II. ANTITUMOR ACTIVITY OF DYNEMICIN A AND ITS TRIACETYL DERIVATIVE

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Dynemicin A showed extremely potent in vitro cytotoxicity against a variety of murine and human tumor cells. In the experimental animal tumor models implanted ip with P388, L1210 leukemias and B16 melanoma cells, dynemicin A administered ip significantly prolonged life-span of tumor-bearing mice with the wide range of activity. This antibiotic administered iv was also active against iv implanted P388 and L1210 leukemias. In the macromolecule biosynthesis of B16 melanoma cells, dynemicin A inhibited DNA synthesis specifically. The triacetyl derivative exhibited similar in vitro and in vivo antitumor activities to those of the parent antibiotic.

Dynemicin A is a new antibiotic isolated from the culture broth of Micromonospora chersina sp. nov. M956-1 (ATCC 53710), which contains the 1,5-diyne-3-ene and anthraquinone subunit in the molecule. The isolation, chemical characterization, structure and some biological activities of dynemicin A have been reported previously1~3). Dynemicin A and its triacetyl derivative have been found to possess broad antimicrobial activity, especially potent against Gram-positive bacteria. On the whole, the latter was 2 to 8 times more active than the former1).

This paper describes the details of the in vitro cytotoxicity against murine and human tumor cells, in vivo antitumor activity in experimental animal tumor models and inhibition of macromolecule biosynthesis of dynemicin A and its triacetyl derivative.

Materials and Methods

Drug Preparation

Dynemicin A, triacetyldynemicin A and esperamicin A1 were prepared at Bristol-Myers Squibb Research Institute according to the procedures described in detail elsewhere4,5). For the in vitro and in vivo tests, they were dissolved in DMSO followed by dilution with sterile 0.9% NaCl or water. Doxorubicin (Kyowa Hakko), mitomycin C (Kyowa Hakko) and vincristine sulfate (Sigma) were used as a reference compound.

Animals

Female CDF1 (Balb/c × DBA/2, 6-week old) and male BDF1 (C57BL/6 × DBA/2, 5-week old) mice purchased from Japan SLC Inc. (Hamamatsu) were used for in vivo antitumor evaluations. Feed and water were provided ad libitum through experiments.

Tumors

In vitro tumor cell lines, B16-F10 (murine melanoma), HCT-116 (human colon carcinoma) and Moser (human colon carcinoma) were obtained from Pharmaceutical Research and Development
Division, Bristol-Myers Squibb Co. (Conn., U.S.A.). P388 (murine leukemia), P388/VCR (a resistant subline of P388 to vincristine), P388/ADM (a resistant subline to doxorubicin), K562 (human myelogenous leukemia) and K562/ADM (a resistant subline to doxorubicin) were kindly provided by Cancer Institute of the Japanese Foundation for Cancer Research (Tokyo). Lymphocytic leukemia P388, lymphoid leukemia L1210 and melanotic melanoma B16 which were used for in vivo experiments were generously provided by Kitasato Institute (Tokyo).

In Vitro Cytotoxicity

B16-F10 and Moser cells were grown in Eagle's minimum essential medium (Nissui), which contains kanamycin (60 μg/ml), supplemented with 10% heat-inactivated fetal calf serum (FCS, Nisseizai) and 2 mM L-glutamine, and HCT-116 cells were grown in McCoy's 5A medium (Gibco) supplemented with 10% FCS, 1.2 mM L-glutamine, essential and non-essential amino acids (Gibco), vitamins (Gibco) and antibiotics (100 μg/ml streptomycin and 100 μg/ml benzylpenicillin) at 37°C under humidified atmosphere in a 5% CO₂ incubator. For P388, P388/VCR, P388/ADM, K562 and K562/ADM, RPMI-1640 medium (Nissui) supplemented with 10% FCS, 2 mM L-glutamine and antibiotics (100 μg/ml streptomycin and 100 μg/ml benzylpenicillin) was used as a growing medium.

Exponentially growing cells were harvested, counted and suspended in the culture medium at 1.5 x 10⁴ (B16-F10), 2.5 x 10⁴ (Moser), 3.0 x 10⁴ (HCT-116), 1.3 x 10⁴ (P388, P388/VCR and P388/ADM), 8.0 x 10⁴ (K562 and K562/ADM) cells/ml, respectively. After planting into wells of a 96-well or 24-well tissue culture plate with test materials, they were incubated for 72 hours (B16-F10, Moser, HCT-116, P388, P388/VCR and P388/ADM) or 48 hours (K562 and K562/ADM). The cytotoxic activities against B16-F10, HCT-116 and Moser cells were colorimetrically determined at 540 nm after staining viable cells with 0.008% neutral red solution while those against P388 and K562 cell lines were determined by directly counting the number of viable cells in a cell counter (Sysmex).

