POTENTIATION OF CYTOTOXICITY AND ANTITUMOR ACTIVITY OF ADENOSINE ANALOGS BY THE ADENOSINE DEAMINASE INHIBITOR ADECYPENOL

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The enzyme adenosine deaminase (ADA) (adenosine aminohydrolase, EC 3.5.4.4), which is widespread in mammalian tissue, is involved in the regulation of the intracellular levels of adenosine and deoxyadenosine, which control a number of important physiological functions and serve as precursors of nucleic acid biosynthesis. ADA inhibitors such as deoxycoformycin are considered to be responsible for alterations in adenosine and deoxyadenosine levels, lymphocytic growth and functions, and also to enhance the chemotherapeutic effects of adenosine analogs which serve as a substrate of ADA[1].

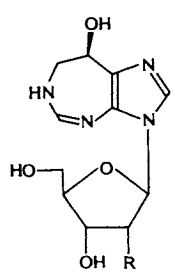
Recently, we found two new ADA inhibitors, adechlorin (2'-chloropentostatin)[2] and adecypenol[3,4] during the screening work for new ADA inhibitors from soil actinomycetes. In this paper, we describe the potentiation by adecypenol of the cytotoxicities of deoxyadenosine and Ara-A (vidarabine) and of the *in vivo* antitumor activity of Ara-A against L1210 leukemia in mice.

The cytotoxicity of adenosine analogs against HeLa S3 cells was examined by the usual method. The cells were grown in a medium supplemented with 10% calf serum. Adecypenol was not cytotoxic to the cultured cells even at 20 μg/ml, however, at 5 μg/ml it potentiated the cytotoxicity of adenine nucleosides as shown in Fig. 2. Deoxyadenosine at 20 μg/ml gave 28% inhibition of growth; on the other hand, 20 μg/ml of deoxyadenosine plus 5 μg/ml of adecypenol gave 90% inhibition. Fig. 3 shows that the mixture of deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine, each 80 μg/ml) reversed about a half of the inhibition by deoxyadenosine alone or deoxyadenosine plus adecypenol. These results coincide with the report that dATP, formed from deoxyadenosine and accumulated in the cells, serves as a feedback inhibitor of ribonucleotide reductase and causes a depletion of other deoxyribonucleotides, and as a result DNA synthesis decreases[5]. Thus, it is considered that the mixture of deoxyribonucleosides restores the DNA synthesis. Next, we examined the potentiation effect of adecypenol and deoxycoformycin on the cytotoxicity and antitumor activity of Ara-A which is used as a chemotherapeutic against Herpes virus disease. The 50% inhibitory concentration (IC_{50}) value of Ara-A against HeLa S3 cells was 27 μg/ml; however, it was decreased to 4.3 and 3.9 μg/ml in the presence of 0.28 μg/ml adecypenol and 0.03 μg/ml 2'-deoxycoformycin, respectively.

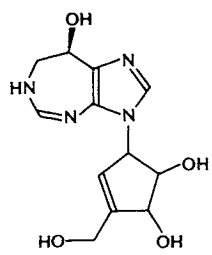
The antitumor activity of adecypenol and its ability to potentiate the antitumor effect of Ara-A were studied in L1210 leukemia in mice. Ara-A (100 mg/kg/day) or adecypenol (5 mg/kg/day) alone did not show significant antitumor activity. However, the combination of the drugs gave a greater extension of life span as shown in Table 1; for example, the optimal combination gave a 94% increase in life span (ILS).

Although the known ADA inhibitors, coformy-

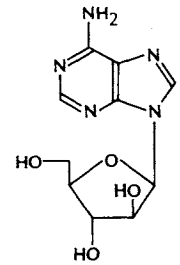
Fig. 1. Structures of adenosine deaminase inhibitors and Ara-A.



