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Effect of ribonucleotide reductase inhibitors on the growth of human colon carcinoma HT-29 cells in culture

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Summary. The effects of ribonucleotide reductase inhibitors on the growth of the human colon carcinoma cell line HT-29 were examined. Inhibitors were chosen for these studies that were specifically directed at each of the subunits of ribonucleotide reductase. The concentrations of drugs required to inhibit the growth of HT-29 cells by 50% (IC_{50}) for hydroxyurea, 2,3-dihydro-lH-pyrazole-[2,3 a]imidazole (IMPY), and 4-methyl-5-amino-l-formyl-isoquinoline thiosemicarbazone (MAIQ) were 206, 996, and 3.2 μM, respectively. Although the IC_{50} for deoxyadenosine alone was >2,000 μM, in the presence of 5 μM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), which protects deoxyadenosine from deamination by adenosine deaminase, it was reduced to 112 μM. The IC_{50} for deoxyguanosine was 1,060 μM. The addition of 8-aminoguanosine to protect deoxyguanosine from phosphorylysis by purine nucleoside phosphorylase did not increase the toxicity of deoxyguanosine in HT-29 cells. The combination of MAIQ or IMPY and deoxyadenosine/EHNA gave strong synergistic inhibition of HT-29 cell growth. The results of these studies indicate that ribonucleotide reductase inhibitors effectively block the growth of human colon carcinoma HT-29 cells and that combinations of inhibitors directed at the individual subunits of reductase result in synergistic inhibition of HT-29 cell growth in culture.

Introduction

Although curative cancer chemotherapy for acute lymphocytic leukemia in children, Hodgkin's disease, histocytic lymphoma, pediatric solid tumors, testicular cancer, and gestational trophoblastic cancer has been demonstrated, colon carcinoma is poorly responsive to current chemotherapeutic agents [14]. It is clear that new approaches must be developed if improvement in the treatment of human colon tumors is to be made. Toward this end, we began an investigation of the effects of antitumor agents directed at the ribonucleotide reductase site on human colon carcinoma cells (HT-29) in culture.

Ribonucleotide reductase, which consits of two non-identical protein subunits (non-heme iron and effector-binding subunits), catalyzes the rate-limiting step in the de novo synthesis of deoxyribonucleoside 5′-triphosphates required for DNA replication [6]. It is expected that drugs directed at ribonucleotide reductase would be useful for cancer chemotherapy [11]. Recent studies have shown that ribonucleotide reductase inhibitors could inhibit mouse leukemia L1210 cell growth [12, 24–26, 28, 33]; moreover combinations of inhibitors that were simultaneously directed at the individual subunits of ribonucleotide reductase resulted in strong synergistic inhibition of L1210 cell growth and synergistic cytotoxicity [24–26]. It was also shown that modulating agents directed at protecting the drugs from inactivation were required [8, 24–26]. In this report, we present data showing that ribonucleotide reductase inhibitors and combinations of specific inhibitors directed at the individual subunits inhibit the growth of human colon carcinoma HT-29 cells in culture.

Materials and methods

Cell culture. HT-29 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 40 mM HEPES (pH 7.4). and 50 μg gentamicin/ml. Cells were plated in 25-cm^2 plastic flasks at a concentration of 1×10^4 cells/ml.

Abbreviations used: MTT, 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide; IMPY, 2,3-dihydro-1H-pyrazole [2,3 a]imidazole; MAIQ, 4-methyl-5-amino-l-formylisoquinoline thiosemicarbazone; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; dAdo, deoxyadenosine; AGuo, 8-aminoguanosine; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-l-piperazine-ethanesulfonic acid; CI, combination index

