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Antiretroviral Effects of 2',3'-Dideoxycytidine and Recombinant Interferon-α-A on the Infection of Anemia-inducing Murine Friend Virus

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ABSTRACT

The anemia-inducing strain of Friend virus (FVA) is a murine retrovirus which stimulates the proliferation of erythroid progenitor cells. The progenitor cells synthesized by FVA-stimulation are unable to proceed with differentiation and accumulate in the spleen resulting in splenomegaly in infected mice. Using FVA-inoculated mice as a model, we have investigated the antiretroviral effects of 2',3'-dideoxycytidine (ddC) and recombinant interferon-α-A (rIFN-α-A) on FVA infection. The extent of the infection was determined by measuring the weights of the spleens. Daily intraperitoneal injection of ddC (100 mg/kg body weight), rIFN-α-A (10 KU/mose) and the combination of both drugs to FVA inoculated mice for 18 days resulted in suppression of the growth of spleens by 15.1%, 52.7% and 61.6%, respectively. When ddC was dissolved in drinking water (0.1 mg/ml) and administered to a group of FVA inoculated mice ad libitum, and rIFN-α-A (10 KU/mouse) was intraperitoneally injected daily to another group of ddC (0.1 mg/ml) drinking mice for 18days, the growth of spleens was suppressed by 38.4% and 83.2%, respectively. These results indicate that administration of ddC via drinking water is more effective in suppressing FVA infection than the daily injection of ddC, and that the combined effects ddC and rIFN-α-A are not synergistic but additive. In order to determine whether ddC treatment alters the characteristic of the progenitor cells with respect to Ca^{++} uptake, Ca^{++} uptake in erythroid cells and the effect of cyclohexyladenosine (CHA) on the Ca^{++} uptake were studied. Ca^{++} uptake in the erythroid progenitor cells was about 20-fold greater than in mouse erythrocytes and the inhibition of Ca^{++} uptake by CHA was the greatest in the progenitor cells from FVA infected mice which were treated with ddC. The inhibition was obviated by theophylline. Results of CHA binding studies showed that the erythroid progenitor cells contain both high and low affinity CHA binding sites, whereas mose erythrocytes contain only the low affinity CHA binding sites.

Key Words: Antiretroviral effects, 2',3'-Dideoxycytidine, Recombinant interferon-α-A, Anemia-inducing murine friend virus

INTRODUCTION

The FVA has been widely used for the preparation of erythroid progenitor cells which are a good model for the studies of the mechanism of erythropoietin (Epo)-mediated erythroid differentiation (Sawyer *et al.*, 1987; Koury *et al.*, 1984). Epo was shown to promote the maintenance of cellular energy level during the in vitro differentiation of the progenitor cells (Kim *et al.*, 1989a; Kim *et al.*, 1991b). The procedures for the partial purification and character-

ization of Epo receptors were developed in this laboratory (Im *et al.*, 1990).

The FVA is a retrovirus which stimulates proliferation of erythroid progenitor cells in mice. However, the progenitor cells are unable to differentiate in the FVA infected mice and accumulate in the spleen resulting in splenomegaly. The weight of the spleens of FVA infected mice becomes more than 10-times larger than that of normal mice in 2~3 weeks. Since the antiretroviral effects of various agents on the FVA infection can be readily determined by simply weighing the spleens of FVA infected mice, this system could be used as an in vivo screening system for potential antiretroviral agents.

Currently, 2',3'-dideoxy nucleosides (ddNs) are being used as antiretroviral agents for the treatment of human immunodeficiency virus (HIV) which causes acquired immunodeficiency syndrome (AIDS) (Sandstorm *et al.*, 1989; Yarchoan *et al.*, 1989; Hirsch and Kaplan, 1989; Mitsuya *et al.*, 1990). ddC is one of the most potent dideoxynucleosides tested against the infection of HIV (Yarchoan *et al.*, 1988; Mitsuya *et al.*, 1987; Mitsuya *et al.*, 1988; Starnes *et al.*, 1987). rIFN-α-A is also an antiretroviral agent against HIV (Hartshorn *et al.*, 1987; Ho *et al.*, 1985). The site of ddC action is known to be at reverse transcriptase leading to DNA chain termination (Yarchoan *et al.*, 1989), but that of rIFN-α-A action is known to be at later stages of viral replication. The inhibition of viral replication by rIFN-α-A is brought by the alteration of the viral assembly and release (Pitha *et al.*, 1981; Aboud *et al.*, 1983). In this study, FVA inoculated mice were used as an *in vivo* model and the activities of ddC and rIFN-α-A were tested individually and in combination to find out whether the combined administration of two antiretroviral agents that act by different mechanisms can increase the efficacy of the drugs.

