Effects of Cimetidine on Various Biological Activities of Human Leucocyte Interferon and on Interferon Production in Lymphocytes

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HIRAI, N., HATTORI, N., HILL, N.O. and OSTHER, K. *Effects of Cimetidine on Various Biological Activities of Human Leucocyte Interferon and on Interferon Production in Lymphocytes.* Tohoku J. exp. Med., 1985, **147** (2), 199-212 — In vitro effects of cimetidine on the antiviral, the antiproliferative and the natural killer (NK) cell stimulating activities of human leucocyte interferon (IFN- α (Le)), and on the Sendai virus induced interferon production in lymphocytes were investigated. The antiviral activity of IFN- α (Le) was dosedependently enhanced by cimetidine, which alone did not demonstrate any antiviral action. The observed titer of the same IFN preparation titrated with 60 μ g/ml of cimetidine was significantly higher ($p < 0.05$) than that without cimetidine. Cimetidine slightly but dosedependently inhibited the growth of Daudi or G-361 cells. The combination effects of these two agents were revealed to be statistically additive or synergistic. The NK cell activity of peripheral blood lymphocytes (PBL) was suppressed after a 16 hr pretreatment with cimetidine. Furthermore, the interferon production in normal lymphocyte cultures was suppressed dosedependently by cimetidine. On the other hand, the augmentation of the NK cell activity by interferon was further potentiated by cimetidine. ——— cimetidine; human leucocyte interferon; antiviral action; antiproliferative action; NK augmenting action

Interferons (IFN) have various biological activities (Stewart II 1979). Based upon the in vitro and in vivo established antiproliferative activity or immunomodulatory effects of IFN, extensive clinical trials of IFN against various malignant tumors are now under investigation.

On the other hand, several recent reports have indicated that cimetidine may

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have probable antitumoral effects (Armitage and Sindner 1979; Tutton and Steel 1979; Tutton and Barkla 1983; Gifford et al. 1981b; Osband et al. 1981). Furthermore, it has been also reported that the combination therapy of IFN- α (Le) and cimetidine in malignant melanoma patients produced more prominent responses than did IFN- α (Le) alone (Borgström et al. 1982; Hill et al. 1983). Thus, cimetidine seems to be beneficial in cancer treatments especially when combined with interferon therapy.

The purpose of the present study is to explore the effects of cimetidine on the interferon system. The effects of cimetidine on the antiviral activity, the antiproliferative activity and the NK augmenting effect of IFN- α (Le) were investigated in vitro. Furthermore, the influence of cimetidine on the Sendai virus induced interferon production in normal peripheral blood lymphocytes (PBL) has also been studied.

MATERIALS AND METHODS

Cell lines

U cells, a human amnion cell line obtained from Dr. K. Cantell, were used for the study of the antiviral activity of IFN- α (Le). Daudi cells, a Burkitt lymphoma cell line, and G-361 cells, a human malignant melanoma cell line, were used to evaluate the antiproliferative activity of IFN- α (Le). K-562 cells, a human myeloid cell line, PLC/PRF/5 cells, a human hepatoma cell line, and G-361 cells were used as the target cells in the assays of the NK cell activity.

Viruses

Vesicular stomatitis virus (VSV) was used as the challenge virus in the assay of the antiviral activity. Sendai virus was used as the inducer of the interferon production in PBL. Both viruses were kind gifts of Dr. K. Cantell.

Interferon

Human leucocyte interferon (HuIFN- α (Le)) was produced from donor buffy coats induced by Sendai virus and partially purified according to the methods described by Cantell et al. (1981). The titer was established by the plaque reduction assay on U cells challenged by VSV referring to the NIH standard interferon- α (Lot No. G023-901-527). The specific activity of IFN- α (Le) preparation used in this study was $1.3\text{--}3.5 \times 10^6$ IU/mg protein. The antigenic properties of the same kind of human leucocyte interferon (IFN- α (Le)) preparation was previously evaluated by Berg et al. (1980), and revealed to contain 99% L-form (IFN- α) and 1% F-form (IFN- β).

