Some Biological Effects of Vincaleukoblastine, an Alkaloid in *Vinca rosea* Linn in Patients with Malignant Disease*

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SUMMARY

The treatment of 22 patients with Vincaleukoblastine is described. The main toxic effects were noted at the sites of injection, on the blood, and on the nervous system. Severe granulocytopenia with little or no depression of platelets was noted in nearly all patients. In fifteen of the 22 patients the administration of Vincaleukoblastine had measurable effects on the malignant process. Partial remissions of short duration, with marked improvement in general clinical condition, were obtained in three children with acute leukemia, in one patient with lymphosarcoma, and in one with breast cancer.

It has been reported by Cutts, Beer, and Noble (2) that extracts of the plant *Vinca rosea*, when injected into rats, led to a marked fall in circulating leukocytes and to a depression of the bone marrow. Subsequently a new alkaloid, Vincaleukoblastine (VLB), was isolated (8) from such extracts; it caused marked hemopoietic effects (1) and affected the growth of experimental tumors (9).

The chemical and physical evidence indicated that VLB had an empirical formula C₄₆H₇₅O₁₉N₄ and was a member of a new class of dimeric alkaloids which contained both indol and dihydroindol moieties (4, 7). Rats given injections of 0.35 mg/kg of VLB developed a severe granulocytopenia which lasted from 3 to 5 days. Lymphocytes were only slightly reduced and platelets relatively unaffected. Extensive depression, particularly of the myeloid elements of the bone marrow, was also noted. Recovery of the hemopoietic system occurred rapidly. In such cases toxic symptoms were minor, consisting of slight temporary weight loss. No intestinal lesions developed even after massive dosage. Folic and folinic acid, thymidine, cytidine, guanine, and vitamin B₁₂ did not prevent the hematological effects.

VLB has also been reported (9) to have carcinostatic activity. This has been demonstrated with L1210 and P-1534 leukemia transplanted in BDF hybrid mice, AKr leukemia transplanted in AK mice, and Sarcoma 180 in Swiss mice. In myeloid leukemia 1RC741 in Fischer rats, survival time was not significantly increased, but a pronounced alteration of the leukemic process in peripheral blood was observed. More extensive observations on the effects of VLB on experimental tumors have been described in the preceding paper (3).

In preliminary reports other workers have shown similar carcinostatic effects on experimental tumors (6) and human leukemia (5).

In view of the above experimental findings VLB has been administered to humans suffering from advanced malignant disease, at the Princess Margaret Hospital in Toronto.

MATERIALS AND METHODS

The following standards were used in selecting cases for treatment: (a) established forms of treatment had already been used and were considered to have no further useful part in the management of the case; (b) diagnosis had been established histologically; and (c) physical signs and/or findings on special examination were such that objective measurement of any beneficial effects would be possible. The 22 patients selected for treatment
included eight with leukemia, five with malignant lymphoma, and nine with other malignant tumors (Table 1).

Apart from any effect which VLB might have on malignant disease, we were at the onset of this study equally concerned with any other of its biological effects. To this end the following protocol was observed when practical.

- Daily hemoglobin, hematocrit, reticulocyte, and white blood cell count, platelet estimation, and differential count; marrow aspiration prior to and 1 week following initiation of treatment.
- Blood pressure immediately following treatment and twice daily; electrocardiogram before and after treatment.
- Cephalin cholesterol flocculation, alkaline phosphatase, and bromsulfalein retention prior to, and 1 week following, treatment; serum electrophoresis by the starch gel method of Smithies (10) prior to, and 1 week following, treatment.
- Photograph of malignant process before and after treatment.
- VLB sulfate was prepared in vials each containing 10 mg. of the crystalline salt. It was dissolved in 10 ml. of distilled water prior to injection. The dosage commonly employed in the study was 0.15 mg/kg, given intravenously for 3 or 4 consecutive days. As noted in Table 1 some patients received, after varying intervals, further injections of VLB in the same or increased dosage.

RESULTS

Local effects.—The following effects were noted in relation to the injection of VLB into antecubital veins. Fairly severe pain was noted in one case when the material gained access to interstitial tissue. Three patients developed thrombophlebitis. In four cases numbness and tingling were noted in the antecubital and forearm areas, sometimes for a week to 10 days following treatment and not necessarily associated with the presence of phlebitis.