It has been reported that Epo promotes Ca^{++} uptake in the erythroid precursor cells (Sawyer *et al.*, 1984; Miller *et al.*, 1988) and Ca^{++} modulates the commitment of erythroid cells to differentiation (Levenson *et al.*, 1980; Bridges *et al.*, 1981), but ddC and ddNs inhibit erythropoiesis (Johnson *et al.*, 1988; Molina *et al.*, 1989), and that adenosine receptor agonists inhibit Ca^{++} uptake in various tissues (Schrader *et al.*, 1975). Therefore, in order to see wether erythroid progenitor cells that accumulate in FVA-infected mice in spite of ddC treatment are altered with respect to untreated FVA-infected progenitor cells, Ca^{++} uptake as a parameter, we investigated the effect of ddC treatment on the Ca^{++} uptake in the erythroid progenitor cells, the effect of cyclohexyladenosine (CHA) on the Ca^{++} uptake, and the binding of CHA to the progenitor cells and mouse erythrocytes.

In this communication we report the suppression of retroviral infection by ddC and rIFN-α-A, the Ca^{++} uptake in the erythroid progenitor cells, and the characterization of CHA binding to the erythroid cells.

MATERIALS AND METHODS

Materials

ddC, CHA, theophylline, bovine serum albumin and Iscove's modified Dulbecco's medium (IMDM) were purchased from Sigma Chemical Company (St. Louis, MO). [^{3}H]CHA (specific radioactivity, 34.4 Ci/mmol) and $^{45}Ca^{++}$ (specific radioactivity, 23.7 mCi/mg) were from duPont-New England Nuclear (Boston, MA). rIFN-α-A was a generous gift from Hoffmann-LaRoche, Inc. (Nutley, NJ).

Effects of ddC and rIFN-α-A on the FVA infection

Male BALB/c mice (23-25 g) were used. Plasma containing FVA was diluted to 1:25 with saline solution and 0.2 ml aliquots were injected to mice through tail veins. For the ddC injection group, ddC (100 mg/kg body weight) and rIFN-α-A (10 KU/mouse), as a single agent and in combination, were intraperitoneally administered daily for 18 days starting one day prior to FVA injection. Saline was administered to the control group of FVA-injected mice. For the ddC drinking group, ddC (0.1 mg/ml) was administered via drinking water ad libitum, and rIFN-α-A (10 KU/mouse) was intraperitoneally injected daily to ddC (0.1 mg/ml) drink-

ing mice for 18 days. After 18 days, the mice were sacrificed, and then spleen weights, hematocrits, hemoglobin contents and body weights were determined. For the determination of hematocrits and hemoglobin contents, blood was collected from the abdominal veins. Hemoglobin contents were determined by measuring absorbance at 527 nm.

Preparation of mice erythrocytes and erythroid progenitor cells

For the preparation of mice erythrocytes, blood was collected from the abdominal veins of mice, and erythrocytes were washed twice with ice cold saline containing 1% bovine serum albumin by centrifugation. Erythroid progenitor cells were prepared from the spleens of FVA infected mice as previously described (Kim *et al.*, 1989a; Kim *et al.*, 1991b).

$^{45}Ca^{++}$ uptake in erythroid cells

In order to determine whether ddC treatment of FVA injected mice alters the Ca^{++} uptake in the erythroid progenitor cells, 20 mg/kg body weight, and 100 mg/kg body weight of ddC were administered to FVA injected BALB/c mice by daily intraperitoneal injection for 3 weeks. After 3 weeks, the mice were sacrificed and erythroid progenitor cells were prepared from the spleens as above. To compare the Ca^{++} uptake in the erythroid progenitor cells with that in mice erythrocytes, erythrocytes were prepared from the blood of normal mice the same way as above, and Ca^{++} uptake was assayed.

For the time course of Ca^{++} uptake into the cells, 200 μl aliquots of cells (2×10^7 cells) in IMDM containing 1% bovine serum albumin were cooled in an ice bath for 15 min and 100 μl of $^{45}Ca^{++}$ (25 μCi/ml) was added. The samples were incubated at 4℃ for 0~60 min. After the incubation, 2 ml of dibutylphthalate was added and centrifuged at 800 ×g for 10 min at 4℃. After the upper layer of the sample was aspirated off, the tubes were carefully washed twice with distilled water, and then dibutylphthalate layer was aspirated off and the inside of the tube was wiped with moistened cotton swabs. The cell pellet was lysed with 0.5 ml of 0.2 M NaOH, neutralized with 60 μl of 2 M HCl, and the radioactivity was determined in a scintillation counter.

For the determination of the effects of CHA and theophylline on the Ca^{++} uptake, 200 μl aliquots of cells (2×10^7 cells) in IMDM containing 1% bovine serum albumin were preincubated for 50 min at 37℃ with 10 μM of CHA or 10 μM of CHA plus 10 μM of theophylline. The preincubated samples were cooled in an ice bath for 15 min. After $^{45}Ca^{++}$ was added to the cooled samples, the samples were incubated for 50 min at 4℃ and the $^{45}Ca^{++}$ uptake was determined the same way as above.