Cimetidine

Cimetidine-HCl (Smith & Kline Lab. Co.), 150 mg/ml solution for clinical use, was diluted in culture medium, pH was adjusted to 7.2-7.4 and used.

Tests for the antiviral activity

A macroplaque reduction assay on U cells challenged by VSV was performed as previously described (Langford et al. 1981). Briefly, 1.2×10^6 of U cells which had grown in a 6 cm culture dish were incubated with various concentrations of IFN and cimetidine. After an overnight incubation, the cells were washed and challenged by 100-150 PFU of VSV for 1 hr. Then, the monolayer was covered with an agar gel and the viral plaque

developed. After staining, the plaques were counted and the reciprocal of the IFN dilution that gives 50% reduction of viral plaque number was calculated as the observed titer.

Tests for the antiproliferative activity

6×10^5 of Daudi cells were cultured with various concentrations of IFN and cimetidine for 3 days and viable cell number was counted in a hemacytometer chamber using 2.5% Trypan Blue solution (GIBCO). G-361 cells, 2×10^4 /well, were cultured in a 24-well culture plate with various concentrations of IFN and cimetidine for 4 days. The medium was refreshed in every 24 hr. Then, they were trypsinized and counted as was done in the Daudi cell experiments. The percentage of cell growth inhibition (relative cell number reduction to control) was calculated.

Tests for the NK cell activity

In each experiment, PBL were prepared from a normal donor blood as previously described (Yamauchi et al. 1979). Then PBL were treated with various concentrations of IFN and cimetidine, in the same time or one by one, for several time lengths and used as the effector cells. The ^{51}Cr releasing cytotoxicity assay was performed as described before (Yamauchi et al. 1979). Briefly, the target cells labelled with $\text{Na}_2 \text{ } ^{51}\text{CrO}_4$ (ICN Chemical & Radioisotope) were washed and seeded in 96-well U plates at a concentration of 1.5×10^4 cells/well with Dulbecco's MEM (DMEM)/5% fetal calf serum. In some experiments using G-361 or PLC/PRF/5 cells, which were monolayer cultures, they were washed after an overnight incubation. Then, the effector cells were added to each well in a concentration of 7.5×10^5 cells/well (E/T ratio=50) and in a final volume of 200 μl /well. The plates were centrifuged at 200 g for 10 min. After a 4 or 16 hr incubation, the plates were recentrifuged and the radioactivity of the 100 μl supernatant from each well was counted by a gamma counter. The NK cell activity was calculated by the following formula:

$$\text{NK cell activity} = \frac{(\text{Experimental} - \text{Spontaneous}) \text{ release}}{(\text{Maximum} - \text{Spontaneous}) \text{ release}} \times 100, (\%)$$

where spontaneous release is the count obtained when target cells were incubated without effector cells, and maximum release is that when they were incubated in the medium containing 2.5% saponin.

Interferon production in PBL cultures

5×10^6 cells/well of PBL were incubated with several concentrations of cimetidine at 37°C for 16 hr. After washed, 6×10^6 cells/well from each group were transferred in to a 24-well culture plate and primed with 100 IU/ml of IFN- α (Le) for 2 hr. Then, 150 HAU/ml of Sendai virus was added to each well and cultured in the final volume of 1.5 ml/well for 24 hr in a 37°C/5% CO_2 atmosphere. The supernatant from each well was centrifuged at 800 g for 10 min, UV-irradiated to inactivate the remaining virus, and kept at -20°C until it was assayed. The IFN yield of each sample was assayed by the plaque reduction assay as mentioned above.

