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PRELIMINARY COMMUNICATIONS

ENHANCEMENT OF THE BIOLOGICAL ACTIVITY OF CORDYCEPIN (3'-DEOXYADENOSINE) BY THE ADENOSINE DEAMINASE INHIBITOR 2'-DEOXYCOFORMYCIN

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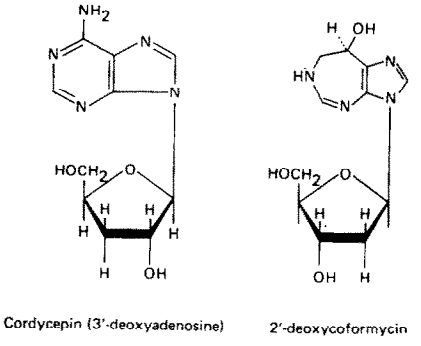
Cordycepin (3'-deoxyadenosine) is a nucleoside antibiotic originally isolated from the culture broth of Cordyceps militaris (LINN.) Link. by Cunningham and his co-workers in 1951 (1). The compound is rapidly converted in vivo to the corresponding mono-, di- and tri-phosphate forms, and exhibits growth-inhibitory properties in both bacterial and mammalian test systems (2). In addition, cordycepin is an effective inhibitor of viral replication, an effect due to its ability to block poly(A) synthesis and thus interfere with the processing and maturation of both cellular and viral mRNA (3). In vivo, however the effectiveness of cordycepin as an antibacterial, antitumor and antiviral agent is limited because of the rapid deamination of the compound to yield 3'-deoxyinosine; the reaction is catalyzed by the widely distributed enzyme adenosine deaminase (4).

From these earlier studies, it is evident that inhibition of the adenosine deaminase-catalyzed inactivation of cordycepin could permit its more efficient conversion to the biologically active nucleotide forms, and thus offer the prospect of enhancing the pharmacological activity of this agent. The purpose of the present communication is to describe the effect of the recently reported adenosine deaminase inhibitor 2'-deoxycoformycin (5) (Fig. 1) on the enzyme-catalyzed conversion of cordycepin to 3'-deoxyinosine and on the antitumor activity of cordycepin in cell culture systems and in vivo.

Adenosine deaminase (Type I) from calf intestinal mucosa, activity 255 units/mg protein, and cordycepin were purchased from Sigma Chemical Co. 2'-Deoxycoformycin was obtained from the Drug Research and Development Branch, National Cancer Institute. For studies of the inhibitory effect of 2'-deoxycoformycin on the adenosine deaminase-catalyzed deamination of cordycepin, the conversion of cordycepin to 3'-deoxyinosine was monitored at 265 nm; the value used for the molar absorbance change was $-8600\ M^{-1}\ cm^{-1}$ (4). For assay of adenosine deaminase activity in murine L1210 cells, 5-day ascites cells were harvested, erythrocytes lysed by osmotic shock, and the cells suspended in 3 volumes of hypotonic phosphate-buffered saline, pH 7.4, and homogenized utilizing a Potter-Elvehjem homogenizer with Teflon pestle. After centrifugation for 30 min at 26,000 x g, the