[^{3}H]CHA biding assay

IMDM was used as the medium for the binding of [^{3}H]CHA to the erythroid cells. For the time-course of [^{3}H]CHA binding to the erythroid cells, 100 μl of [^{3}H]CHA was added to 200 μl aliquots of erythroid cells (2×10^7 cells) and the samples were incubated at 37℃ for the designated time period in the presence (non-specific binding) and absence (total binding) of 500 μM of unlabeled CHA in a final volume of 500 μl. After incubation, the samples were chilled on ice and centrifuged at 800 ×g for 10 min at 4℃. The cells were washed twice with 1 ml of cold IMDM by centrifugation. The cell pellets were lysed with 0.5 ml of 0.2 M NaOH, neutralized with 60 μl of 2 M HCl and the radioactivity of the samples was determined in a scintillation counter. Specific binding was calculated by subtracting the non-specific binding from the total binding.

For the Scatchard analysis of [^{3}H]CHA binding to the erythroid cells, 100 μl of [^{3}H]CHA and 200 μl of solution containing different amount of unlabeled CHA were added to 200 μl aliquots of erythroid cells (2×10^7 cells). The samples were incubated for 30 min at 37℃ and then centrifuged at 800 ×g for 10 min at 4℃. To determine the free CHA concentration, 20 μl aliquot of the supernatant was withdrawn and the radioactivity was determined in a scintillation counter. For the determination of the amount of CHA bound to the cells, the cell pellets were lysed with 0.6 ml of 0.2 M NaOH. After neutralizing the lysed samples with 60 μl of 2 M HCl, the radioactivity of the samples

Table 1. Effects of the treatment of FVA inoculated mice with ddC and rIFV-α-A on the spleen weight, hematocrit, hemoglobin content, and body weight. Data represent mean±S.D. (n=9)

Group	FVA Injection	Treatment	Spleen weight(g)	% Inhibition[7]	Hematocrit(%)	Hemoglobin (g/dl)	Body weight(g)
Normal	−	−	0.110±0.011	−	48.2±2.4	17.0±1.1	24.6±1.7
Control	+	Saline[1]	1.002±0.112	−	35.2±3.0	13.8±1.4	24.3±1.2
ddC	+	ddC[2]	0.867±0.082	15.1	37.3±3.7	15.0±0.9	25.1±2.1
rIFN-α-A	+	rIFN-α-A[3]	0.532±0.055	52.7	38.5±1.8	15.6±1.7	24.1±1.7
ddC+rIFN-α-A	+	ddC+rIFN-α-A[4]	0.453±0.043	61.6	41.7±2.0	16.5±0.7	25.9±1.2
ddC (oral)	+	ddC[5]	0.660±0.070	38.4	39.1±2.6	15.6±1.2	24.9±1.0
ddC+rIFN-α-A (oral)	+	ddC+rIFN-α-A[6]	0.260±0.021	83.2	41.2±2.0	16.5±0.7	24.8±1.6

[1]Salind; [2]ddC (100 mg/kg body weight); [3]rIFN-α-A (10 KU/mouse); [4]ddC(100 mg/kg body weight) plus rIFN-α-A (10 KU/mouse) were administered by I.P. injection; [5]ddC (0.1 mg/ml) in drinking water; [6]ddC (0.1 mg/ml) in drinking water plus daily I.P. injection of rIFN-α-A (10 IU/mouse); [7]% iinhibition was calculated by the following: % inhibition=(1-(spleen weight-0.110)/(1.002-0.110))×100.

was determined in a scintillation counter. The data were plotted according to Scatchard analysis (Scatchard, 1949).

RESULTS

Effects of ddC and rIFN-α-A on FVA infection in mice

The effects of ddC and rIFN-α-A on FVA infection were determined by measuring the weights of the spleens 18 days after ddC, rIFN-α-A, and the combination of the drugs were administered either by daily intraperitoneal injection, or by giving ddC in drinking water and daily intraperitoneal injection of rIFN-α-A to ddC drinking mice which were inoculated with FVA. A typical result of two experiments is shown in Table 1. Compared to uninfected normal mice, after 18 days, FVA infected mice had about 9 times larger spleens. Daily administration of ddC (100 mg/kg body weight), rIFN-α-A (10 KU/mouse), and the combination of both drugs by intraperitoneal injection to FVA inoculated mice, resulted in suppression of spleen growth by 15.1%, 52.7% and 61.6%, respectively. When ddC (0.1 mg/ml) was administered via drinking water, and rIFN-α-A (10 KU/mouse) was administered by daily intraperitoneal injection to the ddC drinking mice for 18 days, the spleen growth was inhibited by 38.4% and 83.2%, respectively. These data show that the administration of ddC via drinking water is more than two fold as effective as the daily intraperitoneal injection of ddC and that the combined effect of ddC and rIFN-α-A is not synergistic but additive. Table 1 shows that both hematocrit and hemoglobin content are significantly lower in the FVA infected control mice than in the uninfected normal mice. Treatment of FVA inoculated mice with ddC and rIFN-α-A increased both hematocrit and hemoglobin content. Increase of hematocrit and hemoglobin content was greatest in the FVA inoculated mice which were treated with combination of ddC and rIFN-α-A. No significant difference in body weight was seen among uninfected normal mice, FVA infected mice and FVA inoculated mice treated with ddC and rIFN-α-A.