Statistical analysis

To compare the mean, Student's *t*-test was employed, and the differences with *p* values below 0.05 were considered to be significant. The analysis of the combination effects of two agents was performed as following. In each combination, the expected value, the combination index (CI), and the combined standard error (SE) were calculated following the theory described by Spector et al. (1982). The combination effect was considered to be synergistic when CI/SE was more than +2.0, to be additive when CI/SE was between -2.0 and +2.0, and to be antagonistic when CI/SE was less than -2.0.

TABLE 1. *Influence of cimetidine on the antiviral activity of HuIFN- α (Le) in U amnion cells challenged with VSV**

	Log titers of interferon Cimetidine concentration ($\mu\text{g/ml}$)			
	0 (control)	0.6	6	60
Exp. 1	5.90 (0.980†)	6.10 (0.997)	6.64 (0.983)	6.67 (0.987)
Exp. 2	5.72 (0.999)	6.07 (0.993)	6.24 (0.997)	6.26 (0.999)
Exp. 3	5.61 (0.997)	5.97 (0.999)	5.89 (0.997)	6.10 (0.997)
Mean $\pm$ S.D.	5.75 $\pm$ 0.15	5.98 $\pm$ 0.18	6.26 $\pm$ 0.38	6.33 $\pm$ 0.30‡
[U/ml]	[5.6 $\times 10^5$]	[6.0 $\times 10^5$]	[1.8 $\times 10^6$]	[2.1 $\times 10^6$]
S.D.	0.15	0.18	0.38	0.30

* U amnion cells were incubated with interferon and cimetidine overnight, washed and challenged with VSV.

† The correlation coefficient in each titration is given in the parenthesis.

‡ The mean value of log titers is significantly different ($p < 0.05$) from that of control.

TABLE 2. *Plaque reduction assay performed on U amnion cells using interferon and cimetidine**

Dilution of HuIFN- α (Le) (concentration)	Plaque counts in interferon/cimetidine combination Cimetidine concentration ($\mu\text{g/ml}$)			
	0 (control)	0.6	6	60
1 : 1.2 $\times 10^5$ (12.5 IU/ml)	38-42-42 (41)	28-27-30 (28)	23-18-25 (22)	16-18-15 (16)
1 : 4.0 $\times 10^5$ (3.8 IU/ml)	76-68-74 (73)	46-45-54 (48)	44-42-43 (42)	40-45-47 (44)
1 : 1.2 $\times 10^6$ (1.3 IU/ml)	98-102-102 (101)	87-85-78 (83)	71-75-74 (73)	65-70-75 (70)
1 : 4.0 $\times 10^6$ (0.4 IU/ml)	136-123-126 (128)	107-108-105 (107)	100-94-99 (98)	92-100-96 (96)
virus control (no IFN)	153-164-157 (158)	159-160-156 (158)	156-162-159 (159)	158-155-160 (158)
Log titer	5.72	6.07	6.24	6.26
[U/ml]	[5.3 $\times 10^5$]	[1.2 $\times 10^6$]	[1.7 $\times 10^6$]	[1.8 $\times 10^6$]
r	0.999	0.993	0.997	0.999

* Actual plaque counts from experiment 2 in TABLE 1 are shown and the average plaque numbers are given in parenthesis.

† Human leucocyte interferon (HuIFN- α (Le)), specific activity ; 3.5 $\times 10^6$ IU/mg protein, undiluted sample at 1.5 $\times 10^6$ IU/ml.

r, correlation coefficient.

RESULTS

Influence of cimetidine on the antiviral activity of IFN- α (Le)

Effects of cimetidine on the antiviral activity was explored by titrating the same IFN- α (Le) preparation in the presence of cimetidine on U amnion cells challenged by VSV. Table 1 summarizes the results of three titrations and in Table 2, the actual plaque counts in one of these titrations are shown. The observed titer of the same IFN preparation was increased according to the increment of the cimetidine concentration in each titration (Table 1), whereas the plaque counts on the virus control dish (no interferon) was not affected by cimetidine (Table 2) indicating that cimetidine alone does not induce any antiviral state at all in the U cell cultures. The mean of IFN titers assayed with 60 $\mu\text{g/ml}$ of cimetidine was significantly higher ($p < 0.05$) than that without cimetidine (Table 1).