General effects.—The most constant systemic effect was weight loss, noted in all cases. In some instances this amounted to only a few pounds, but in other cases patients lost 8–12 pounds and in one case 16 pounds. Nausea occurred in four cases and was commonly at its worst the morning following administration of VLB. One patient had a brief period of vomiting following treatment, and another had diarrhea. Stomatitis and pharyngitis were seen only in association with marked leukopenia. Severe gastrointestinal bleeding occurred in two patients known previously to have peptic ulceration. This was not associated with thrombocytopenia or prothrombin deficiency. One female patient had temporary, partial epilation.

Symptoms referable to the nervous system were a feature in four of the cases. One patient had paresthesia of the tongue and dizziness for about a day. An odd behavior pattern unaccompanied by abnormal neurological signs was a feature in three cases. One patient seemed unable to understand spoken questions and walked along the corridor undressed. Another wandered about aimlessly and on two occasions urinated on his neighbor’s bed. Disorientation and agitated depression occurred in an elderly man in association with infection and fever.

Hematological effects.—A significant fall in hemoglobin was noted in eighteen patients. Acute leukemia was associated with progressive anemia before treatment, but even in patients with such conditions as cancer of the breast, with an apparently normal marrow, the average drop following VLB was 2 gm. per cent. This was not associated with an increase of serum bilirubin or urobilin in urine. Depression of the reticulocytes was usual, being most marked about 7–10 days following initiation of treatment. This was followed by a compensatory rise in reticulocytes and an increasing level of hemoglobin. In two patients a more severe drop in hemoglobin was related to bleeding from peptic ulcer.

In all patients a significant drop in the total leukocyte count was observed within 24–48 hours of the first injection of VLB. In twelve patients a marked leukopenia developed, with counts below 1,000 per cu. mm. and frequently 300–700 per cu. mm. The maximum depression was noted between the 5th and 9th day following initial treatment. In children this marked leukopenia was invariably associated with agranulocytosis. Where the marrow was not primarily involved by disease the agent affected mainly the granulocytic series. Following the leukopenia there was a rapid rise to levels above those noted prior to treatment, as illustrated in Case No. 22 (Chart 1).