In Vivo Antitumor Activity

Murine leukemias P388 and L1210 were implanted ip with 10⁶ and 10⁵ cells per mouse or implanted iv with 5 x 10⁵ and 10⁴ cells per mouse, respectively. Murine melanoma B16 was implanted ip with 0.5 ml of 10% tumor brei (on day 0). The graded doses of test materials were administered once a day on days 1 to 3 (Q1D x 3) or iv, on days 1, 5 and 9 (Q4D x 3) after tumor implantation. Death or survival of the drug- and vehicle-treated animals was recorded daily during the observation period of 45 days and median survival time (MST) was calculated for each of the test (T) and control (C) groups. A T/C value over 125% is considered significant antitumor effect.

Inhibition of Macromolecule Biosynthesis

B16-F10 cells harvested and washed with the culture medium described above were planted into wells of a 24-well tissue culture plate as 1.0 ml aliquot of 5 x 10⁵ (for DNA and RNA synthesis) or 1 x 10⁴ (for protein synthesis) cells/ml. The plates were pre-incubated for 16 hours at 37°C in a 5% CO₂ incubator. After that, the cells were exposed to test materials for 45 minutes at 37°C and then incubated with 7.4 kBq [methyl-³H]thymidine, 7.4 kBq [2-¹⁴C]uridine or 29.6 kBq L-[4,5-³H]leucine for 30 minutes. After gently washing with cold 5% TCA solution, the radio-activity incorporated into the acid-insoluble fraction was determined in a liquid scintillation counter ( Aloka, LSC-701).

Acute Toxicity in Mice

Acute toxicity of dynemicin A was determined in male ddY mice weighing 20 to 24g after a single ip administration of graded doses of the antibiotic to groups of 5 to 8 mice. LD₅₀ value was calculated according to the method of Van der Waerden on day 10.

Results

In Vitro Cytotoxicity

Both dynemicin A and its triacetyl derivative demonstrated extremely potent cytotoxicity against
Table 1. *In vitro* cytotoxicity against murine and human tumor cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B16-F10</td>
</tr>
<tr>
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</tr>
<tr>
<td>Triacetyldynemicin A</td>
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<tr>
<td>Mitomycin C</td>
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</tr>
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<td>Vincristine sulfate</td>
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</table>


In Vivo Antitumor Activity

As shown in Tables 2 and 3, dynemicin A administered ip gave significant chemotherapeutic activity against ip implanted P388 and L1210 leukemias. Although the maximum T/C values of dynemicin A were 10,000~60,000 times superior to those of mitomycin C in terms of IC₅₀ values. Dynemicin A was interestingly as active against drug-resistant sublines P388/VCR, P388/ADM and K562/ADM as against the corresponding sensitive cells.

Table 2. Antitumor activity against P388 leukemia (ip) in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>MST (day)</th>
<th>T/C (%)</th>
<th>Average weight change on day 4 (g)</th>
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<td>135</td>
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<td>12.0</td>
<td>120</td>
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Vehicle control — 10.0 — +2.4

Table 3. Antitumor activity against L1210 leukemia (ip) in mice.

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<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>MST (day)</th>
<th>T/C (%)</th>
<th>Average weight change on day 4 (g)</th>
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Vehicle control — 8.0 — +1.3

* Q1D x 3, ip.
* b Median survival time.

murine and human tumor cells, having IC₅₀ values ranging from 0.0027 to 4.1 ng/ml, which indicate that they have similar or stronger cytotoxic potential than esperamicin A₁ (Table 1). Against human tumors such as Moser and K562 cells, their activities were 10,000~60,000 times superior to those of mitomycin C in terms of IC₅₀ values. Dynemicin A was interestingly as active against drug-resistant sublines P388/VCR, P388/ADM and K562/ADM as against the corresponding sensitive cells.

In Vivo Antitumor Activity

As shown in Tables 2 and 3, dynemicin A administered ip gave significant chemotherapeutic activity against ip implanted P388 and L1210 leukemias. Although the maximum T/C values of dynemicin A were
Table 4. Antitumor activity against B16 melanoma (ip) in mice.

<table>
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<tr>
<th>Compound</th>
<th>Dosea (mg/kg/day)</th>
<th>MSTb (day)</th>
<th>T/C (%)</th>
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<td>Vehicle control</td>
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<td></td>
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</tr>
</tbody>
</table>

a Q4D × 3, ip.
b Median survival time.