Influence of cimetidine on the antiproliferative activity of IFN- α (Le)

Table 3 shows the combination cell growth inhibitory effects of IFN- α (Le) and cimetidine on Daudi cells. IFN alone suppressed the cell growth of this cell line in about 26% at 2 IU/ml, and 40% at 5 IU/ml. On the other hand, the growth of Daudi cells was inhibited approximately 11% by 0.6 $\mu\text{g/ml}$, 12% by 6 $\mu\text{g/ml}$, and 16% by 60 $\mu\text{g/ml}$ of cimetidine alone. As both IFN and cimetidine showed the cell growth inhibitory effects, the combination effects of these two agents were analyzed as mentioned above. As indicated in Table 3 the observed

TABLE 3. *Influence of cimetidine on the antiproliferative activity of HuIFN- α (Le) on Daudi cells**

HuIFN- α (Le) concentration (IU/ml)	Growth inhibition rates (%)† Cimetidine concentration ($\mu\text{g/ml}$)			
	0	0.6	6	60
0	—	11.3 $\pm$ 6.6	12.2 $\pm$ 8.5	16.2 $\pm$ 12.5
2	25.6 $\pm$ 3.9	36.7 $\pm$ 0.2	41.7 $\pm$ 6.4	46.0 $\pm$ 2.2
(expected‡)	—	34.0	34.7	37.7
(CI/SE§)	—	+0.77 [A]	+1.43 [A]	+1.72 [A]
5	39.5 $\pm$ 3.2	49.7 $\pm$ 3.2	52.3 $\pm$ 8.8	60.3 $\pm$ 6.4
(expected)	—	46.3	46.9	49.3
(CI/SE)	—	+1.25 [A]	+1.15 [A]	+2.55 [S]

* 6×10^5 cells were cultured with interferon and/or cimetidine. Cell growth inhibition rates were evaluated on Day-3. The mean of the control cell growth (without IFN or cimetidine) was 853.7%.

† Mean $\pm$ s.d. of 6 experiments is given.

‡ The expected value was calculated in each combination of interferon and cimetidine following the method of Spector et al. (1982).

§ CI/SE was calculated in each combination to evaluate the mode of combination effect. [A], additive; [S], synergistic.

TABLE 4. *Influence of cimetidine on the antiproliferative activity of HuIFN- α (Le) on G-361 cells**

HuIFN- α (Le) concentration (IU/ml)	Growth inhibition rates (%)† Cimetidine concentration (μ g/ml)		
	0	3	30
Experiment 1 (n=3)			
0	—	6.9 $\pm$ 6.9	10.8 $\pm$ 10.2
100	27.2 $\pm$ 4.0	49.2 $\pm$ 3.3	69.1 $\pm$ 5.7
(expected‡)	—	32.2	35.5
(CI/SE§)	—	+4.00 [S]	+4.82 [S]
Experiment 2 (n=4)			
0	—	-3.2 $\pm$ 5.4	15.3 $\pm$ 3.4
100	16.6 $\pm$ 5.0	26.5 $\pm$ 7.2	38.0 $\pm$ 4.0
(expected)	—	14.0	29.4
(CI/SE)	—	+2.20 [S]	+2.24 [S]
Experiment 3 (n=4)			
0	—	1.0 $\pm$ 5.4	8.8 $\pm$ 7.8
500	53.3 $\pm$ 5.6	57.0 $\pm$ 3.6	65.4 $\pm$ 3.6
(expected)	—	53.3	57.4
(CI/SE)	—	+0.91 [A]	+2.31 [S]

* 2×10^4 cells were cultured with interferon and/or cimetidine. Viable cells were counted and the cell growth inhibition rates were evaluated. The means of the control cell growth were 373.5% (exp. 1), 613.2% (exp. 2) and 809.7% (exp. 3).