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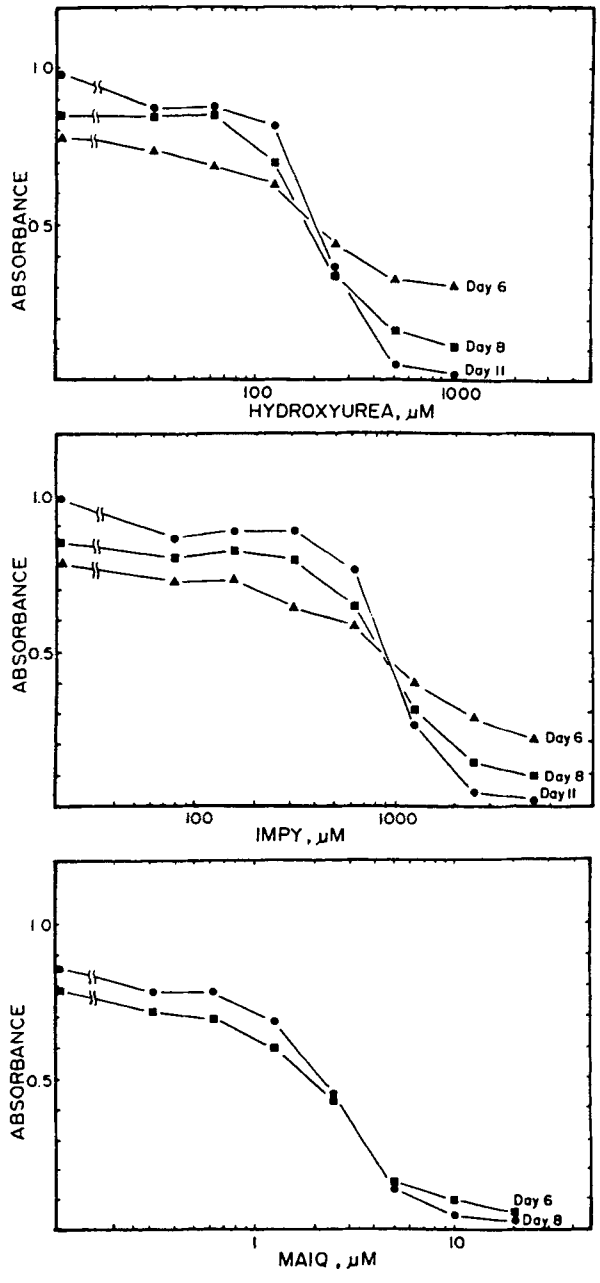

Fig. 1. Effects of hydroxyurea, IMPY, and MAIQ on HT-29 cell growth. HT-29 cells were inoculated on day 0 and the inhibitors were added on day 2. The MTT assay was caried out on the days indicated. Mean values of triplicate assays are shown on a semi-log scale

MTT assay. A modification of the microculture tetrazolium (MTT) assay was used as previously described by Alley et al. [1]. Single-cell suspensions were obtained by trypsinization of monolayer cultures, and cell counts were performed with a Model ZBI Coulter Counter. The HT-29 cells (1,500/well) were plated into 96-well tissue-culture plates (Costar, Cambridge, Mass.) in 0.15 ml complete culture medium with 0.1% $NaHCO_3$. The plates were incubated for 2 days in a humidified CO_2 incubator (5% CO_2/95% air) at 37° C, after which 50 μl of drug solutions diluted with complete RPMI 1640 medium were added to culture wells in triplicate. Unless otherwise stated MTT (15 μl of 5 mg/ml in phosphate-buffered saline without calcium) was added to each well after 8 days of culture and was incubated at 37° C for 4 h. The medium was removed from wells and 150 μl dimethylsulfoxide (DMSO) was added to each well to solubilize the formazan crystals. After mixing by pipetting, the absorbance of each well was measured using a microplate reader (Dynatęch MR700, Alexandria, Va.) in the dual mode at 490 nm (test filter) and 630 nm (reference filter). The IC_{50} was defined as being the concentration of drug required to reduce the absorbance ($A_{490}-A_{630}$) by 50% as compared with the values for control cells, which received no drug. All cultures, at each drug concentration and drug combination, were set up in triplicate.

[^{3}H]-Thymidine incorporation into DNA. Cellular growth in the presence or absence of ribonucleotide reductase inhibitors was also determined by measuring [^{3}H]-thymidine incorporation into DNA. After 8 days of culture, [^{3}H]-thymidine (0.1 μCi, 61 Ci/mmol) was added to each well and incubated at 37° C for 4 h. The medium was removed from each well and the cells were washed with phosphate-buffered saline without calcium. Methanol was added to each well to fix the cells. Following washing with water, 0.3 *M* NaOH was added to solubilize the DNA. The radioactivity incorporated into DNA was measured using a MARK V model 5303 Tm Analytic scintillation counter. All cultures, at each drug concentration, were set up in triplicate.

