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RAPID COMMUNICATION

POTENTIATION OF THE CELL GROWTH INHIBITORY EFFECT OF β INTERFERON BY MOPIDAMOLE

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It was suggested that the antitumor effect of the interferons is based in part on their ability to stimulate increased cAMP production. We have explored the interaction of human fibroblastic β interferon (HFIF) with a cAMP decomposition inhibitory pyrimido-pyrimidine derivative, Mopidamole (RA-233) in cultures of neoplastic and normal cell lines. Mopidamole potentiated the growth inhibitory effect of HFIF in cultures of ES-1 malignant melanoma cells, LNCaP prostatic carcinoma cells, RT-4 transitional carcinoma cells, HT-29 colon adenocarcinoma cells and in diploid fibroblast cells.

Hope is high in the medical community that interferons would prove to be effective anticancer agents. To date, these expectations were only partly fulfilled (1-6). However, most of the clinical trials included only limited number of patients and the questions always arose, whether higher doses would have been more effective. On the other hand, it was difficult to extend the dose range because of limited supply of drugs and high incidence of side effects at higher doses. Development of recombinant interferons introduced new hope for eventual availability of adequate supply, however, the question of glycosylation particularly of recombinant β and ∂ interferon is still unresolved.

We have studied human fibroblastic β interferon (HFIF) produced in our Institute, because of its relative lack of side effects in man (7). Interferons appear to exert their growth inhibitory effect in part by acting on adenylate cyclase and increasing the synthesis of cyclic nucleotides (8-11). The tumor growth inhibitory role of cAMP has been reviewed by Braun et al (12).

In a series of studies we have investigated cAMP phosphodiesterase inhibitors as intracellular cAMP increasing agents and have shown that related to this function they inhibit platelet aggregation and tumor metastasis (13-15). We wanted to explore whether these agents potentiate the anticancer activity of HFIF and increase its therapeutic index. In this study we employed the cAMP phosphodiesterase inhibitor, Mopidamole (RA-233), a pyrimido-pyrimidine derivative (13)

MATERIALS AND METHODS

The normal and neoplastic cell lines investigated are listed in Table I. Media and methods of cell culture and of daily cell counting have been reported previously (16,17) (and are indicated in Table I). Drugs are added during the lag phase of cell growth ($T_{0\ hr}$ for cell suspension cultures and $T_{24\ hr}$ for adherent cultures). Each experiment was done with quadruplicate samples. Each 24 hour cultures were trypsinized and cell counts determined

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TABLE I

Cell line	Origin	Doubling time hrs.	Media, monolayer (M) or Suspension (S) culture
ES-1	Malignant melanoma	36	RPMI[1]1640 M
DAUDI	Burkitt's B cell lymphoma	24	RPMI 1640 S
HT-29	Adenocarcinoma	28	MEM[2] M
RT-4	Transitional cell carcinoma of bladder	48	MEM M
LNCaP	Prostatic carcinoma	70	RPMI 1640 M
BG-27	Diploid foreskin fibroblasts	48	MEM M

[1]RPMI = Roswell Park Memorial Institute medium 1640 developed in our Institute and supplied by Grand Island Biologicals Inc., Buffalo, NY. +5% fetal bovine serum + L-glutamine (15)

[2]MEM = Minimal essential medium (Grand Island Biologicals, Inc.) + 10% fetal bovine serum (15, 17).

using a Coulter counter, model ZMI. Mean and standard deviations are given. Viability was measured by the Trypan blue exclusion method. At the concentrations used no non-viable but morphologically intact cells were seen.

RESULTS

Table II shows an example of the degree of inhibition of cell growth (as percentage of growth rate in control cultures) at T_{72} hrs after

TABLE II

EFFECT OF HFIF AND RA-233 ON IN VITRO CELL GROWTH

Cell lines	Drugs and Concentrations	Population increase % of control ± S.D.	% of control expected if additive
DAUDI	0.001 mg/ml RA-233	91 ± 3	
	50 ref. U/ml HFIF	53 ± 4	48
	RA-233 + HFIF	36 ± 2	
ES-1	0.01 mg/ml RA-233	66 ± 4	
	50 ref. U/ml HFIF	48 ± 3	32
	RA-233 + HFIF	13 ± 5	
LNCaP	0.1 mg/ml RA-233	80 ± 2	
	100 ref. U/ml HFIF	76 ± 2	61
	RA-233 + HFIF	32 ± 3	
RT-4	0.1 mg/ml RA-233	86 ± 2	
	100 ref. U/ml HFIF	58 ± 4	50
	RA-233 + HFIF	37 ± 5	
HT-29	0.1 mg/ml RA-233	16 ± 2	
	100 ref. U/ml HFIF	75 ± 4	12
	RA-233 + HFIF	2 ± 1	
BG-27	0.1 mg/ml RA-233	65 ± 3	
	100 ref. U/ml HFIF	86 ± 3	56
	RA-233 + HFIF	33 ± 5	