† Mean $\pm$ s.d. is given.

‡ & § The expected value and CI/SE in each combination were calculated as indicated in the legend of TABLE 3. [A], additive; [S], synergistic.

cell growth inhibition rate was higher than the expected value in each combination of IFN- α (Le) and cimetidine. However, only in the combination of 5 IU/ml of IFN and 60 μ g/ml of cimetidine, the combination effect was revealed to be synergistic statistically, and to be additive in the others.

The results of three experiments on the G-361 cells were shown in Table 4. Against this cell line, 3 μ g/ml of cimetidine did not show remarkable cell growth inhibitory effect, whereas 30 μ g/ml of cimetidine inhibited the growth in approximately 12% in the mean. IFN- α (Le) suppressed the growth of this cell line in about 20% at 100 IU/ml, and 53% at 5,000 IU/ml. When both agents were combined, the observed cell growth inhibition rate in each combination was much higher than the expected value. Except one experiment with 5,000 IU/ml of IFN- α (Le) and 3 μ g/ml cimetidine where it was found to be additive, the combination effect of each concentration of IFN and cimetidine was revealed to be synergistic, statistically (Table 4). Mock interferon did not show any cell growth inhibitory effect at all against these two cell lines (data not shown).

TABLE 5. *Augmentation of natural killer cell activity of PBL pretreated with cimetidine by HuIFN- α (Le)**

HuIFN- α (Le)	%NK cell activity of PBL pretreated with cimetidine†			
	no cimetidine	0.3 μ g/ml	3 μ g/ml	30 μ g/ml
I. no interferon	21.7 $\pm$ 2.1	19.4 $\pm$ 3.2‡	18.0 $\pm$ 2.1‡‡	20.1 $\pm$ 2.7 ^{ns}
II. 50 IU/ml (II-I§)	23.3 $\pm$ 5.9 (1.7)	25.7 $\pm$ 5.2 (6.3)	25.5 $\pm$ 3.7 (7.5**)	26.5 $\pm$ 4.9 (6.3*)
III. 500 IU/ml (III-I§)	32.0 $\pm$ 3.0 (10.3)	33.6 $\pm$ 2.7 (14.2*)	30.9 $\pm$ 1.5 (12.9*)	34.0 $\pm$ 3.6 (13.9*)
IV. 5000 IU/ml (IV-I§)	35.6 $\pm$ 3.9 (14.0)	37.2 $\pm$ 3.0 (17.8*)	36.8 $\pm$ 4.0 (18.8**)	39.7 $\pm$ 3.4* (19.6**)

* After pretreated with or without cimetidine for 16 hr, PBL were treated with or without HuIFN- α (Le) for 2 hr. The assay of the NK cell activity was done at E/T ratio of 50 by 4 hr ⁵¹Cr releasing cytotoxicity test against K-562 target cells.

† Mean $\pm$ s.d. (%) of twelve experiments.

§ %NK above no interferon treatment by each concentration of interferon was calculated by subtracting the %NK without interferon from that with interferon.

‡ $p < 0.05$; ‡‡, $p < 0.01$ when compared with the value without cimetidine or interferon.

*, $P < 0.05$; **, $p < 0.01$ when compared with the value without cimetidine.

Influence of cimetidine on the NK augmentation activity of IFN- α (Le)

Table 5 summarizes the NK cell activity of PBL after a 16 hr pretreatment with or without three concentrations of cimetidine followed by a 2 hr incubation with IFN- α (Le). Compared to untreated controls, the NK cell activity was suppressed by the cimetidine treatment (for 16 hr). The mean NK cell activity found at 0.3 μ g/ml ($p < 0.05$) and at 3 μ g/ml ($p < 0.01$) was significantly lower than the control, respectively. The viability of PBL after the 16 hr treatment with each concentration of cimetidine was more than 98% and not impaired when tested by the dye exclusion technique using Trypan Blue solution. By the 2 hour treatment with IFN, the NK cell activity was augmented dosedependently in each experiment group. The magnitude of augmentation, evaluated by the NK cell activity above no IFN treatment indicated in parenthesis in Table 5 was found to be statistically higher in each cimetidine pretreated group, except one, than that without cimetidine pretreatment.