Effects of CHA and theophylline on the Ca^{++} uptake in mouse erythrocytes and erythroid progenitor cells

Time course of Ca^{++} uptake in the erythroid progenitor cells and mouse erythrocytes was studied. Ca^{++} uptake in the erythroid progenitor cells reached the plateau in about 50 min

Table 2. Ca^{++} uptake in mouse erythrocytes and erythroid progenitor cells. Data represent mean±S.D. of triplicate

Cells	Treatment	$^{45}Ca^{++}$ uptake		
		Control nmol/2×7^{7} cells	CHA (10 μM)	CHA+Theophyline (10 μM+10 μM)
Erythrocyte	−	0.218±0.028 (100%)	0.210±0.02 (96%)	0.216±0.032 (99%)
Progenitor cells	Saline*	4.88±0.4 (100%)	4.20±0.16 (86%)	4.62±0.26 (95%)
Progenitor cells	ddC* (20 mg/kg)	5.04±0.22 (100%)	4.38±0.26 (87%)	4.84±0.06 (96%)
Progenitor cells	ddC* (100 mg/kg)	5.28±0.18 (100%)	4.02±0.32 (76%)	5.02±0.16 (95%)

*Saline or ddC were administered daily to FVA infected mice by I.P. for 3 weeks.

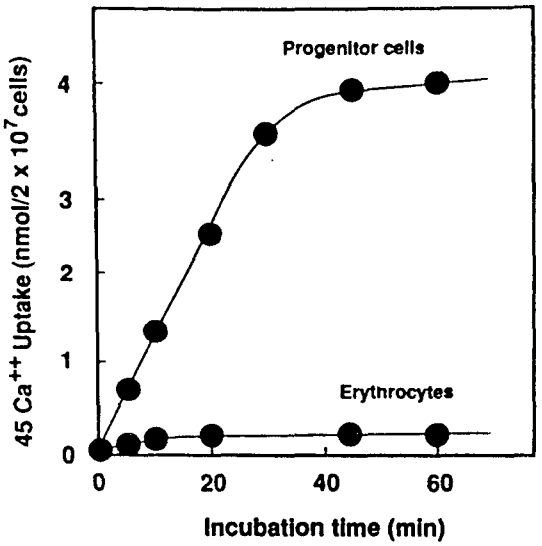

Fig. 1. Time-co urse of Ca^{++} uptake in the erythroid progenitor cells and mouse-erythrocytes. Aliquots (200 μl) of cells (2×10^7 cells) in IMDM containing 1% bovine serum albumin were chilled on ice and 100 μl of Ca^{++} (25 μCi/ml) was added. After the samples were incubated at 4°C, 2 ml of dibutylphthalate was added and centrifuged, the supernatant was aspirated off and the tube was carefully washed with distilled water. Then the dibutylphthalate was completely removed by suction, and the inside walls of the tubes were wiped with moistened cotton swabs. The cell pellet was lysed with 0.6 ml of 0.2 M NaOH, neutralized with 2 M HCl, and then the radioactivity was determined in a scintillation counter.

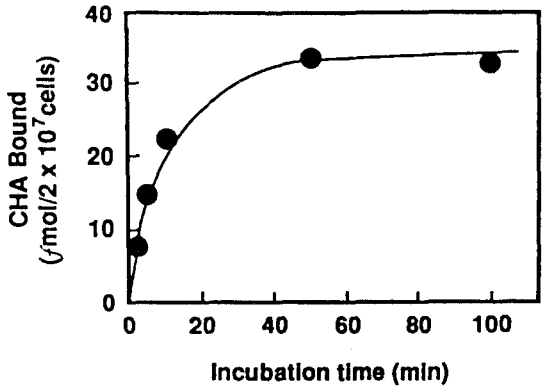

Fig. 2. Time-course of [^{3}H]CHA binding to erythroid progenitor cells. [^{3}H]CHA (100 μl) was added to 200 μl aliquot of erythroid progenitor cells (2×10^7 cells) in IMDM and the samples were incubated at 37°C in the presence (non-specific binding) and absence (total binding) of 500 μM of unlabeled CHA in a final volume of 500 μl. After incubation, the samples were chilled on ice and centrifuged at 800×g for 10 min at 4°C. The cells were washed twice with 1 ml of cold IMDM by centrifugation. The cell pellets were lysed with 0.6 ml of 0.2 M NaOH, neutralized with 2 M HCl and the radioactivity of the samples was determined in a scintillation counter. Specific binding was calculated by subtracting the non-specific binding from the total binding.

at 4°C, and the Ca^{++} uptake in the erythroid progenitor cells was more than 20-fold greater than in the erythrocytes (Fig. 1). Table 2 shows the effect of CHA, and CHA plus theophylline in the Ca^{++} uptake in the erythrocytes and the erythroid progenitor cells which were obtained from FVA infected mice without or with ddC treatment. CHA had almost no effect on the Ca^{++} uptake in the erythrocytes, but Ca^{++} uptake in the erythroid progenitor cells was partially inhibited by CHA. The inhibition was most pronounced in the progenitor cells from FVA infected mice which were treated with 100 mg/(kg body weight) of ddC. Theophylline obviated the inhibitory activity of CHA. These results suggest that treatment of FVA infected mice with ddC alters the property of the erythroid progenitor cells for the Ca^{++} uptake, and that adenosine receptors may play a role in the regulation of Ca^{++} uptake in the erythroid progenitor cells.