Coformycin R=OH
Deoxycoformycin R=H
Adechlorin R=Cl



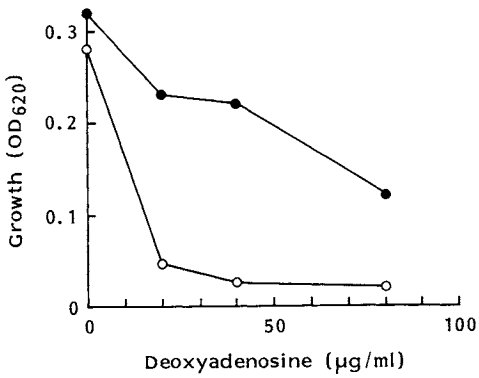
Adecypenol



Ara-A

Fig. 2. Potentiation of the cytotoxicity of deoxyadenosine by adecypenol.

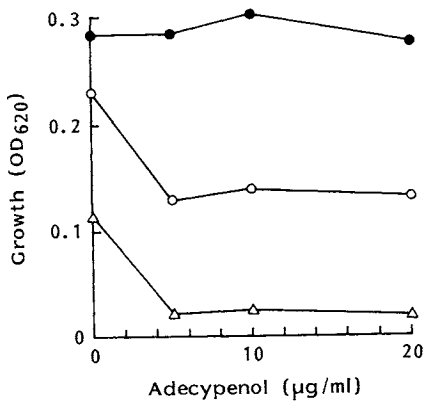
● None, ○ 5 μg/ml adecypenol.



A cell suspension (200 μl, 4×10^4 cells/ml) in EAGLE's minimal essential medium (Gibco) supplemented with 10% calf serum was dispensed into a well of a 96-well microplate and incubated at 37°C in a 5% CO_2-95% air atmosphere. After 1 day incubation a drug solution (5 μl) was added to the suspension. The incubation was continued for additional 3 days and then the cells were subjected to Giemsa stain. The OD_{620} was measured with a multiwell scanning spectrophotometer.

Fig. 3. Reversion of the cytotoxicity in the presence of deoxyadenosine and adecypenol by other deoxyribonucleosides.

● None, △ 80 μg/ml deoxyadenosine, ○ 80 μg/ml deoxyribonucleosides. Deoxyribonucleosides consisted of deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine.



cin, 2'-deoxycoformycin and adechlorin, have a sugar moiety (ribose, deoxyribose or 2'-chloro-2'-deoxyribose, respectively) and are tight-binding inhibitors of ADA[1,2], adecypenol has a cyclopentene moiety instead of a sugar and is a semi-tight-binding inhibitor[3,4]. The cyclopentene moiety is also a structural moiety of neplanocin A, a cyclopentenyl analog of adenosine, which is a specific inhibitor of RNA methylation but is not an RNA synthesis inhibitor and not utilized for transcription[6]. SIAW and COLEMAN[7] reported that deoxycoformycin is phosphorylated to give mono-, di- and triphosphate derivatives, and that the triphosphate derivative is incorporated into cellular DNA. It is considered that this may result in unusual organ toxicities which have been associated with the use of high levels of this drug[7]. Recently, 2'-deoxycoformycin therapy at low doses in hairy cell leukemia[8] and adult T-cell leukemia[9~11] has been reported to give good results.

Table 1. Combination effect of adecypenol and Ara-A on L1210 leukemia in mice.

Drug	Dose (mg/kg/day)	ILS (%)	Body weight (g)
Adecypenol	5	0	19.2
Ara-A	100	17	18.2
Adecypenol/Ara-A	5/100	Toxic	
	2/100	94	14.4
	0.4/100	53	17.2
	5/25	44	17.9
	2/25	42	19.4
	0.4/25	28	19.3

Mean survival time and weight at day-6 of control mice (5 mice in a group) was 7.2 ± 0.4 days and 20.6 ± 0.8 g. CDF_1 mice were injected intraperitoneally with 1×10^5 L1210 cells on day-0 and administered drugs intraperitoneally twice a day (9 a.m. and 6 p.m.) on days 1~3 and 5~8.

The above results indicate that the semi-tight-binding ADA inhibitor adecypenol is also effective in potentiating *in vivo* antitumor activity of Ara-A. Further *in vitro* and *in vivo* studies using adecypenol are of interest because it is expected that the compound is not phosphorylated and has low toxicity *in vivo*.

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