In Fig. 1, the NK cell activity of PBL treated with various concentrations of IFN- α (Le) and/or cimetidine for 16 hr. The mean NK cell activities of PBL treated with cimetidine but without IFN were slightly lower than that without cimetidine nor IFN, respectively. On the other hand, the NK cell activity was dosedependently augmented by IFN at each concentration of cimetidine selected, and that of IFN/cimetidine combination group was more pronouncedly enhanced compared to IFN alone (Fig. 1). At 3,000 IU/ml of IFN- α (Le), the means $\pm$ s.d.

Fig. 1. Augmentation of NK cell activity of PBL treated with 0 (○), 0.3 (●), 3 (△) and 30 (▲) $\mu\text{g/ml}$ of cimetidine and IFN- α (Le) for 16 hr. The assay of NK cell activity was done at an E/T ratio of 50 and by a 4 hr cytotoxicity test at 37°C. The NK cell activities indicated in the figure are the mean values of eight experiments.
* $p < 0.05$; † $p < 0.01$ when compared with that without cimetidine treatment.

of the NK cell activities with cimetidine were $54.3 \pm 4.4\%$ (at 0.3 $\mu\text{g/ml}$), $57.9 \pm 6.6\%$ (at 3 $\mu\text{g/ml}$) and $60.9 \pm 4.0\%$ (at 30 $\mu\text{g/ml}$), which were significantly ($p < 0.05$ at 0.3 $\mu\text{g/ml}$; $p < 0.01$ at 3 and 30 $\mu\text{g/ml}$) higher than the activity without cimetidine ($49.6 \pm 2.1\%$), respectively.

The cimetidine effect on the NK cell activity was also tested against two other cell lines, G-361 and PLC/PRF/5 cells, in 5 experiments indicated in Fig. 2. In these experiments, PBL were pretreated with or without 3 $\mu\text{g/ml}$ of cimetidine followed by the treatment with 5,000 IU/ml of IFN- α (Le). The NK cell activity of PBL pretreated with cimetidine alone was also slightly suppressed against those cell lines. The means $\pm$ s.d. of the augmentation of NK cell activity above no IFN treatment in PBL pretreated with cimetidine against K-562, G-361 and PLC/PRF/5 target cells were $27.5 \pm 3.1\%$ ($p < 0.001$), $23.2 \pm 2.4\%$ ($p < 0.01$) and $32.2 \pm 2.6\%$ ($p < 0.05$), and significantly higher than the activity without cimetidine pretreatment, ($12.7 \pm 1.7\%$, $17.0 \pm 2.8\%$ and $26.8 \pm 4.3\%$) respectively.

The IFN activity (the antiviral activity) of the supernatant from the 16 hr PBL culture with each concentration of cimetidine in several experiments was tested by using CPE inhibition assay on GM cells challenged by VSV. No (less

Fig. 2. Augmentation of NK cell activity of PBL pretreated with cimetidine by IFN- α (Le). After pretreated with or without 3 μ g/ml of cimetidine for 16 hr, normal PBL was treated with or without 5,000 IU/ml of IFN for 5 hr. The assay was done at an E/T ratio of 50 and by a 16 hr cytotoxicity test. NK cell activity above no IFN was estimated by the difference in NK cell activity between "with" and "without" IFN treatment.