In 21 of the 22 patients VLB therapy had no significant effect on platelet counts even when thrombocytopenia was present prior to treatment. In a patient with lymphosarcoma a fall in platelets...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Disease</th>
<th>Duration</th>
<th>Previous treatment</th>
<th>VLB dosage (mc/kg)</th>
<th>Toxic effects</th>
<th>Hematological effects</th>
<th>Effect on malignant disease</th>
<th>Over-all effect of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>M</td>
<td>Acute leukemia</td>
<td>10 mo.</td>
<td>Prednisone, aminopterin, 6-mercaptopurine (favorable remissions)</td>
<td>1) 0.15, 0.05, 0.15</td>
<td>Pain and paresthesia in forearm</td>
<td>Hb, 10.8–6.1 gm. W.B.C., 32,000–5,000 per cu. mm. Blast cells, 73–34%</td>
<td>Spleen smaller</td>
<td>Partial remission, 4 weeks</td>
</tr>
<tr>
<td>2</td>
<td>1½</td>
<td>M</td>
<td>Acute leukemia</td>
<td>18 mo.</td>
<td>Prednisone, aminopterin, 6-mercaptopurine (favorable remissions)</td>
<td>1) 0.15×3</td>
<td>Nil</td>
<td>Hb, 14.5–9.6 gm. W.B.C., 6,600–700 per cu. mm.</td>
<td>Spleen and liver smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>F</td>
<td>Acute leukemia</td>
<td>1 yr.</td>
<td>Prednisone, aminopterin, 6-mercaptopurine (favorable remissions)</td>
<td>1) 0.15×3 interval 2 weeks</td>
<td>1) Nil</td>
<td>1) Hb, 10.3–6.5 gm. W.B.C., 20,000–5,000 per cu. mm. Blast cells, 76–42%</td>
<td>1) Spleen and liver smaller</td>
<td>1) No improvement</td>
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<td></td>
<td></td>
<td>2) Nil</td>
<td>2) W.B.C., 119,000–1,250 per cu. mm. Blast cells 75–15%</td>
<td>2) Nil</td>
<td>2) No improvement</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>F</td>
<td>Acute leukemia</td>
<td>2 yr.</td>
<td>Prednisone, aminopterin, 6-mercaptopurine (favorable remissions)</td>
<td>1) 0.15×3</td>
<td>Nil</td>
<td>Hb, 11–6.5 gm. W.B.C., 2,500–200 per cu. mm. Blast cells 45–4%</td>
<td>Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>5</td>
<td>1½</td>
<td>M</td>
<td>Acute leukemia</td>
<td>2 yr.</td>
<td>Prednisone, aminopterin, 6-mercaptopurine (favorable remissions)</td>
<td>1) 0.15×3 interval 2 mo.</td>
<td>1) Phlebitis</td>
<td>1) Hb, 9.0–5.9 gm. W.B.C., 3,000–400 per cu. mm. Blast cells, 45–5%</td>
<td>1) Spleen smaller</td>
<td>1) Partial remission, 6 weeks</td>
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<td></td>
<td>2) Paresthesia in forearm</td>
<td>2) Hb, 9.4–5.0 gm. W.B.C., 2,000–500 per cu. mm. Blast cells 30–12%</td>
<td>2) Spleen smaller</td>
<td>2) No improvement</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>F</td>
<td>Leukosarcoma</td>
<td>21 mo.</td>
<td>External radiation, aminopterin, prednisone, 6-mercaptopurine</td>
<td>1) 0.15×3 interval 8 weeks</td>
<td>Nil</td>
<td>1) Hb, 12.2–5.7 gm. W.B.C., 4,000–550 per cu. mm. Blast cells 33–9%</td>
<td>1) Liver and spleen smaller</td>
<td>1) Partial remission, 6 weeks</td>
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<td></td>
<td>2) 0.15×3</td>
<td>2) Hb, 11.1–5.8 gm. W.B.C., 32,000–400 per cu. mm.</td>
<td>2) Liver and spleen smaller</td>
<td>2) No improvement</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>M</td>
<td>Acute leukemia</td>
<td>4 mo.</td>
<td>Prednisone, 6-mercaptopurine (partial remissions)</td>
<td>0 15×1</td>
<td>Nil</td>
<td>W.B.C., 900–450 per cu. mm.</td>
<td>Nil</td>
<td>Death on day following treatment attributed to disease</td>
</tr>
<tr>
<td>Patient</td>
<td>Age (yr)</td>
<td>Sex</td>
<td>Disease</td>
<td>Duration</td>
<td>Previous treatment</td>
<td>VLB dosage (mg/m²)</td>
<td>Toxic effects</td>
<td>Hematological effects</td>
<td>Effect on malignant disease</td>
<td>Overall effect of therapy</td>
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<tr>
<td>8</td>
<td>31</td>
<td>M</td>
<td>Chronic myeloid leukemia in blast crisis</td>
<td>4 mo.</td>
<td>External radiation, Myleran, 6-mercaptopurine (poor response to 6-mercaptopurine)</td>
<td>1) 0.15X3  2) 0.15X1  3) 0.15X1  4) 0.15X1</td>
<td>Weight loss 10 lb.</td>
<td>Hb, 14.0 8.3 gm, W.B.C., 15,000-8,200 per cu. mm.</td>
<td>Liver, spleen, and lymph nodes smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>M</td>
<td>Hodgkin's</td>
<td>7 yr.</td>
<td>External radiation, alkylating agents, lamine Ferm</td>
<td>0.15X4</td>
<td>Weight loss 8 lb., bleeding from duodenal ulcer</td>
<td>Hb, 8.9 4.8 gm, W.B.C., 20,000-2,500 per cu. mm.</td>