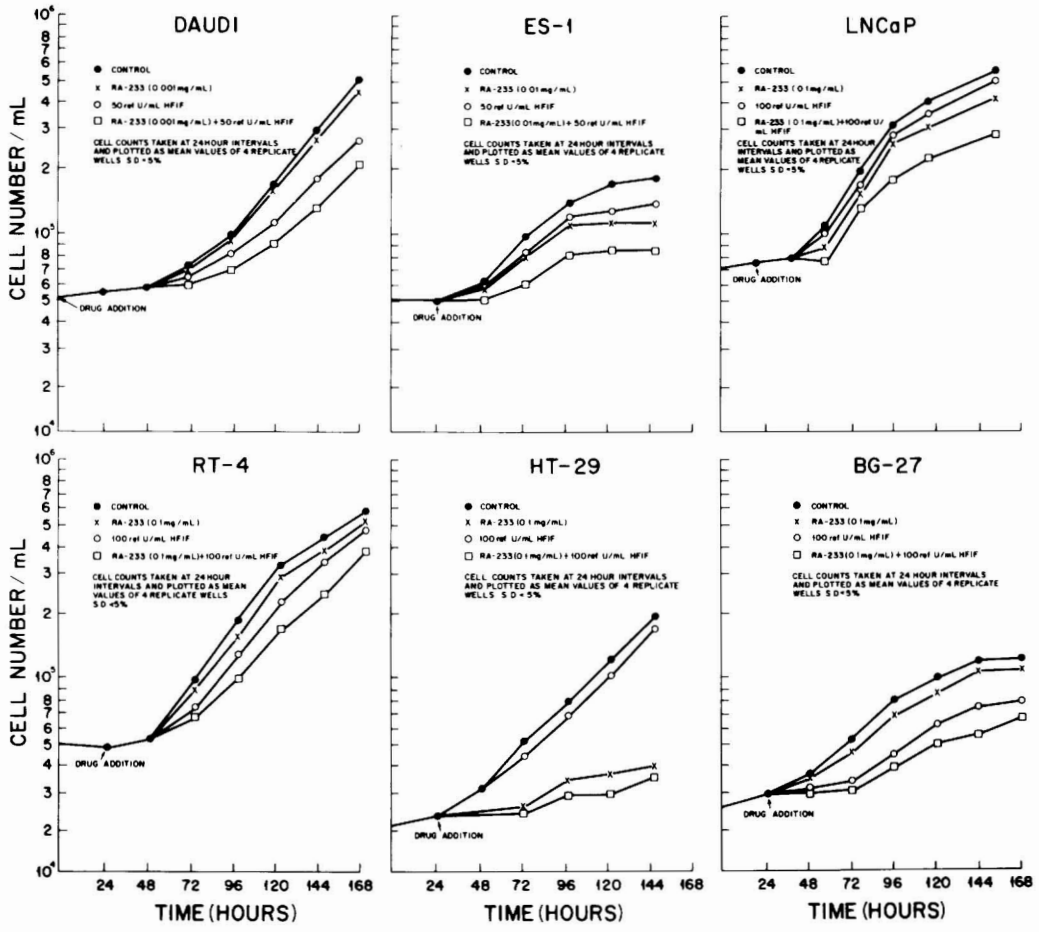

FIGURE 1.

addition of drugs. Figure 1 shows means of actual cell counts from 24 to 168 hours of culture. Standard deviations were less that 5% and for this reason are not indicated. Drugs were added at T_0 hrs in the suspension cultures and at T_{24} hrs for adherent cultures. In order to study the effect of combination treatment, we attempted to find drug concentrations for each agent which would produce growth inhibition close to 50%. At the concentrations used (0.001-0.1 mg/ml) RA-233 produced inhibition in the range of about 10-80% of the control growth rate. HFIF at the 50-100 U/ml level produced inhibition of about 25-50% of the control growth rate. When RA-233 and HFIF were combined in doses used in the above mentioned experiments, the resulting growth inhibition was mostly greater than the inhibition calculated assuming an additive effect. It thus appears that RA-233 potentiates the effect of HFIF in most cell lines.

DISCUSSION

Inhibition in the BG-27 diploid foreskin fibroblast cell line was in the same range as in the five neoplastic cell lines. However, the degree of "normality" in any established, continuously growing cultured cell line is open to question. These experiments measured direct inhibitory drug effect and did not consider effects on host immunity. Interferons were shown to increase NK cell activity and phagocyte activity of macrophages (18). In preliminary experiments (19), we found that RA-233 potentiates the NK cell activating effect of inter-

ferons possibly also by increasing cAMP levels in NK cells. It may be these immunologic functions of interferons which add to their chemotherapeutic effect and confer specificity on their anticancer activity in vivo. It is possible that RA-233 and other related cAMP phosphodiesterase inhibitors will contribute to increasing the therapeutic index and decreasing the cost of interferon therapy. There is a need for clinical studies to test interferons in combination with these agents as well as with other anticancer drugs.

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