Table 5. Antitumor activity against iv implanted P388 and L1210 leukemias in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosea (mg/kg/day)</th>
<th>MSTb (day)</th>
<th>T/C (%)</th>
<th>Average weight change on day 4 (g)</th>
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<td>+0.8</td>
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<td>L1210 leukemia (iv):</td>
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</tr>
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</table>

a Q1D × 3, iv.
b Median survival time.

not high in both leukemias, the wide range of activity was seen and the antitumor activity was approximately 16 ~ 32 times more potent than that of mitomycin C in terms of minimum effective dose. Similar anti-P388 leukemia activity was determined for the triacetyl derivative of dynemicin A. Both dynemicin A and its triacetyl derivative were also active against ip implanted B16 melanoma with maximum T/C values of 144 ~ 156% (Table 4).

When administered iv, dynemicin A demonstrated quite promising anti-leukemic activities against both iv implanted P388 and L1210 leukemias with maximum T/C values of 178 and 171%, respectively, the potency being approximately 8 ~ 16 times stronger than that of doxorubicin (Table 5).

Inhibition of Macromolecule Biosynthesis

Dynemicin A strongly inhibited the incorporation of [methyl-3H]thymidine into the acid-insoluble fraction of B16-F10 cells with IC₅₀ value of 2.2 ng/ml while the IC₅₀ values for the incorporation of [2-¹⁴C]uridine and L-[4,5-³H]leucine were 8.5 and 14 μg/ml, respectively. These results indicate that the inhibitory effect of dynemicin A on DNA synthesis was approximately 4,000 and 6,000 times stronger than that on RNA and protein synthesis, respectively.

Acute Toxicity in Mice

When administered ip, dynemicin A demonstrated delayed-type toxicity in non-tumor-bearing mice.
The first death of mice received 10 mg/kg of dynemicin A, which was the highest dose tested, was seen at
3 days post-administration. The LD$_{50}$ value was determined to be 0.58 mg/kg ip. Therefore, the acute
toxicity of dynemicin A in mice was approximately 20 times less than that of esperamicin A$_1$ (LD$_{50}$
value: 0.026 mg/kg ip).

**Discussion**

Dynemicin A is a novel antitumor antibiotic produced by *M. chersina* sp. nov. M956-1 (ATCC
53710) isolated from a soil sample collected in Gujarat State, India$^1$. According to the structural
studies$^2$,$^3$, dynemicin A is described to be an entry in the family of diynene antitumor antibiotics such as
esperamicins$^5$,$^7$ and calicheamicin$^8$. Since these antibiotics are receiving increasing attention due to their
extremely potent antitumor activity$^9$,$^{10}$, the *in vitro* and *in vivo* antitumor activities of dynemicin A were
determined in comparison with its triacetyl derivative. Both dynemicin A and the triacetyl derivative
achieved extremely potent *in vitro* cytotoxicity against murine and human tumor cell lines representing a
variety of histological types. Dynemicin A administered ip also showed significant *in vivo* antitumor
activity in mice against ip implanted P388 and L1210 leukemias and B16 melanoma with the wide range
of activity. Similar antitumor results were obtained for the triacetyl derivative against both P388 leukemia
and B16 melanoma. In the present experiments, one of the important characteristics for dynemicin A is
antileukemic activity against iv implanted murine leukemias. Dynemicin A administered iv was 8~16
times more active against iv implanted P388 and L1210 leukemias than doxorubicin. Although dynemicin
A gave more potent *in vitro* cytotoxicity than esperamicin A$_1$ in all cell lines tested except in B16-F10, the
acute toxicity in mice was approximately 20 times less than that of esperamicin A$_1$.

Long et al.$^{11}$ revealed in their alkaline elution studies that esperamicins in very low concentrations
was capable of producing both single and double strand DNA breaks, and Zein et al.$^{12}$ reported
that calicheamicin interacted with double-helical DNA in the minor groove and caused site-specific
double-stranded cleavage. Recently, Sugiura et al.$^{13}$ described a possible mechanism of dynemicin A for
DNA intercalation and cleavage. In the present experiments, dynemicin A specifically inhibited the
incorporation of radio-labelled thymidine into the acid-insoluble fraction of B16-F10 cells. This result
indicates that dynemicin A is a potent inhibitor of DNA synthesis. The above evidence suggest that
dynemicin A has specific and unique interaction with DNA molecule and this may cause strong *in vitro*
cytotoxicity and *in vivo* antitumor activity.

**Acknowledgment**

The authors wish to thank Drs. H. Kawaguchi and M. Konishi of their institute for kind suggestions and
couragement to this work.

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