<td>Lymph nodes and other palpable masses smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>M</td>
<td>Lymphosarcoma chronic lymphatic leukemia</td>
<td>4 mo.</td>
<td>External radiation, alkylating agents</td>
<td>1) 0.15X4 interval 6 weeks  2) 0.15X4</td>
<td>Weight loss 12 lb., thrombocytopenia</td>
<td>Hb, 9.5 7.6 gm, W.B.C., 15,000-3,000 per cu. mm.</td>
<td>1) Liver, spleen, and lymph nodes smaller 2) Liver, spleen, and lymph nodes smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>M</td>
<td>Lymphosarcoma</td>
<td>5 mo.</td>
<td>External radiation, alkylating agents, prednisone</td>
<td>1) 0.15X3  2) 0.18X5  3) 0.2X4  4) 0.15X5</td>
<td>Weight loss, 16 lb., thymus, emesis, diarrhea</td>
<td>Hb, 13-8.8 gm, W.B.C., 4,500-350 per cu. mm.</td>
<td>1) Marked tumor regression 2) Marked tumor regression 3) Marked tumor regression 4) Nil</td>
<td>Partial remission, 4 weeks</td>
</tr>
<tr>
<td>12</td>
<td>47</td>
<td>M</td>
<td>Lymphosarcoma</td>
<td>4 yr.</td>
<td>External radiation, alkylating agents, prednisone</td>
<td>0.15X1</td>
<td>Weight loss, 8 lb.</td>
<td>Hb, 10.6-7.1 gm, W.B.C., 10,000-1,700 per cu. mm., Platelets, 155,000-60,000 per cu. mm.</td>
<td>Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>13</td>
<td>44</td>
<td>M</td>
<td>Giant follicle lymphoma, hydronephrosis, uremia</td>
<td>5 mo.</td>
<td>External radiation</td>
<td>0.15X3</td>
<td>Weight loss, 12 lb., nausea</td>
<td>Hb, 8.4 6.2 gm, W.B.C., 1,500-350 per cu. mm.</td>
<td>Spleen, lymph nodes, and other tumor masses smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>Patient</td>
<td>Age (yr.)</td>
<td>Sex</td>
<td>Disease</td>
<td>Duration</td>
<td>Previous treatment</td>
<td>VLR dosage (mg/kg)</td>
<td>Toxic effects</td>
<td>Hematological effects</td>
<td>Effect on malignant disease</td>
<td>Overall effect of therapy</td>
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<tr>
<td>14</td>
<td>48</td>
<td>F</td>
<td>Malignant thymoma</td>
<td>8 mo.</td>
<td>External radiation</td>
<td>1) 0.15X3 interval 3 weeks</td>
<td>1) Weight loss, 11 lb., burning and hyperesthesia antecubital areas and forearms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>F</td>
<td>Retinoblastoma with metastases</td>
<td>9 mo.</td>
<td>External radiation</td>
<td>0.15X3</td>
<td>2) Nil</td>
<td>2) Similar effect</td>
<td>2) Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>16</td>
<td>54</td>
<td>M</td>
<td>Melanocarcinoma</td>
<td>1 yr.</td>
<td>Surgery, external radiation</td>
<td>0.2X4</td>
<td>2) Nil</td>
<td>2) Nil</td>
<td>No improvement</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>35</td>
<td>F</td>
<td>Carcinoma cervix uteri with pulmonary metastases</td>
<td>2½ yr.</td>
<td>External radiation</td>
<td>0.15X3</td>
<td>Nausea, paresthesia of tongue, dizziness</td>
<td>Hb, 9.2-8.9 gm. W.B.C., 7,000-500 per cu. mm.</td>
<td>Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>F</td>
<td>Carcinoma esophagus with skin metastases</td>
<td>5 yr.</td>
<td>Surgery, external radiation</td>
<td>0.15X4</td>
<td>Partial epilation</td>
<td>Hb, 14.3-12.8 gm. W.B.C., 8,000-3,200 per cu. mm.</td>
<td>Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>19</td>
<td>51</td>
<td>M</td>
<td>Carcinoma bronchus with metastases to liver and subcutaneous tissues</td>
<td>6 mo.</td>
<td>External radiation</td>
<td>1) 0.15X4 interval 7 days</td>
<td>Bleeding from peptic ulcer, mental changes</td>
<td>Hb, 11-5.7 gm. W.B.C., 12,000-1,600 per cu. mm.</td>
<td>1) Metastatic nodes smaller</td>
<td>No improvement</td>
</tr>
<tr>
<td>20</td>
<td>91</td>
<td>M</td>
<td>Carcinoma skin, neck</td>
<td>2 yr.</td>
<td>External radiation</td>
<td>0.15X4</td>
<td>Mental changes</td>
<td>Hb, 10.7-10.3 gm. W.B.C., 7,200-250 per cu. mm.</td>
<td>Nil</td>
<td>No improvement</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>F</td>
<td>Choriocarcinoma with pulmonary metastases</td>
<td>3 mo.</td>
<td>Surgery</td>
<td>0.15X3</td>
<td>Nil</td>
<td>1) Hb, 12.4-12.3 gm. W.B.C., 4,800-3,200 per cu. mm.</td>
<td>No improvement</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>45</td>
<td>F</td>
<td>Carcinoma breast with metastases chest wall</td>
<td>2½ yr.</td>
<td>Surgery, external radiation</td>
<td>0.15X4</td>
<td>Nausea, vomiting, mental changes</td>
<td>Hb, 14-10.5 gm. W.B.C., 6,600-350 per cu. mm.</td>
<td>Marked tumor regression</td>
<td>Partial remission, 6 weeks</td>
</tr>
</tbody>
</table>
from 160,000 per cu. mm. to 60,000 per cu. mm. was observed.