Determination of deoxyribonucleoside triphosphate pools in HT-29 cells. Exponentially growing HT-29 cells were treated with trypsin, pooled and distributed to individual flasks, and incubated for 24 h. The drugs were added and the cells were incubated for another 2 h. The culture medium was poured off, and the cells were released from the flasks by trypsin treatment, washed in phosphate-buffered saline and resuspended at a cell concentration of 1×10^8 cells/ml. The deoxyribonucleoside triphosphate (dNTP) pools in control and drug-treated HT-29 cells were determined by the method of Garrett and Santi [15] as modified by Tanaka et al. [29].

Metabolism of [^{14}C]-cytidine in HT-29 cells. HT-29 cells (log phase, 1×10^5 cells/ml) were incubated in the presence or absence of drug(s) for 2 h at 37° C. [^{14}C]-Cytidine (2 or 2.5 μCi/flask, 400 mCi/mmol was added and the cells were incubated for 1 additional h at 37° C. The cells were harvested by trypsin treatment, resuspended in complete culture medium, counted, and washed with phosphate-buffered saline. They were subjected to a modified Schmidt-Thannhauser procedure [27] to separate the acid-soluble, RNA, and DNA fractions. Aliquots of these fractions were taken for radioactivity measurements by liquid scintillation counting. The acid-soluble fraction was neutralized with KOH, the $KClO_4$ was removed by centrifugation, and the supernatant fluid was lyophilized. The lyophilized material was dissolved in TRIS buffer (pH 8.5) and treated with snake venom to convert the nucleotides to nucleosides. [^{14}C]-Deoxycytidine was separated from cytidine on Dowex-1-borate columns [10].

Drugs. IMPY and MAIQ were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, through the assistance of Dr. Nancita R. Lomax. IMPY was dissolved in water and diluted (7.5-fold) with complete RPMI 1640 medium. MAIQ was solubilized in 100% DMSO and diluted into complete RPMI 1640 medium to 0.4% DMSO before its addition to cell cultures. Hydroxyurea, deoxyadenosine, and deoxyguanosine were purchased from Sigma Chemical Company (St. Louis, Mo.). EHNA was obtained from Burroughs-Wellcome (Research Triangle Park, N. C.) and 8-aminoguanosine was purchased from Calbiochem-Behring (La Jolla, Calif.). Hydroxyurea and EHNA were dissolved in water; deoxyadenosine and deoxyguanosine, in water, were warmed to obtain clear solutions; 8-aminoguanosine was solubilized in 50 m*M* NaOH, then neutralized by HCl. These drugs were diluted (7.5-fold) with complete RPMI 1640 medium before their addition to the cultures.

Materials. The human colon carcinoma HT-29 cell line was purchased from American Type Culture Collection (Rockville, Md.). MTT, DMSO, HEPES, and gentamicin were obtained from Sigma Chemical Company (St. Louis, Mo.). RPMI 1640 medium, fetal calf serum, and sodium bicarbonate were purchased from Grand Island Biological Company (Grand Island, N. Y.). [^{3}H]-Thymidine (61 Ci/mmol was obtained from ICN Radiochemicals (Costa Mesa, Calif.), and [^{14}C]-cytidine (400 mCi/mmol) was purchased from Research Products International Mount Prospect, Ill.

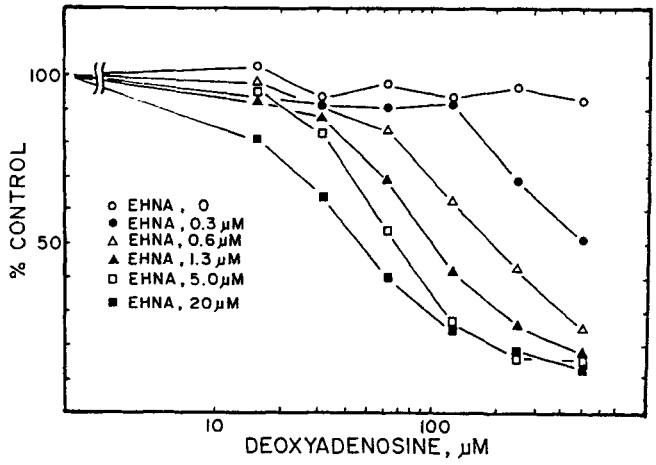

Fig. 2. Effect of EHNA on deoxyadenosine toxicity. Various concentrations of deoxyadenosine with various concentrations of EHNA as indicated were added to HT-29 cell culture on day 2. The ordinate represents the percentage of control absorbance. Mean values of triplicate assays are shown on a semi-log scale

Table 1. Effect of ribonucleotide reductase inhibitors on HT-29 cell growth in culture