Binding of CHA to the erythroid progenitor cells

The time course of CHA binding to the

Table 3. Dissociation constants and maximum binding of [^{3}H]CHA to mouse erythrocytes and erythroid progenitor cells. Data represent mean±S.D. of triplicate

Cells	Low or high affinity binding	Kd(nM)	Bmax*
Erythrocyte	Low	16.6±1.3	$4.8\pm0.9\times10^5$
Progenitor cells	High	0.56±0.3	$5.5\pm0.5\times10^4$
	Low	73.3±0.71	$4.2\pm0.4\times10^5$

*Bmax: Maximum number of binding sites per cell.

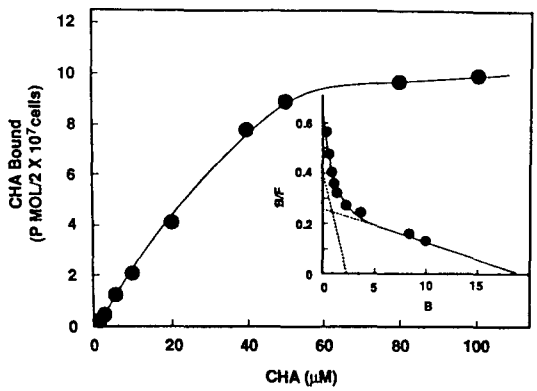

Fig. 3. Characterization of [^{3}H]CHA binding to erythroid progenitor cells. To 200 μl aliquots of erythroid progenitor cells, 100 μl of [^{3}H]CHA and 200 μl of IMDM containing varying amounts of unlabeled CHA were added and the samples were incubated for 30 min at 37°C. The incubated samples were chilled on ice and centrifuged at 800 ×g for 10 min at 4°C. To determine the free CHA concentration, 20 μl aliquot of the supernatant was withdrawn and the radioactivity was determined in a scintillation counter. For the determination of the amount of CHA bound to the cells, the remaining supernatant was aspirated off and the cell pellets were lysed with 0.6 ml of 0.2 M NaOH, neutralized with 2 M HCl and the radioactivity of the samples was determined in a scintillation counter. The data are plotted according to Scatchard (inset).

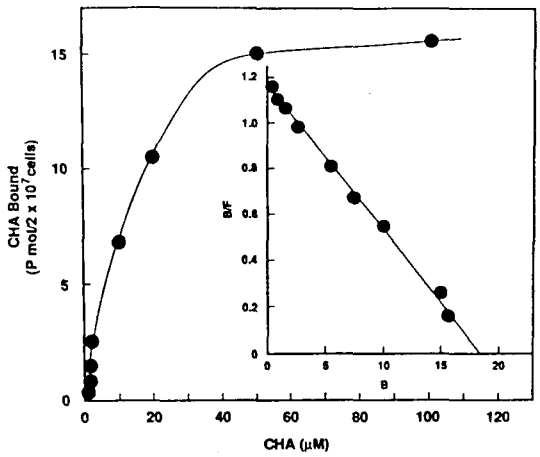

Fig. 4. Characterization of [^{3}H]CHA binding to mouse erythrocytes. Experimental conditions were the same as for Fig. 3.

erythroid progenitor cells showed that the binding reached the plateau in about 40 min at 37°C (Fig. 2). The saturation binding of CHA to the erythroid progenitor cells was attained at 60 μM CHA (Fig. 3), and Scatchard analysis of the binding revealed that the progenitor cells contain high and low affinity binding sites (Fig. 3 inset). At the high affinity binding site, the Kd and the maximum number of binding sites per cell are 0.56 nM and 5.5×10^4, respectively. At the low affinity binding site, the Kd and the maximum number of binding sites per cell are 73.3 nM and 4.2×10^5, respectively.

Binding of CHA to mouse erythrocytes

CHA binding to mouse erythrocytes also

reached the plateau in about 40 min at 37°C (data not shown). The maximum binding of CHA to mouse erythrocytes reached at 50 μM CHA (Fig. 4). Scatchard analysis of the binding revealed that mouse erythrocytes have only the low affinity CHA binding sites (Fig. 4 inset). The Kd and the maximum number of CHA binding sites per cell are 16.6 nM and 4.8×10^5, respectively. The parameters of CHA binding to mouse erythrocytes and erythroid progenitor cells are summarized in Table 3.

