Oncostatic Effects of *Alangium vitiense* Extracts (ICIG-EORTC 1131, 1186, and 1207) on Lymphoid Murine Tumors

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**ABSTRACT**

A total alkaloid and two purified alkaloid extracts of *Alangium vitiense* were found to be oncostatic for L1210 leukemia; the total alkaloid exerted a noticeable activity, and the purified extracts exerted a borderline activity. These two purified extracts are noticeable oncostatic for two other lymphoid neoplasias in mice, P388 leukemia and Gardner lymphosarcoma. These compounds are not active on Warner myelomonocytic leukemia WEHI3 or on B16 melanoma.

**INTRODUCTION**

In 1974 we submitted to ICIG-EORTC² screening (2) a total alkaloid extract (ICIG-EORTC 1131) of *Alangium vitiense*. Because significant oncostatic activity was observed, Husson et al. (4) extracted 2 pure alkaloids termed "Fraction 3" (ICIG-EORTC 1186) and "Fraction 4" (ICIG-EORTC 1207), respectively, which we submitted to the same screening. It is the object of this paper to describe the effects of our 3 extracts on the growth of several murine tumors.

**MATERIALS AND METHODS**

The preparation of these extracts will be published separately (4).

The general formula of *A. vitiense* alkaloids is given in Chart 1; it is similar to that of tubulosine (the isolation of which was reported by Popelak et al.) (7), a compound that was shown to inhibit the biosynthesis of DNA and protein, but that does not inhibit the biosynthesis of RNA (3).

**Lethal Toxicity.** The acute LD₅₀ was determined as follows. The drug was administered to C57BL/6 × DBA/2 F₁, (hereafter called B6D2F₁) mice at doses of 5, 25, 125, and 625 mg/kg (Table 1). After the percentage of deaths that occurred in less than 10 days after single doses was observed, an assessment was made of the doses required to affect the progression of L1210 leukemia. The lethal toxicity of the total alkaloid extracts and of Fraction 3 corresponds to Table 1, Horizontal Column E, and that of Fraction 4 corresponds to Table 1, Horizontal Column D.

**Second Test for Lethal Toxicity and Primary Assay on L1210 Leukemia to Determine the “Optimal Dose.”** For each compound, 34 B6D2F₁ mice were inoculated with 1 × 10⁶ L1210 leukemia cells i.p. on Day 0. At Days 1, 5, and 9, according to the results of the first test, 24 mice were given i.p. injections of the doses determined by the previous test, as presented in Table 1. The doses were 125, 50, 20, and 8 mg/kg for total alkaloid extract and for Fraction 3, whereas doses of 250, 100, 40, and 16 mg/kg were administered for Fraction 4.

The mortality of the animals was studied, and the autopsy indicated whether the death was due to leukemia or the toxic effects of the drug. This assay determined the size of the dose to be used in the secondary assay on mice carrying L1210 leukemia, which is conventionally one-third of the LD₅₀ determined by the present test.

**Secondary Assay on L1210 Leukemia.** The results of treatment with these 3 extracts are expressed as T/C × 100, where T represents the median survival of the treated group of 10 mice, and C represents the median survival of the control group of 10 mice. This ratio expresses the PS.

If the difference between the treated animals and the controls is statistically significant by Wilcoxon's test and if PS > 1.25, the agent tested is considered active.

**Other Tumors.** Six more murine tumors were tested for the 2 pure alkaloids studied. Four of the 6 were tumors of lymphoid nature: P388, EδG2, and EγK1 leukemias (the last 2 neoplasias were induced by Gross virus) and 6C3H/EDOG Gardner lymphosarcoma. The others were WEHI3 Warner myelomonocytic leukemia and B16 melanoma.