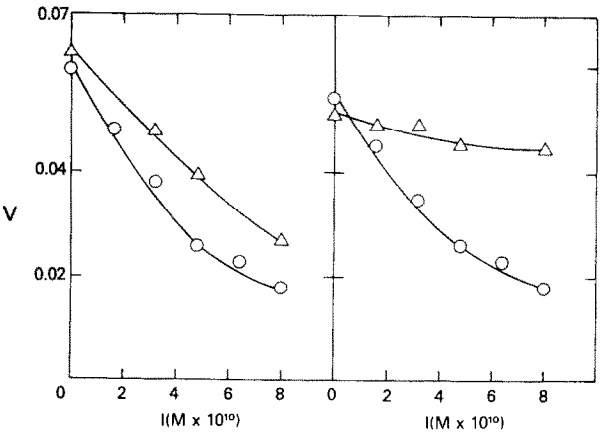


Fig. 1 (left): Structural formulas of cordycepin and 2'-deoxycoformycin. Fig. 2 (right): Inhibition by 2'-deoxycoformycin of the adenosine deaminase-catalyzed conversion of cordycepin to 3'-deoxyinosine. Cuvettes contained potassium phosphate buffer, pH 7.4, 50 µmoles; cordycepin, 0.06 µmole; and enzyme and inhibitor as indicated, in a total volume of 1 ml. V = Δ A/min at 265 nm, 37°. I = concentration of 2'-deoxycoformycin. Fig. 2A (left): Comparison of inhibitory activity of 2'-deoxycoformycin against adenosine deaminase from calf intestine and from L1210 murine leukemia cells. The inhibitor was incubated with the enzyme for 5 min prior to the addition of substrate. Δ: Calf intestinal adenosine deaminase, 0.0073 units; O: Adenosine deaminase from L1210 leukemia cell extract, 0.0070 units. Fig. 2B (right): Effect of preincubation with enzyme on inhibitory activity of 2'-deoxycoformycin. Cuvettes contained adenosine deaminase from L1210 leukemia cell extract, 0.0060 units. Δ: Reaction started by the addition of enzyme. O: Inhibitor incubated with enzyme for 5 min prior to the addition of substrate.

supernatant was utilized immediately for enzyme assays. Specific activity ranged from 0.07 to 0.11 µmole cordycepin deaminated/min/mg extract protein. Kinetic studies were carried out at 37°, utilizing a Gilford multiple sample absorbance recorder. Details of reaction conditions are given in the legend for Fig. 2.

When compared under the same reaction conditions, 2'-deoxycoformycin was found to be almost equally effective as an inhibitor of crude adenosine deaminase activity in the supernatant fraction from L1210 ascites cells and of highly purified adenosine deaminase from calf intestine (Fig. 2A); when the inhibitor was preincubated with enzyme for 5 min prior to addition of cordycepin, the I_{50} values observed with these two enzyme preparations were 4.5×10^{-10} and 6.9×10^{-10} respectively. Under these conditions, the Michaelis constant for cordycepin was 90 µM. Preincubation of enzyme and inhibitor was found to be essential for maximal inhibitory activity (Fig. 2B). In the light of the latter observation, 2'-deoxycoformycin was given 10 minutes prior to the administration of cordycepin in the in vivo studies described below.

To determine the effects of cordycepin, 2'-deoxycoformycin, and combinations of these two agents in mammalian cell culture systems, the compounds were evaluated for growth inhibitory effects against the murine leukemia cell lines L1210 and P388 and the human lymphoblastic leukemia cell line CEM; experimental conditions are described in Table 1. When added together, cordycepin and 2'-deoxycoformycin resulted in marked inhibition of cell growth at the lowest doses studied (cordycepin 0.1 µg/ml and

2'-deoxycoformycin 1 μg/ml)(Table 1). When cordycepin was added alone, a drug concentration three to four orders of magnitude higher (10 - 100 μg/ml) was required to produce a comparable effect. 2'-Deoxycoformycin when used alone at levels of 1 μg/ml and 10 μg/ml was without growth inhibitory effect.

Table 1. EFFECT OF 2'-DEOXYCOFORMYCIN (2'-DCF; 1 μg/ml) ON INHIBITION BY CORDYCEPIN OF GROWTH OF THREE MAMMALIAN TUMOR CELL LINES IN SUSPENSION CULTURE*

	Percent inhibition of cell growth at 48 hr					
Cordycepin Concentration (μg/ml)	L1210 Minus 2'-DCF	L1210 Plus 2'-DCF	P388 Minus 2'-DCF	P388 Plus 2'-DCF	CEM Minus 2'-DCF	CEM Plus 2'-DCF
0.1	19	77	< 1	62	4	59
1	24	77	< 1	82	< 1	74
10	45	80	39	84	32	93
100	85	82	82	83	66	93

