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.

Recently, we found two new ADA inhibitors, adechlorin (2'-chloropentostatin) and adecypenol during the screening work for new ADA inhibitors from soil actinomycetes. In this paper, we describe the potentiation effect of adecypenol on the cytotoxicity and antitumor activity of Ara-A which is used as a chemotherapeutic agent against Herpes virus disease. The 50% inhibitory concentration (IC50) 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, coformyc-
Fig. 2. Potentiation of the cytotoxicity of deoxyadenosine by adecypenol.

- None, ○ 5 μg/ml adecypenol.

![Graph showing growth (OD620) against deoxyadenosine (μg/ml)]

A cell suspension (200 μl, 4 × 10⁴ 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% CO₂ - 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 OD₆₂₀ 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, deoxyctydine and thymidine.

![Graph showing growth (OD620) against adecypenol (μg/ml)]

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg/day)</th>
<th>ILS (%)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adecybenol</td>
<td>5</td>
<td>0</td>
<td>19.2</td>
</tr>
<tr>
<td>Ara-A</td>
<td>100</td>
<td>17</td>
<td>18.2</td>
</tr>
<tr>
<td>Adecybenol/Ara-A</td>
<td>5/100 Toxic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/100</td>
<td>94</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>0.4/100</td>
<td>53</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>5/25</td>
<td>44</td>
<td>17.9</td>
</tr>
<tr>
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<td>42</td>
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<tr>
<td></td>
<td>0.4/25</td>
<td>28</td>
<td>19.3</td>
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</table>

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. CDF₁ mice were injected intraperitoneally with 1 × 10⁵ 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.

References