The tumor inoculum was 5 × 10⁶ cells for EδG2 leukemia and 1 × 10⁶ for the other tumors, except for B16 melanoma for which it was 0.5 ml of the tumor homogenate. The cells were injected i.p. for P388, EδG2, and EγK1 leukemias and s.c. for other tumors at Day 0, and the chosen dose of the...
Table 1
Reference table for the screening of oncostatics

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% of observed mortality&lt;sup&gt;a&lt;/sup&gt; from Days 1–10 (first test: search for toxicity at the following doses)</th>
<th>Doses (mg/kg) used in the second test: (simultaneous determination of acute LD₅₀ and the primary assay on L1210 leukemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>625 mg/kg 125 mg/kg 25 mg/kg 5 mg/kg Occurrence</td>
<td>1500 600 240 96 1250 500 200 80 625 250 100 40 125 50 20 8 50 20 8 3,2 25 10 4 1,6 10 4 1,6 0,64 5 2 0,8 0,32</td>
</tr>
<tr>
<td>0 0 0 0 A</td>
<td>0 0 0 0 B</td>
<td>100 0 0 0 C</td>
</tr>
</tbody>
</table>

<sup>a</sup> The mortality for 1 compound corresponds to 1 of the A to I occurrences.

<sup>b</sup> The lethal toxicity of the total alkaloid extract and of Fraction 3 was that of Horizontal Column E, and the lethal toxicity of Fraction 4 was that of Horizontal Column D.

Table 2
Secondary assay of total and purified alkaloid extracts on L1210 leukemia

<table>
<thead>
<tr>
<th>ICIG-EORTC No.</th>
<th>Optimal dose (mg/kg)</th>
<th>PS (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1131 (total extract)</td>
<td>30</td>
<td>164</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1181 (Fraction 3)</td>
<td>8</td>
<td>127</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1207 (Fraction 4)</td>
<td>50</td>
<td>125</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Chart 2. Graphic determination of the acute LD₅₀ of extracts of A. vitiense.

RESULTS

The lethal toxicities of the 3 preparations on normal mice are indicated in Table 1. The dose for the L1210 leukemia test was determined, from the results of the first assay on such animals, to be one-third of the LD₅₀ (indicated on Chart 2); hence, the chosen doses were 30 mg for the total alkaloid extract, 8 mg for Fraction 3, and 50 mg for Fraction 4.

As can be seen in Table 2, the total alkaloid extract at 30 mg/kg is significantly active in L1210 lymphoid leukemia (PS, 164%). A significant although borderline effect was also found with the purified alkaloid fractions: PS, 127% for Fraction 3, and 125% for Fraction 4 with 50 mg/kg. However, as can be seen in Table 3, the 2 extracted Alkaloid Fractions 3 and 4 are noticeably active on 2 other tumors of lymphoid nature, P388 leukemia and Gardner.

Table 3
Oncostatic effects of purified extracts of A. vitiense on murine tumors other than L1210 leukemia

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Nature</th>
<th>ICIG 1186 (Fraction 3)</th>
<th>ICIG 1207 (Fraction 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388</td>
<td>Lymphoid leukemia</td>
<td>192</td>
<td>169</td>
</tr>
<tr>
<td>GC3HED/OG GARDNER</td>
<td>Leukosarcoma</td>
<td>169</td>
<td>169</td>
</tr>
<tr>
<td>E₇D₇</td>
<td>Leukemia induced by Gross virus</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>E₉K₁</td>
<td>Leukemia induced by Gross virus</td>
<td>106</td>
<td>74</td>
</tr>
<tr>
<td>WEHI3 Warning</td>
<td>Myelomonocytic leukemia</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>B16</td>
<td>Melanoma</td>
<td>117</td>
<td>105</td>
</tr>
</tbody>
</table>

<sup>a</sup> NS, not significant.
lymphosarcoma. They are not active on the Gross virus-induced lymphoid leukemia, on the WEHI3 Warner myelomonocytic leukemia, and on the B16 melanoma.

**DISCUSSION**

In conclusion, not only do these extracts seem to be significantly oncostatic (although moderately so in most tumors on which they are active), but also their effect has been detected only on lymphoid tissue neoplasias. This possible specificity and the fact that they are active on P388 leukemia make these extracts valuable for completing studies.

Although there are many chemotherapy compounds active on lymphoid neoplasias (1) and although long-term survival of acute lymphoid leukemia patients (5) and of lymphosarcoma patients (6) occurs in about 50% of the patients, there are still disease types (5, 6) that are poorly sensitive either to available chemotherapy or to the chemoimmunotherapy of the present day; hence, there is an interest in such a series of agents active on lymphoid neoplasias.

Preclinical toxicity in monkeys has already been studied; this study showed excellent tolerance (data not published).

This observation will allow us to conduct a Phase 1 trial in humans, followed by a Phase 2 trial in cancer patients resistant to other alkaloids.

**REFERENCES**

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