Fig. 3. Interferon production in PBL cultures treated with cimetidine for 16 hr. After the treatment with 0, 0.3, 3 and 30 μ g/ml of cimetidine for 16 hr, 6×10^6 of PBL were primed with 100 IU/ml of IFN- α (Le) for 2 hr, 150 HAU/ml of Sendai virus was added, and the cells were cultured for 24 hr at 37°C. After UV irradiation, the IFN yields in the culture supernatants were assayed by the plaque reduction method.

than 4 IU/ml) interferon activity was detectable at all.

Influence of cimetidine on the interferon production in PBL

The effect of cimetidine was also tested on the IFN production in normal PBL cultures induced by Sendai Virus. As shown in Fig. 3, the IFN yield induced by Sendai virus in PBL treated with selected concentrations of cimetidine was suppressed dosedependently when compared to that without cimetidine treatment. The IFN production in PBL treated with 3 $\mu\text{g/ml}$ ($p < 0.01$) and 30 $\mu\text{g/ml}$ ($p < 0.001$) of cimetidine were significantly lower than that without cimetidine.

DISCUSSION

Our results indicated that the presence of cimetidine can dosedependently potentiate the antiviral state in U cells induced by IFN- α (Le) (Tables 1 and 2), and that IFN- α (Le) and cimetidine have additive or synergistic combined inhibitory effects on the growth of two human malignant cell lines (Tables 3 and 4). Also, it was suggested that cimetidine may potentiate the NK augmenting activity of IFN- α (Le) (Table 5, Fig. 1 and 2), whereas cimetidine alone seems to suppress the NK cell activity of PBL in accordance with the reduction of IFN production in PBL treated with cimetidine (Fig. 3).

Cimetidine is one of the most widely used histamine H-2 receptor antagonists, for instance, in the treatments of peptic ulcers and acute gastrointestinal bleedings (Freston 1982). Recently, this drug was reported to have possible antitumoral effects in mice as well as in humans as mentioned above. Lately, Borgeström et al. (1982) and Hill et al. (1983) have reported that partial and complete responses were obtained in some of the patients with malignant melanomas by an IFN- α (Le) treatment combined with cimetidine administration, whereas IFN- α (Le) alone did not seem to benefit such patients. The mechanisms of the antitumoral action of cimetidine may be generally considered to be due to its immunomodulatory action including the reduction of the suppressor cell activity (Ogden et al. and Hill 1980; Meretey et al. 1981; Siegel et al. 1982). However, other reports, suggesting that cimetidine has suppressed the growth of human colorectal tumors xenografted on the immunodeprived mice (Tutton and Steel 1979; Tutton and Barkla 1983) seem to imply the possibility that cimetidine may also have a direct inhibitory effect on the growth of these target cells.

Our results have suggested that the cimetidine can directly inhibit the growth of at least two human malignant cell lines in vitro. When a higher concentration (600 $\mu\text{g/ml}$) of cimetidine was tried, Daudi cells were completely killed after a few days (data not shown). On the other hand, Gifford et al. (1981a) reported that cimetidine did not have any cytotoxic effect on a mouse cell line used in their laboratory. However, the concentration of cimetidine used in their experiments

was not mentioned.

Regarding to the concentration levels of cimetidine used in this study, the lower doses (0.6, 3, and 6 $\mu\text{g/ml}$) are known to be pharmacological levels and the higher ones (30 and 60 $\mu\text{g/ml}$) may be considered to be over doses in vivo (Sawyer et al. 1981). As shown in our results, the growth of Daudi cells was inhibited in the lower doses as well as in higher one, and as for G-361 cells, it was suppressed in the higher dose but not in the lower one. Thus, cimetidine is considered to have a direct cell growth inhibitory effect at least on some malignant cells even at pharmacological doses, whereas the sensitivity of the cells to this cimetidine effect seems to differ from cell line to cell line.