DISCUSSION

With the expectation that the combined administration of antiretroviral drugs that act by different mechanisms may provide greater effects against retroviral infection, we investigated the effects of ddC and rIFN-α-A on the infection of FVA using FVA inoculated mice as an in vivo model. Our results (Table 1) show that the antiretroviral effect of the combined administration of the drugs is additive, not synergistic. It has been reported that the effect of the combination of ddC and rIFN-α-A on the in vitro HIV-1 replication is synergistic (Vogt *et al.*, 1988). Recently, it also showed that when 3'-azido-3'-deoxythymidine (AZT) and rIFN-α-A/D were administered in combination to Rouscher murine leukemia virus (RLV) inoculated mice, virus-induced splenomegaly was synergistically suppressed (Ruprecht *et al.*, 1990). It is not clear why ddC and rIFN-α-A inhibits HIV-1 replication synergistically, and AZT and rIFN-α-A/D synergistically suppress the splenomegaly in RLV infected mice, whereas ddC and rIFN-α-A suppress the spleen growth of FVA infected mice additively. Since the rate of anabolic conversion of ddC to 2',3'-dideoxycytidine-5'-triphosphate (ddCTP) is critically important for ddC to be an active antiviral agent (Cooney *et al.*, 1986) and the extent of the anabolism, thus the antiretroviral activity, of a certain ddN varies among cell lines (Waqar *et al.*, 1984; Balzarini *et al.*, 1988), perhaps the difference may be due to that the phosphorylation pattern of ddC in FVA infected mice may be different from that in HIV-1 cells and that of AZT in RLV infected mice. Alternatively, the action mechanism of rIFN-α-A in FVA infected mice may be different from those in HIV-1 cells and in RLV infected mice.

Table 1 shows that the administration of ddC via drinking water is about 2-fold more effective than by daily intraperitoneal injection against FVA infection. This may be attributed to the fact that ddC is rapidly cleared from plasma and the effective terminal half life ($t_{1/2}$) of ddC in mice is only 67 min, and that oral bioavailability of ddC in mice is very high (Kelley *et al.*, 1987). Because of the rapid elimination and lack of accumulation of ddC, frequent administration of ddC is required for more effective antiretroviral activity.

Our data (Table 1) show that the treatment of FVA infected mice with ddC and rIFN-α-A increases both hematocrit and hemoglobin content while suppressing the splenomegaly and increasing both hematocrit and hemoglobin content, and prevent the mice from becoming anemic. However, our recent data (unpublished results) show that rIFN-α-A strongly inhibits the Epo-mediated differentiation of the erythroid progenitor cells, and it has shown that ddC inhibits erythropoiesis (Johnson *et al.*, 1988). Therefore, the suppression of the splenomegaly and the increases in the hematocrit and the hemoglobin content by ddC and rIFN- -A are most likely due to the inhibition of FVA infection by the drugs.

It has been shown that adenosine receptor agonists inhibit Ca^{++} uptake in various tissues (Schrader *et al.*, 1975; Wu *et al.* 1982). Our results (Fig. 1 and Table 2) show that (i) Ca^{++} uptake in the erythroid progenitor cells is about 20-fold greater than in mouse erythrocytes; (ii) an adenosine A1 receptor agonist, CHA, inhibits the Ca^{++} uptake in the erythroid progenitor cells but CHA has almost no effect on the Ca^{++} uptake in mouse erythrocytes; and, (iii) the inhibition of Ca^{++} uptake by CHA is abolished by an adenosine antagonist theophylline. These suggest that the erythroid progenitor cells have functioning adenosine A1 receptors and the Ca^{++} uptake may be in part regulated by the receptor.

In contrast, mouse erythrocytes appear to have no significant number of functioning

adenosine A1 receptor. Table 2 also shows that the inhibition of Ca^{++} uptake by CHA is most pronounced in the erythroid progenitor cells from FVA infected mice which were treated with 100 mg/(kg body weight) of ddC. This suggests that the characteristic of the erythroid progenitor cells in terms of Ca^{++} uptake is altered by ddC treatment. Previously it was found that Epo stimulates the Ca^{++} uptake in erythroid progenitor cells and increases the intracellular Ca^{++} level of the cells (Sawyer and Krantz, 1984; Miller *et al.*, 1988). Based on the finding, it has been proposed that Ca^{++} metabolism may contribute to the differentiation of erythroid progenitor cells (Sawyer and Krantz, 1984). Since the Ca^{++} uptake in the erythroid progenitor cells is far greater than in mouse erythrocytes, it is plausible that Ca^{++} may play a role in the differentiation of the erythroid progenitor cells.

CHA is not only involved in the Ca^{++} uptake in the erythroid progenitor cells, but also known to inhibit the albuterol enhanced Epo production (Ueno *et al.*, 1988), and theophylline, an adenosine antagonist, attenuates Epo production (Bakris *et al.*, 1990).

Therefore, to see whether there is any difference between the binding of CHA to mouse erythrocytes and to erythroid progenitor cells, we carried out the characterization of CHA binding to both cell types. Our Data that the erythroid progenitor cells have both high and low affinity binding sites (Table 3, Fig. 3) but mouse erythrocytes have only the low affinity sites Table 3, Fig. 4), and that CHA inhibits the Ca^{++} uptake in the erythroid progenitor cells, but has almost no effect on the Ca^{++} uptake in the mouse erythrocytes (Table 2) suggest that the high affinity CHA binding sites in the erythroid progenitor cells may be involved in the inhibition of the Ca^{++} uptake.