Inhibitor	Subunit specificity	IC_{50} (μ*M*)	
		MTT assay	[^{3}H]-dThd uptake
Hydroxyurea	NHI	206± 62 (13)[b]	215
IMPY	NHI	996±260 (13)	910
MAIQ	NHI	3.2±0.7 (10)	–
dAdo/EHNA[a]	EB	112± 55 (14)	65
dGuo	EB	1,060	–

[a] EHNA was used at a concentration of 5 μ*M*
[b] Number of different experiments
NHI, non-heme iron subunit; EB, effector-binding subunit

Analysis of multiple drug effects. The method described by Chou and Talalay [5] was used to analyze the effects of multiple drugs and to determine synergism, summation and antagonism.

Results

Effect of ribonucleotide reductase inhibitors directed at the non-heme iron subunit of reductase

The effects of hydroxyurea, IMPY, and MAIQ (which were directed at the non-heme iron subunit) on human colon carcinoma HT-29 cell growth were determined using the MTT assay. HT-29 cells were inoculated on day 0, then the drugs were added on day 2. At 6, 8, and 11 days after inoculation, the MTT assay was carried out. The results of a typical experiment are shown in Fig. 1. The IC_{50} values measured for hydroxyurea on days 6, 8 and 11 were 315, 210, and 200 μ*M*, respectively. The corresponding values for IMPY were 1250 (day 6), 970 (day 8), and 900 μ*M* (day 11), and those for MAIQ were 2.7 (day 6) and 2.6 μ*M* (day 8). There was no great difference between IC_{50} values assayed on day 8 and those measured on day 11. The IC_{50} value for MAIQ did not appear to depend on the day of assay; therefore, an 8-day culture duration was selected for the rest of the experiments.

Effect of deoxyadenosine on HT-29 cell growth

Deoxyadenosine has been shown to be cytotoxic to cells. However, our previous studies using mouse leukemia L1210 cells showed that the presence of EHNA was required to protect deoxyadenosine from deamination by adenosine deaminase [24]. At concentrations as high as 2,000 μ*M*, deoxyadenosine alone had no inhibitory effect on HT-29 cell growth. In the presence of EHNA, the concentration of deoxyadenosine required to inhibit HT-29 cell growth was markedly decreased with increasing concentration of EHNA. The data from a typical experiment are shown in Fig. 2. Deoxyadenosine (250 μ*M*) with 5 μ*M* EHNA inhibited HT-29 cell growth by 82.8%, although 5 μ*M* EHNA alone inhibited cell growth by only 14.2%. The IC_{50} for deoxyadenosine in the presence of 5 μ*M* EHNA was 112±55 μ*M*.

Effect of deoxyguanosine on HT-29 cell growth

The effect of deoxyguanosine on HT-29 cell growth was examined. Deoxyguanosine inhibited the growth of HT-29 cells in culture with an IC_{50} of 1,060 μ*M*. 8-Aminoguanosine was added to the cultures to protect deoxyguanosine from phosphorylysis [17] and to lower the IC_{50}; however at concentrations as high as 100 μ*M* 8-aminoguanosine did not potentiate the effect of deoxyguanosine (data not shown).

Summary of IC_{50} data

The IC_{50} data obtained for the reductase inhibitors in 10–14 multiple experiments are summarized in Table 1.

Effect of ribonucleotide reductase inhibitors an [^{3}H]-thymidine incorporation into DNA

The ability of ribonucleotide reductase inhibitors to inhibit HT-29 cell growth and [^{3}H]-thymidine incorporation into DNA based on IC_{50} values are summarized and compared in Table 1. The combination of deoxyadenosine and EHNA also had marked inhibitory activity against human colon carcinoma HT-29 cells.