Although the precise mechanisms of IFN actions are not established, several reports (Kerr and Brown 1978; Zilberstein et al. 1978) have indicated that several intracellular proteins induced by the IFN molecules bound to the receptors on the cell membrane may inhibit the viral replications. Therefore, it is now considered that the antiviral activity of interferons may be probably generated by these proteins inside the target cells. Moreover, in a recent study (Creasey et al. 1983), the level of (2'-5') oligoadenylate synthetase, one of these intracellular proteins, showed an inversed correlation to the relative number of melanoma cells in S phase. Hence, it is possible that the antiproliferative activity of IFN may be also generated by the same kind of intracellular proteins induced by IFN molecules.

Our results indicated that cimetidine seems to potentiate both of the antiviral and the antiproliferative activities of IFN- α (Le) in an additive or synergistic manner. In other experiments, where the target cells were pretreated with cimetidine prior to the IFN treatment, we also observed that the cimetidine effects to potentiate both IFN activities retained (but less pronounced) for 2 to 5 days after the drug had been removed from the culture medium (data not shown).

It is possible that both of the antiviral and the antiproliferative activities of IFN might be generated by the same intracellular pathway, at least partially, as mentioned above. If so, cimetidine might play a role in accelerating or increasing quantitatively the development of such intracellular proteins inside the target cells.

As for the effects of cimetidine on the NK cell activity, recent studies have indicated that cimetidine may suppress the NK cell activity of PBL against K-562 cells (Láng et al. 1981; Reiz-Argüelles et al. 1982; Nair et al. 1983.) However, with respect to the concentration levels of cimetidine, there seem to be considerable differences in these reports. Especially at the pharmacological doses of cimetidine, Ruiz-Argüelles et al. (1982) reported a clear suppression of the NK cell activity, but Láng et al. (1981) did not observe any prominent suppression, and Nair et al. (1983) only tried the over doses of cimetidine.

In regards to our results, we found that the NK cell activity was slightly suppressed at considerable pharmacological concentrations (0.3 and 3 $\mu\text{g/ml}$), when PBL was preincubated with cimetidine for 16 hr (Table 5). However, the

degree of suppression was not so prominent as Ruiz-Argüelles et al. (1982) reported.

It is known that endogenous as well as exogenous IFN is of importance for the NK cell activity. Trinchieri et al. (1977) indicated that lymphocytes incubated with tumor cell lines for a few hours in vitro released IFN into the culture medium. It has also been observed that anti-IFN antiserum added directly to the NK assay system reduced the NK cell activity remarkably (Dr. Kurt Berg, personal communication). Our results of IFN production in PBL pretreated with cimetidine indicated a remarkable dosedependent suppression of IFN production by these cells (Fig. 3). Based upon these experimental results, we anticipate that the suppressive effect of cimetidine on the NK cell activity may be due to a reduction of the endogenous IFN production in the effector PBL treated with cimetidine.

Besides the antiproliferative activity, one of the most widely recognized non-antiviral actions of IFN is to augment the NK cell activity by acting on the maturation process of NK cells from pre-NK cells (Saksela et al. 1979). We found that either the pretreatment with cimetidine or the presence of this drug enhanced the activity of IFN- α (Le) on the NK cell system. Cimetidine is known to have various immunomodulatory effects including the reduction of the suppressor cell activities as mentioned above. Based upon the fact that the NK cell system is also controlled, at least partially, by suppressor T cells, (Herberman 1982), we suppose that the enhanced NK augmenting activity of IFN- α (Le) induced by cimetidine is probably due to the cimetidine related reduction of the suppressor cell activity on this immunosurveillance system. Another possibility may be that the immunomodulatory effects of IFN might involve the same cellular mechanisms (Stewart II 1979), and that cimetidine might have the same effect on the NK augmenting activity as on the antiviral and antiproliferative activities of IFN- α (Le).

Our in vitro observations seem to sustain the possibility that the combination of IFN and cimetidine, tried preliminarily by Borgeström et al. (1982) and Hill et al. (1983) may be beneficial in the cancer treatments. Further studies including in vivo trials against various malignant tumors are needed to elucidate this possibility.

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