REFERENCES

Aboud M and Hassan Y: *Accumulation and breakdown of RNA-deficient intracellular virus particles in interferon-treated NIH 3t3 cells chronically producing moloney murine leukemia virus. J Virol 45: 489-495 1983*

Bakris GL, Sauter ER, Hussey JL, Fisher JW, Gaber AO and Winsett R: *Effects of theophylline on erythropoietin production in normal subjects and in patients with erythrocytosis after renal transplantation. N Engl J Med 323: 86-90 1990*

Balzarini J, Pauwels R, Baba M, Herdewijin P, DeClercq E, Broder S and Johns DG: *The in vitro and in vivo anti-retrovirus activity, and intracellular metabolism of 3'-azido-2',3'-dideoxythymidine and 2',3'-dideoxycytidine are highly dependent on the cell species. Biochem Pharmacol 37: 897-903 1988*

Bridges K, Levenson R, Housman D and Cantley L: Calcium regulates the commitment of murine erythroleukemia cells to terminal erythroid differentiation. *J Cell Biol 90: 542-544 1981*

Cooney DA, Dalal M, Mitsuya H, McMahon JB, Nadkarni M, Balzarini J, Broder S and Johns DG: *Initial studies on the cellular pharmacology of 2',3'-dideoxycytidine, an inhibitor of HTLV-III infectivity. Biochem Pharmacol 35: 2065-2068 1986*

Hartshorn KL, Vogt MW, Chou T-C, Blumberg RS, Byington R, Schooley RT and Hirsh MS: *Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha A interferon. Antimicrob Agents Chemother 31: 168-172 1987*

Hirsch MS and Kaplan JC: *Treatment of human immunodeficiency virus infections. Antimicrob Agents Chemother 31: 839-843 1987*

Ho DD, Hartshorn KL, Rota TR, Andrews CA, Kaplan JC, Schooley RT and Hirsh MS: *Recombinant human interferon alpha A suppresses HTLV-III replication in vitro. Lancet 1: 602-604 1985*

Im JH, Lee SJ and Kim HD: *Partial purification and characterization of erythropoietin receptors from erythroid progenitor cells. Arch Biochem Biophys 278: 486-491 1990*

Johnson M, Caiazzo T, Molina JM, Donahue R and Groopman J: *Inhibition of bone marrow myelopoiesis and erythropoiesis in vitro by antiretroviral nucleoside derivatives. Br J Haematol 70: 27-141 1988*

Kelley JA, Litterst CL, Roth JS, Vistica DT, Poplack DG, Cooney DA, Nadkarni M, Balis, FM, Broder S and Johns: *The disposition and metabolism of 2',3'-dideoxycytidine, an in vitro inhibitor of human T-lymphotrophic virus type III infectivity, in mice and monkeys. Drug Metab Dispo 15: 595-601 1987*

Kim HD, Tsai Y-S, Lee SJ, Im JH, Koury MJ and Sawyer ST: *Metabolic development in erythropoietin-dependent maturation of erythroid cells; In the red cell: 7th Ann Arbor Conference, Progress in Clinical*

and Biological Research (ed GD Brewer.)Vol. 319, pp491-504, 1989a

Kim HD, Koury MJ, Lee SJ, Im JH and Sawyer ST: *Metabolic adaptation during erythropoietin-mediated terminal differentiation of mouse erythroid cells. Blood 77; 387-392 1991b*

Koury MT, Sawyer ST and Bondurant MC: *Splenic erythroblasts in anemia-inducing Friend virus: A source of cells for studies of erythropoietin mediated differentiation. J Cell Physiol 121: 526-532 1984*

Levenson R, Housman D and Cantley L: *Amiloride inhibits murine erythroleukemia cell differentiation; Evidence for Ca^{2+} requirement for commitment. Proc Natl Acad Sci USA 77: 5948-5952 1980*

Miller BA, Scaduto RC. Jr., Tillotson DL, Botti JJ, Cheung JY: *Erythropoietin stimulates a rise in intracellular free calcium concentration in single early human erythroid precursors. J Clin Invest 82: 309-315 1988*

Mitsuya H, Yarchoan R and Broder S: *Molecular targets for AIDS therapy. Science 249: 1533-1543 1990*

Mitsuya H, Jarrett RF, Matsukura M, Veronese FDM, DeVico AL, Sarngadharan MG, Johns DG, Reitz MS and Broder S: *Long-term inhibition of human T-lymphotropic virus type III/lymphadenopathy-associated virus (human immunodeficiency virus) DNA synthesis and RNA expression in T cells protected by 2',3'-dideoxynucleotides in vitro. Proc Natl Acad Sci USA 84: 2033-2037 1987*

Mitsuya H and Broder S: *Inhibition of infectivity and replication of HIV-2 SIV in helper T cells by 2',3'-dideoxynucleosides in vitro. AIDS Res Retroviruses 4: 107-113 1988*

Molina JM and Groopman JE: *Bone marrow toxicity of dideoxyinosine. N Engl J Med 321: 1478 1989*

Pitha PM, Bilello JA and Riggin CH: *Effect of interferon on retrovirus replication. Tex Rep Biol Med 41: 603-609 1981*