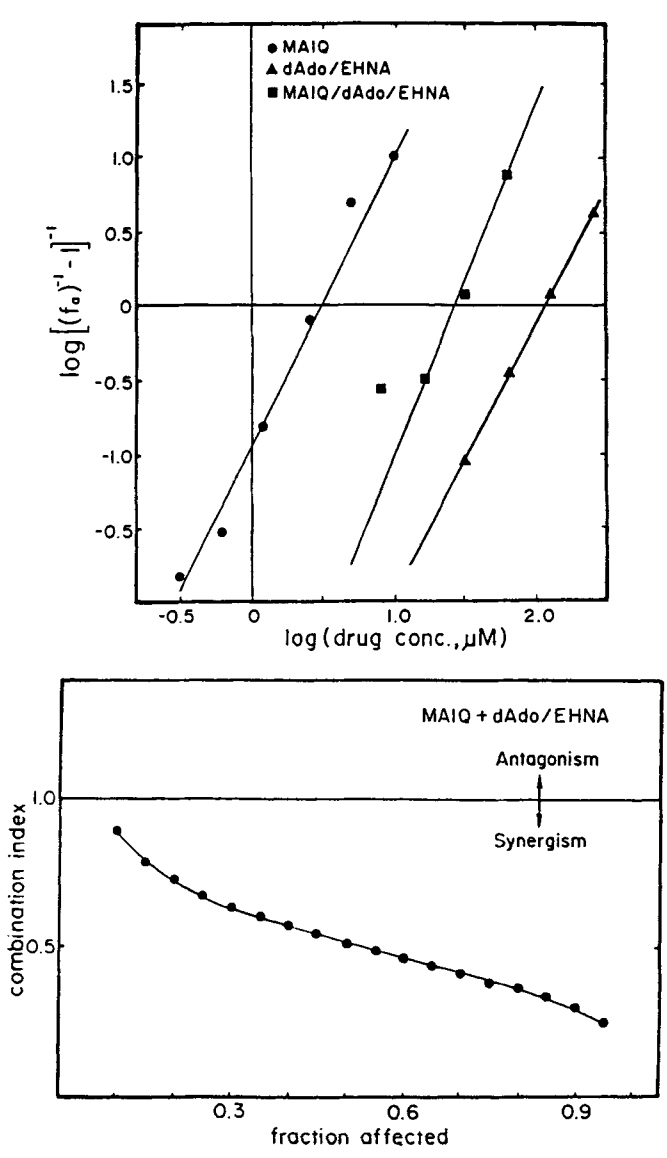

Fig. 3. Effect of combinations of MAIQ and deoxyadenosine/EHNA on HT-29 cell growth. The data for MAIQ and deoxyadenosine (dAdo)/EHNA (5 μ*M*) and their mixtures (molar ratio, 1:25) were plotted according to the median-effect equation. Plots of combination index (CI) with respect to the fraction affected (*fa*) for the inhibitory effect of a mixture of MAIQ and dAdo/EHNA (molar ratio, 1:25) on HT-29 cell growth were obtained by step-by-step calculation. When CI <1, synergism is indicated; when CI = 1, summation is indicated; and when CI >1, antagonism is indicated

