine and gemcitabine.

Key Words: Cytarabine; Deoxynucleoside analogs; Modulation.

*Correspondence: I. Hubeek, Department of Pediatric Hematology/Oncology, VU University Medical Center, Amsterdam, The Netherlands; E-mail: isabelle.hubeek@vumc.nl.

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INTRODUCTION

1-β-D-arabinofuranosylcytosine (ara-C, cytosine arabinoside) plays a central role in the treatment of acute leukemias, particularly in acute myeloid leukemia (AML).[1] The effectiveness of ara-C depends on the formation of its active metabolite, ara-CTP by the enzyme deoxycytidine kinase (dCK).[2,3] Ara-C cytotoxicity results from DNA synthesis arrest due to direct inhibition of DNA polymerase alpha and incorporation of ara-CTP into DNA, causing chain termination. Ara-CTP levels correlate with prognosis and improved clinical outcome has been associated with an increased area under the ara-CTP accumulation and retention curve.[4] Resistance to ara-C is a major cause of treatment failure in AML.[5] Studies on the modulation of ara-C resistance are therefore warranted. 2-Chlorodeoxyadenosine (cladribine, 2-CdA), 9-β-D-arabinosyl-2-fluoroadenine (fludarabine, F-ara-A) and 2′2′-difluorodeoxycytidine (gemcitabine, dFdC) may be able to enhance intracellular ara-CTP levels by inhibition of ribonucleotide reductase (RR) activity. RR is a rate-limiting enzyme in DNA synthesis and converts ribonucleoside diphosphates into deoxyribonucleoside diphosphates.[6,7] Inhibition of RR decreases deoxycytidine triphosphate (dCTP) pools, causing a competitively higher incorporation of ara-CTP into DNA, as compared to dCTP. In addition, dCK activity is up-regulated via a feedback mechanism, thereby augmenting cellular ara-CTP levels. In this study we tried to enhance ara-C induced cytotoxicity by combining ara-C with 3 deoxynucleoside analogs (cladribine, fludarabine and gemcitabine) in the leukemic cell line HL60.

MATERIALS AND METHODS

The human leukemic cell line HL60 (promyelocytic leukemia), was cultured at 37°C in a 5% CO_2 humidified atmosphere in RPMI 1640 (Dutch modification, Gibco, Uxbridge, UK), supplemented with 10% fetal calf serum (FCS) and 1% glutamine (Gibco laboratories, Irvine, UK)[8] and was growing exponentially during all experiments. HL60 cells were co-incubated with ara-C (Cytosar®; Pharmacia & Upjohn, The Netherlands) and each of the modulators (Leustatin®, Ortho Biotech, USA; Fludara®; Schering AG, Germany; Gemzar®, Eli Lily) Drugs were combined at the ratios of their respective IC50s. Drugs were mixed and serially diluted. Cytotoxicity was determined using the 4 day MTT assay. After 4 days of incubation in 5% CO_2 humidified air at 37°C, 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT; Sigma, St Louis MO, USA) was added and cells were incubated for another 4 hours. Subsequently, formazan crystals formed were dissolved in acidified isopropanol. The optical density (OD) is linearly related to the number of viable cells.[9] Control wells, containing HL60 cells with culture medium but no drugs were used to determine the control cell survival. Wells with culture medium only were used as blank. The IC50 value, the drug concentration needed to inhibit cell growth by 50% was calculated by extrapolation from the growth inhibition curve. Drug interactions were determined with the software program Calcusyn (Biosoft, UK). The combination index (CI) method is based on the median drug effect equation of Chou-Talalay.[10] CI < 1, CI ± 1 and >1 indicate synergism, additive effect and antagonism, respectively. The averaged CI at EC_{50}, EC_{75} and EC_{90} were used for each of the

Table 1. Drug interactions between ara-C and the different modulators in the HL60 cell line.

	Drug ratio	Mean CI (± SD)	Description
Ara-C + Cladribine	1:1.25	0.76 (±0.14)	Moderate synergism
Ara-C + Fludarabine	1:85	1.29 (±0.11)	Moderate antagonism
Ara-C + Gemcitabine	1:0.44	0.82 (±0.04)	Moderate synergism

Values given are the mean combination index (CI ± SD) of three experiments.

combinations tested. The average CI (±SD) of three experiments is given for each of the combinations.

RESULTS

We observed moderate synergism between ara-C/cladribine and ara-C/gemcitabine. In contrast, the interaction between ara-C/fludarabine was moderately antagonistic (Table 1).

DISCUSSION

In this in vitro study we showed that the cytotoxicity of ara-C can be succesfully enhanced in the HL60 cell line by the RR inhibitors cladribine and gemcitabine. dCK activity is feedback inhibited by intracellular dCTP, depletion or reduction of normal dCTP levels by inhibiting RR can enhance ara-C cytotoxicity. Inhibition of RR is one of the mechanisms of action of these drugs. Iwasaki et al have previously shown that inhibition of RR with gemcitabine favored ara-C incorporation in replicating DNA.[11] Gandhi and coworkers showed that co-administration of cladribine and ara-C resulted in maximum inhibition of DNA synthesis, accumulation and higher concentrations of ara-CTP.[12] But for fludarabine, only sequential incubation with fludarabine followed by ara-C has been shown to enhance ara-CTP accumulation.[13,14] Apparently fludarabine exposure should precede ara-C exposure in order to maximally deplete dCTP and to enhance ara-C cytotoxicity. Possibly, this precluded synergism in a simultaneous incubation, since co-incubation of ara-C/fludarabine decreased the cytotoxicity of ara-C (moderate antagonism). In conclusion, these results suggest that not only RR inhibition, but also direct effects on e.g. DNA may play a role in this interaction and these drugs might by useful for combination therapy with ara-C. We are currently testing these ara-C combinations ex vivo in leukemic blasts from childhood AML patients.

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