*Cordycepin and 2'-deoxyadenosine in physiological saline, 0.1 ml, were added to cells in log phase growing in 5 ml volume of RPMI 1630 (L1210 and P388 cells) or 1640 medium (CEM cells) with 10% fetal calf serum. 2'-Deoxycoformycin alone (1 and 10 μg/ml) showed no effect on cell growth. Cells were counted at 24 and 48 hr; all experiments were performed at least twice in duplicate, with the values shown being mean values at 48 hr from duplicate culture bottles from a single representative experiment. Initial (zero time) cell counts were 10^5 L1210 cells/ml, 2 x 10^5 P388 cells/ml and 3 x 10^5 CEM cells/ml. Final (48 hr) cell counts in control (saline-treated) cultures in the experiment shown were 9.1 x 10^5 L1210 cells/ml, 16.0 x 10^5 P388 cells/ml and 12.5 x 10^5 CEM cells/ml.

For the assessment of antitumor activity in vivo, cordycepin, 2'-deoxycoformycin, and combinations of the two agents were evaluated in CDF_1 male mice bearing the P388 leukemia (Table 2). Cordycepin alone was only marginally active in this test system, but resulted in significant prolongation of survival when administered in combination with 2'-deoxycoformycin. 2'-Deoxycoformycin alone was without antitumor activity.

Table 2. EFFECT OF 2'-DEOXYCOFORMYCIN (2'-DCF: 0.5 mg/kg) ON ANTITUMOR ACTIVITY OF CORDYCEPIN IN MALE CDF_1 MICE BEARING P388 ASCITES LEUKEMIA*

Drug	Cordycepin dose range tested (mg/kg)	Optimal daily dose (mg/kg)	Mean survival at optimal dose (days±S.E.)
Controls (saline)	-	-	11.4±0.3
Controls (2'-DCF)	-	-	11.0±0.4
Cordycepin	1.25 - 300	25	14.5±0.8
Cordycepin + 2'-DCF	1.25 - 300	2.5	21.0±0.6

*Mice received 10^6 tumor cells i.p. Drug treatment was started 24 hr later by the i.p. route and was continued once daily for 10 days. In experiments with cordycepin: 2'-DCF combinations, 2'-DCF was administered 10 min prior to cordycepin. Treatment groups consisted

of 10 mice each. The experiment was repeated 3 times, with the results shown being a single representative experiment.

Discussion. The unusually potent inhibitory activity of 2'-deoxycoformycin toward adenosine deaminase (Fig. 2A), and the time-dependent increase in inhibition observed on preincubation of the enzyme and inhibitor (Fig. 2B), are similar to the results obtained by Cha and his co-workers (6) in a careful and detailed study of the kinetics of inhibition of human erythrocytic adenosine deaminase by the closely related compound coformycin, utilizing adenosine rather than cordycepin as substrate. As the latter investigators point out, the non-steady state nature of the inhibition by coformycin when studied under conventional enzyme assay conditions, and the unusually high inhibitory potency of the compound, render the determination of meaningful inhibition constants difficult. On the basis of the present studies, however, 2'-deoxycoformycin, with I_{50} values in the range 10^{-10} to 10^{-9}M, appears to be at least as potent an inhibitor of adenosine deaminase as is coformycin.

Plunkett and Cohen (7) recently showed that the toxicity of cordycepin toward L-cells in culture is enhanced significantly by the addition of the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl) adenine; in vivo studies with the latter combination were not reported. In the present studies, utilizing the combination of cordycepin and the considerably more potent adenosine deaminase inhibitor 2'-deoxycoformycin, we have been able to demonstrate not only inhibition of the deamination of cordycepin in vitro, but also increased activity of this agent in an experimental tumor system in vivo. Furthermore, since 2'-deoxycoformycin retains its potent adenosine deaminase-inhibitory activity in vivo, it offers the possibility of significant enhancement of the pharmacological activity not only of cordycepin as reported here, but also of several other adenosine analogs, whose in vivo activity is presently limited by rapid metabolic inactivation.

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