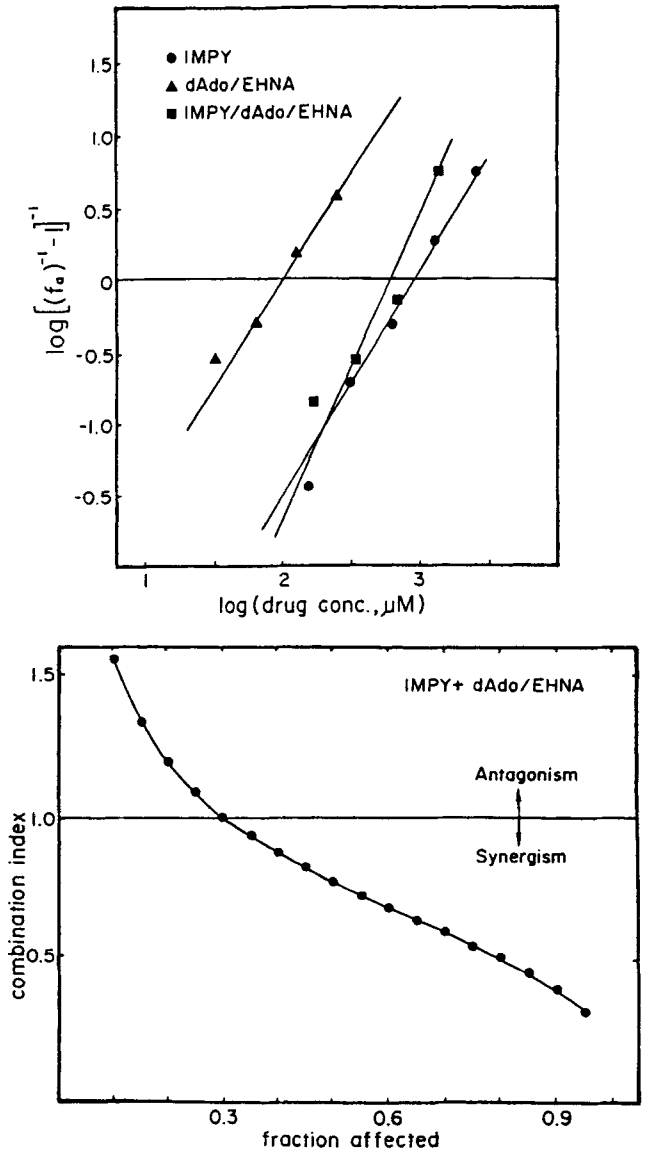

Fig. 4. Effects of combinations of IMPY and deoxyadenosine (dAdo) EHNA on HT-29 cell growth. The data for IMPY and dAdo/EHNA (5 μ*M*) and their mixtures (molar ratio, 40:1) were plotted according to the median-effect equation. Plots of CI with respect to the fraction affected (*fa*) for the inhibitory effect of a mixture of IMPY and dAdo/EHNA (molar ratio, 40:1) on HT-29 cell growth were obtained by step-by-step calculation. When CI <1, synergism is indicated; when CI = 1, summation is indicated; and when CI >1, antagonism is indicated

Effects of combinations of MAIQ and dAdo/EHNA on HT-29 cell growth

The effects of the combinations of inhibitors directed at the individual subunits of ribonucleotide reductase were examined. MAIQ (1.25 μ*M*) and deoxyadenosine (31.25 μ*M*)/EHNA (5 μ*M*) gave only 13% and 8% inhibition of HT-29 cell growth, respectively, but their combination inhibited cell growth by 55%. To quantitate the effects of the combinations, the data were analyzed by the method of Chou and Talalay [5]. The ratio of MAIQ to deoxyadenosine was 1:25. In median-effect plots, the dose-effect relationships of MAIQ and dAdo/EHNA were parallel, but the plot of their mixture was upwardly concave, which indicated that MAIQ and deoxyadenosine/EHNA were mutually nonexclusive inhibitors (i.e. they have different modes of action or act independently). The data for a typical experiment are shown in Fig. 3. The combination index indicated that the effects of MAIQ and dAdo/EHNA were markedly synergistic at all values of the fraction affected (fa).

Table 2. Effect of ribonucleotide reductase inhibitors on 2′-deoxyribonucleoside 5′-triphosphate levels in HT-29 cells

Treatment[a]	dCTP	dTTP (% control)[b]	dATP	dGTP
None	100	100	100	–[c]
MAIQ, 2.5 μ*M* (A)	76	54	90	–
dAdo, 62,5 μ*M* (B)	70	60	134	–
A + B	48	52	113	–

[a] HT-29 cells were treated with drug for 2 h at 37° C

[b] The concentrations of dCTP, dTTP, dATP in the control, untreated cells were 11.1 ± 1.8, 8.3 ± 1.8, and 6.1 ± 1.6 pmol 10^{-6} cells ml^{-1}, respectively

[c] The concentration of dGTP was too low, even in the control cells, to be measured by the integrator

Effects of combinations of IMPY and dAdo/EHNA on HT-29 cell growth

Human colon carcinoma HT-29 cells were incubated at a range of drug concentrations in IMPY (39.1–2,500 μ*M*), dAdo (3.9–250 μ*M*) EHNA (5 μ*M*), or their mixture at a constant molar ratio of 40 : 1. Analysis of the results by the median-effect plot (Fig. 4) indicated that IMPY and dAdo/EHNA were mutually nonexclusive inhibitors. The combination index for the effects of IMPY and dAdo/EHNA indicated moderate antagonism at low fraction affected (fa) values (fa <0.3) and marked synergism at high fa values (fa >0.3).

Effects of combinations of MAIQ and dAdo/EHNA on dNTP pools in HT-29 cells

HT-29 cells were incubated in culture for 2 h in the presence of MAIQ, dAdo/EHNA, and MAIQ/dAdo/EHNA. The nucleotide pool fraction was prepared from control and drug-treated cells and treated with periodate to degrade the ribonucleoside 5′-triphosphates; dNTP levels were determined by high-pressure liquid chromatography. The data are shown in Table 2. With this short-term incubation (2 h), MAIQ reduced the deoxycytidine 5′-triphosphate (dCTP) and thymidine 5′-triphosphate (dTTP) pool levels by 24% and 46%, respectively; dAdo/EHNA reduced the dCTP and dTTP levels by 30% and 40%, respectively. In the presence of a combination of MAIQ and dAdo/EHNA, dCTP and dTTP levels were decreased to 48% and 52% of control levels, respectively. In HT-29 cells, the deoxyguanosine 5′-triphosphate (dGTP) levels were not measurable, even in control cells.

