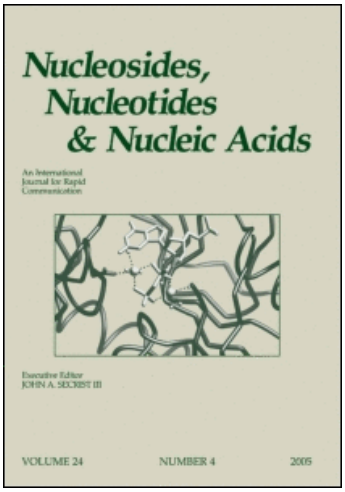

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Amidox, an Inhibitor of Ribonucleotide Reductase, Potentiates the Action of Ara-C in HL-60 Human Promyelocytic Leukemia Cells

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ABSTRACT

Amidox (3,4-dihydroxybenzamidoxime), a new polyhydroxy-substituted benzoic acid derivative, is a potent inhibitor of the enzyme ribonucleotide reductase (RR), which catalyses the de novo synthesis of DNA. RR is considered to be an excellent target for cancer chemotherapy. In the present study we investigated the antineoplastic effects of Amidox alone and in combination with Arabinofuranosylcytosine (Ara-C) in HL-60 human promyelocytic leukemia cells. In growth inhibition experiments Amidox yielded an IC_{50} of 30 μM, colony formation was inhibited at an IC_{50} of 20 μM as determined by a soft agar assay. Exposure of the cells to 75 and 100 μM Amidox for 24 hours was shown to significantly decrease intracellular dCTP, dGTP and dATP pools, whereas dTTP concentration increased, as determined by HPLC. The combination of Amidox with Ara-C yielded more than additive cytotoxic effects both in growth inhibition assays and in soft agar assays. We could show that—after

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preincubating the cells with 75 and 100 μM Amidox and subsequent exposure to Ara-C—intracellular Ara-CTP levels increased by 576% and 1143%, respectively. In conclusion, Amidox might offer an additional option for the treatment of leukemia and thus be further investigated in vitro and in vivo.

Key Words: Amidox; Ara-C; Ribonucleotide reductase; Combination chemotherapy.

INTRODUCTION

Ribonucleotide reductase (EC 1.17.4.1) (RR) catalyzes the rate limiting step in the de-novo synthesis of deoxyribonucleoside triphosphates (dNTPs). Since its activity was shown to be correlated to the proliferation rate in tumor cells,[1,2] it is an excellent target for cancer chemotherapy.

A newer group of RR inhibitors are benzohydroxamic acid derivatives.[3] Amidox (3,4-dihydroxybenzamidoxime) inhibits RR by interfering with the iron binding subunit of the enzyme. It was shown that combining inhibitors of RR with Arabinofuranosylcytosine (Ara-C), a widely used drug for the treatment of leukemia, can yield synergistic effects.[4–7] Ara-C has to be phosphorylated to Ara-CTP to exert its effects; Ara-CTP is then incorporated into DNA which leads to strand breaks, DNA fragmentation and apoptosis in malignant tumor cells. The enzyme deoxycytidine kinase catalyzes this phosphorylation step of Ara-C, and is itself feedback inhibited by dCTP. As Amidox might deplete dCTP pools by inhibiting RR, we suggested that combining Amidox with Ara-C might increase the metabolism of Ara-C, thus leading to synergistic growth inhibitory and cytotoxic effects.

MATERIALS AND METHODS

Suspension culture growth and soft agar colony formation inhibition were studied using either Amidox (synthesized and gift of Dr. Bart van't Riet) alone or in combination with Ara-C.

Measurements of intracellular dNTP concentrations after exposure to Amidox for 24 hours was achieved by isolating dNTPs followed by separation and detection using HPLC. Variations in intracellular Ara-CTP concentrations after subsequent exposure to Amidox and Ara-C were detected by HPLC too.

RESULTS

Growth Inhibition of HL-60 Cells by Ara-C and Amidox

Incubation of HL-60 cells with Amidox alone for four days yielded an IC_{50} value of 30 μM. Combination of Ara-C (5, 10, 20, 30 nM) with 25 μM Amidox yielded more than additive effects in all concentrations.

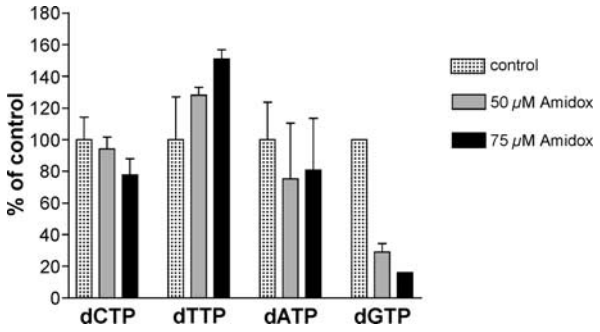

Figure 1. Concentration of intracellular dNTPs in HL-60 cells after 24 hours incubation with Amidox as measured by HPLC.

Clonogenic Assay

Amidox alone yielded an IC_{50} value of 13 μM after 7 days of incubation.

The combination of 0.5 and 0.75 nM Ara-C with 5, 10 and 20 μM Amidox yielded more than additive effects.

Determination of Deoxyribonucleoside Triphosphates

Incubation with 50 and 75 μM Amidox decreased dCTP concentrations to 94.1 and 77.8 % of control values, respectively (Fig. 1). The intracellular concentrations of dATP and dGTP were also decreased to 75.3 and 80.9 % and 29.0 and 16.0 %, respectively. Intracellular dTTP pools experienced an increase to 128.0 and 150.9 % after treatment with Amidox.

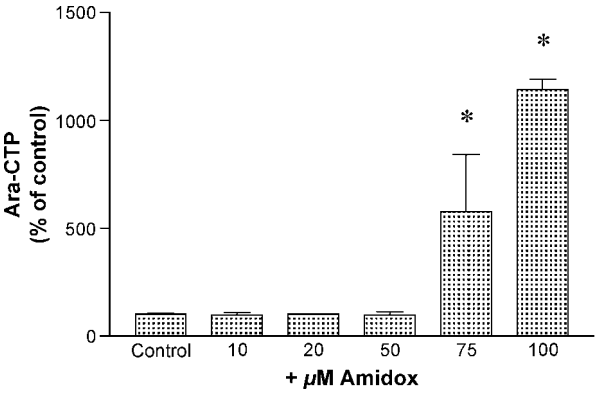

Figure 2. Ara-CTP concentrations after treatment with Amidox in HL-60 leukemia cells (*significantly different from control).

Ara-CTP Concentrations After Amidox Exposure

Treatment of HL-60 cells with 75 and 100 μM Amidox caused a significant increase of intracellular Ara-CTP pools to 576 and 1143% of control values, respectively (Fig. 2).

DISCUSSION

Amidox effectively inhibited growth and colony formation of HL-60 cells, and exerted a more than additive effect on inhibition of growth and colony formation in combination with Ara-C in HL-60 cells. In addition we could show that Amidox was able to influence the concentrations of deoxyribonucleoside triphosphates and to significantly increase the formation of Ara-CTP, the effective metabolite of Ara-C, in HL-60 cells. Therefore Amidox might be further investigated in vitro and in vivo for the treatment of acute or chronic myeloid leukemia, both as a single agent and in combination with other potent antineoplastic substances.

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