Ruprecht RM, Chou TC, Chipty F, Sosa MG, Mullaney S, O'Brien L and Rosas D: *Interferon- and 3'-azido-3'-deoxythymidine are highly synergistic in mice and prevent viremia after acute retrovirus exposure. J Acquired Immune Deficiency Syndr 3: 591-600 1990*

Sandstorm E: *Antiviral therapy in human immunodeficiency virus infection. Drugs 38: 417-450 1989*

Sawyer ST and Krantz SB: *Erythropoietin stimulates $^{45}Ca^{2+}$ uptake in Friendvirus-infected erythroid cells. J Biol Chem 259: 2769-2774 1984*

Scatchard GA: *The attractions of proteins for small molecules and ions. N.Y. Acad Sci 51: 660-672 1949*

Schrader J, Rubio R and Berne RM: *Inhibition of slow action potentials of guinea pig atrial muscle by adenosine; A possible effect on Ca^{2+} influx. J Mol Cell Cardiol 7: 427-433 1975*

Starnes MC and Cheng Y-C: *Cellular metabolism of 2',3'-dideoxycytidine, a compound active against human immunodeficiency virus in vitro. J Biol Chem 262: 988-991 1987*

Ueno M, Brookins J, Beckman B, Fisher JW: *A_1 and A_2 adenosine receptor regulation of erythropoietin production. Life Sci 43: 229-237 1988.*

Vogt MW, Durno AG, Chou T-C, Coleman LA, Paradis TJ, Schooley RT, Kaplan JC and Hirsch MS: *Synergistic interaction of 2',3'-dideoxycytidine and recombinant interferon- -A on replication of human immunodeficiency virus type 1. J Infect Dis 158: 378-385 1988*

Waqar MA, Evans MJ, Manly KF, Hughes RG and Huberman JA: *Effects of 2',3'-dideoxynucleosides on mammalian cells and viruses. J Cell Physiol 121: 402-408 1984*

Wu PH, Phillis JW and Thierry DL: *Adenosine receptor agonists inhibit K^+-evoked Ca^{2+} uptake by rat brain cortical synaptosomes. J Neurochem 39: 700-708 1982*

Yarchoan R, Mitsuya H, Myers CE and Broder S: *Clinical pharmacology of 3'-azido-2'3'-dideoxythymidine (zidovudine) and related dideoxynucleosides. N Engl J Med 321: 726-738 1989*

Yarchoan R, Perno CF, Thomas RV, Klecker RW, Allain J-P, Wills RJ, McAtee N, Fischl MA, Dubinsky R, McNeely MC, Mitsuya H, Pluda JM, Lawley TJ, Leuther M, Safai B, Collins JM and Broder S: *Phase I studies of 2',3'-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and altering with zidovudine (AZT). Lancet 1: 76-80 1988*

=국문초록=

Anemia-inducing Murine Friend Virus 감염에 대한 2',3'-dideoxycytidine 및 Interferon-α-A의 항retrovirus효과

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Anemia-inducing strain of Friend virus (FVA)는 적혈구 progenitor cell의 증식을 촉진하는 생쥐 retrovirus의 일종이다. FVA에 감염된 생쥐는 생성이 촉진된 progenitor cell이 분화되지 못하고 비장내에 축적되므로서 비장비대를 초래한다. 이에 본 실험에서는 FVA에 감염된 생쥐의 비장비대를 지표로 사용하여 2',3'-dideoxycytidine (ddC) 및 interferon-α-A (rIFN-α-A)의 항retrovirus효과를 측정하였다. 매일 ddC (100 mg/kg) 및 rIFN-α-A (10 KU /mouse)를 각각 단독 또는 병용하여 18일간 복강내 투여시 비장의 비대가 각각 15.1%, 52.7%, 61.6% 억제되었다. 또 다른 실험군으로 ddC를 식수중에 용해하여 (0.1 mg/ml) 경구로 18일간 투여시, 그리고 ddC의 경구투여와 병용하여 rIFN-α-A을 위와 마찬가지 용량으로 복강내 투여시, 비장비대를 각각 38.4% 및 83.2% 억제하였다. 이 결과는 ddC의 투여시 복강내 주사보다는 경구투여가 더 유효하며, ddC와 rIFN-α-A는 병용투여시 상가적인 효과가 있음을 제시한다.

ddC 투여시 progenitor cell의 특성상 변화를 검토하기 위해, Ca^{++} uptake [^{3}H]cyclohexyladenosine (CHA) bindng 실험을 실시하였다. CHA bindng 실험결과 성숙된 적혈구에서는 저친화성의 결합부위 하나뿐인데 반해, progenitor cell에서는 고친화성과 저친화성의 두가지 결합부위를 나타내었다. Ca^{++} uptake 측정결과 성숙된 적혈구에 비해 대조군의 정상적인 progenitor cell은 약 20배 증가를 나타내었으며, ddC를 연속투여한 군에서도 유사한 결과를 나타내었다. 이때 CHA에 의한 Ca^{++} uptake의 억제효과를 측정한 바, ddC 100 mg/kg 투여군의 경우 76%로 대조군의 86% 보다 억제효과가 크게 나타났으며, 이들 모두는 adenosine 길항약인 theophylline의 전처치시 대조군과 유사하게 회복되었다.