Effects of combinations of MAIQ and dAdo/EHNA on [^{14}C]-cytidine metabolism in HT-29 cells

HT-29 cells were incubated in culture for 2 h in the presence of MAIQ, dAdo/EHNA, and MAIQ/dAdo/EHNA. [^{14}C]-Cytidine was added and the cells were incubated for 1 additional h. Using the Schmidt-Thannhauser procedure [27], the nucleotide pool, RNA, and DNA fractions were separated and analyzed. As shown in Table 3, short-term incubation of HT-29 cells with the ribonucleotide reductase inhibitors had relatively little effect on the incorporation of [^{14}C]-cytidine into RNA. The total uptake of cytidine into HT-29 cells was also not affected (data not shown). The formation of [^{14}C]-deoxycytidine from cytidine via the ribonucleotide reductase step and its incorporation into DNA were inhibited in the presence of MAIQ, dAdo, or combinations of MAIQ and dAdo. According to the method of Webb [32], in HT-29 cells at the concentrations of MAIQ and dAdo used, it appears that only additive effects were seen using the combination MAIQ/dAdo (in terms of inhibition at the ribonucleotide reductase site).

Table 3. Conversion of [^{14}C]-cytidine to deoxycytidine and its incorporation into RNA and DNA in HT-29 cells in the presence and absence of ribonucleotide reductase inhibitors

Experiment		% control[a]	
		RNA	dCyd + DNA
1	Control	100	100
	MAIQ, 1.25 μ*M* (A)	100	58
	dAdo, 30 μ*M* (B)	115	70
	45 μ*M* (C)	116	62
	60 μ*M* (D)	118	57
	A + B	116	50
	A + C	109	49
	A + D	127	40
2	Control	100	100
	dAdo, 45 μ*M* (E)	91	61
	MAIQ, 0.62 μ*M* (F)	82	79
	1.25 μ*M* (G)	82	56
	2.50 μ*M* (H)	92	42
	E + F	107	51
	E + G	90	40
	E + H	72	25

[a] The control values for experiment 1 were: RNA, 51,000; dCyd + DNA, 8,850 cpm/10^6 cells. In experiment 1, the amount of [^{14}C[-cytidine added was 2.5 μCi/flask. In experiment 2, the control values were: RNA, 27,900; dCyd + DNA, 7,400 cpm/10^6 cells. The amount of [^{14}-C]-cytidine added was 2.0 μCi/flask in experiment 2. The flasks were set up in triplicate

Discussion

The deoxyribonucleoside triphosphates (dNTPs) required for DNA replication are generated de novo through the ribonucleotide reductase reaction. The ribonucleotide reductase step has been shown to be rate-limiting in the de novo synthesis of dNTPs. Furthermore, ribonucleotide reductase activity is low in resting cells high in rapidly growing tumor cells, and increases markedly during the transition from the late G_1 to the S-phase of the cell cycle [13, 31]. Thus, it is reasoned that ribonucleotide reductase

is an appropriate site for enzyme-targeted chemotherapy. Hydroxyurea, the only drug in clinical use that is directed at ribonucleotide reductase, has problems of chemical stability and rapid plasma clearance [20]. Clinically, it has some activity in leukemia and limited, albeit not clearly established, activity in solid tumors [20]. We have previously shown that ribonucleotide reductase inhibitors are potent cytotoxic agents in mouse leukemia L1210 cells in culture [12, 24–26, 33]. In the present study, we demonstrated that ribonucleotide reductase inhibitors blocked the growth of human colon carcinoma HT-29 cells in culture. Our results, obtained by MTT assay with 8-day culture duration, showed a good reproducibility of IC_{50} values. Moreover, there was good correlation between the IC_{50} values measured by the MTT assay and by those determined based on [^{3}H]-thymidine incorporation into DNA, since IC_{50} values for hydroxyurea, IMPY, and dAdoEHNA (5 μM) were 215, 910, and 65 μM, respectively, as measured by [^{3}H]-thymidine uptake.

Among the ribonucleotide reductase inhibitors studied, MAIQ, a thiosemicarbaxone derivative that inhibits the non-heme iron subunit [11, 23], had the highest activity against HT-29 cells based on concentration; this is in line with previous observations in other cell lines. IMPY appeared to be less active than hydroxyurea, but the former is much more stable than the latter. In the presence of EHNA[1], deoxyadenosine was the only potent inhibitor of the effector-binding subunit studied against HT-29 cells. In general, 2- to 3-fold higher concentrations of the ribonucleotide reductase inhibitors were needed ot obtain 50% inhibition of HT-29 cell growth as compared with L1210 cell growth [7]. 8-Aminoguanosine, which is a purine nucleoside phosphorylase inhibitor [17], had no effect on deoxyguanosine cytotoxicity in HT-29 cells. Our previous studies showed that 100 μM 8-aminoguanosine markedly potentiated the inhibitory effect of deoxyguanosine on L1210 cells [25]. It is possible that the difference in the activities of 8-aminoguanosine between human colon carcinoma HT-29 cells and mouse leukemia L1210 cells is attributable to lower kinase activity, which salvages deoxyguanosine, or higher purine nucleoside phosphorylase activity in the HT-29 cells.

The goals of combination chemotherapy are to generate a greater antitumor effect than the sum of single drug's effects without mutually potentiating their side effects on the host and to reduce the probability of drug-resistant tumor cells' development [3, 16, 18, 19, 22]. Ribonucleotide reductase has two nonidentical subunits, each of which can be specifically and independently inhibited by the known ribonucleotide reductase inhibitors. The non-heme iron and effector-binding subunits are encoded by different genes [30], which are differentially expressed [9, 21]. Combinations of drugs directed at these subunits have resulted in synergistic inhibition of L1210 cell growth [24]. Our results showed that the combination of MAIQ and deoxyadenosine/EHNA gave a strong synergistic inhibition of HT-29 cell growth. MAIQ is the most active inhibitor of both the non-heme iron subunit of the holoenzyme and cell growth, and these effects can be further potentiated by its use in combination with deoxyadenosine/EHNA directed at the effector-binding subunit. The combination of IMPY and deoxyadenosine/EHNA also showed synergism at high fraction-affected (fa) values, although moderate antagonism occurred at low fa values.

Studies of HT-29 cells in short-term culture in the presence of the ribonucleotide reductase inhibitors showed that the combination of MAIQ/dAdo resulted in decreases in the dCTP and dTTP pools within 2 h (dGTP concentrations were too low to measure). Likewise, the metabolism of [^{14}C]-cytidine via the ribonucleotide reductase step was inhibited in an additive manner in the presence of MAIQ/dAdo. The effects were relatively specific, as these agents had no effect on the total uptake of cytidine into the cells and relatively little influence on the incorporation of cytidine into RNA.

The present data show that ribonucleotide reductase is an appropriate target in human HT-29 cells; combinations of agents directed at its subunits can be expected to result in synergistic inhibition of HT-29 cell growth. Furthermore, drug resistance at the ribonucleotide reductase site is specific for the inducing agent; that is, cell lines resistant to hydroxyurea or IMPY retain sensitivity to deoxyadenosine/EHNA, whereas those resistant to deoxyadenosine/EHNA remain sensitive to hydroxyurea or IMPY [2, 4, 7].

These results indicate a possibility that combination chemotherapy regimens using ribonucleotide reductase inhibitors could be applied in the treatment of human cancers, especially colon cancers that are not curable by current chemotherapy protocols. At this point, neither MAIQ nor deoxyadenosine has been studied as a single agent in the treatment of human cancer; thus the use of these agents in combination chemotherapy protocols in humans is not yet timely. At high concentrations, deoxyadenosine would be expected to show effects at metabolic sites independent of ribonucleotide reductase and could contribute to immunosuppression and increased uric acid levels in patients. However, as shown in these studies, when this drug is used in combinations with EHNA and IMPY or MAIQ, its effective concentration may be lowered. Additional preclinical studies are required to determine whether these aspects warrant phase I trials.

[1] EHNA was required for the inhibition of serum and cellular adenosine deaminase activity, thereby enabling the achievement of higher levels of dATP in the cell. EHNA was chosen over deoxycoformycin as the adenosine deaminase inhibitor because it is a relatively potent yet reversible inhibitor of adenosine deaminase, and the concentration range of its effectiveness was not as narrow as that of deoxycorformycin.

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