In patients with leukemia the administration of VLB was followed within 5-7 days by a phase of hypoplasia in the marrow. At this stage an increased percentage of smudge cells and other degenerate, atypical cells could be seen. When remission was obtained this hypoplastic phase was followed initially by an erythroid and then a myeloid response (Figs. 5, 6, and 7). When remission was not obtained in leukemic patients, the hypoplastic marrow phase was followed immediately by reinfiltiration with blast cells. In patients with carcinoma where the marrow was not involved by disease, a phase of hypoplasia was noted within 3-7 days. This was rapidly followed by erythroid and myeloid hyperplasia. Throughout, the megakaryocytes showed no change in appearance and no significant change in numbers.

**Effect on other systems.**—No significant change was noted in fluid balance. Uric acid crystals were noted following treatment in several patients with leukemia and lymphoma, but there was no associated hematuria, and there was no evidence of urinary obstruction as indicated by oliguria. One patient with hydronephrosis and moderate uremia prior to treatment showed an increase in blood urea which quickly returned to normal. Another, with normal renal function, had a temporary rise of blood urea to 60 mg. per cent. In the lymphomas and leukemias a slight to marked increase of uric acid excretion was noted.

No changes in pulse or blood pressure were noted immediately following treatment or thereafter. No significant alteration was noted in electrocardiogram except for temporary changes associated with severe gastrointestinal bleeding in two patients.

In several cases liver function was disturbed prior to treatment, as shown by cephalin cholesterol flocculation and increase in bromsulphalein retention. The findings were not altered subsequently by VLB therapy. In one patient a negative cephalin cholesterol flocculation became positive following treatment. No significant change was noted in serum electrophoretic pattern as a result of treatment, although most patients showed some abnormality in pattern prior to treatment.

**Effect on malignant disease.**—It became apparent early in the study that VLB had, in some cases, a rapid and sometimes striking effect on malignant disease. Fourteen of the 22 patients showed objective signs of improvement in their disease. This was characterized by decrease in size of lymph nodes, spleen, liver, and tumor masses. In only five cases, however, could it be said that treatment improved the clinical condition of the patient.

In all the leukemia patients the spleen, when palpable, decreased in size during the course of therapy. Also observed was a shrinkage in the size of the liver and of enlarged lymph nodes. In three of the eight patients with acute leukemia partial remissions were obtained. Accompanying the disappearance of blast cells from the peripheral blood was a decrease of blasts in the marrow, with subsequent rise in hemoglobin and return of normal myeloid components in both marrow and peripheral blood. The remissions in these cases were of 4 and 6 weeks' duration.

**Effects on the costal margin, and the spleen, 8 cm. Hemoglobin was 10.5; red cells, 3.95 million per cu. mm. with 33 per cent blast cells; white cells, 4,300 per cu. mm. with 8 per cent blast cells.**}

**Figure 1.**—Effect of VLB on peripheral blood of patient No. 92—carcinoma of breast. Platelets were normal in number and not affected by treatment.

**Females, age 7 years, Case No. 6, lymphosarcoma.**—This patient developed cervical lymphadenopathy 31 months prior to admission. Initially she was considered as having a localized lymphosarcoma. Bone marrow was later shown to be involved. External radiation, aminopterin (4-aminopteroylglutamic acid), prednisone, and 6-mercaptopurine had all given favorable remissions. Relapse of disease occurred 1 week prior to admission.

Physical examination revealed one small submental lymph node measuring $3 \times 1.5$ cm. The liver was enlarged 7 cm. below the costal margin, and the spleen, 8 cm. Hemoglobin was 12.2 gm. per cent; W.B.C., 5,950 per cu. mm. with 33 per cent blast forms; platelets, 140,000 per cu. mm. Marrow was hypercellular with almost complete replacement of normal elements by blast forms.

She received VLB 0.15 mg/kg on 3 consecutive days. The changes in peripheral blood are recorded on Chart 2. The submental node was found to be smaller 2 days following the first injection, and 8 days following the initiation of therapy it was 1 cm. in diameter. The liver was 4 cm. below the costal margin and the spleen 2 cm. At this time marrow was hypoplastic with an increase in erythroid elements.

The submental node, liver, and spleen became smaller over the next 2 weeks. The marrow remained hypoplastic with a
relative increase of erythroid and myeloid elements. Blast cells in the marrow fell to 32 per cent the 8th week after commencement of therapy.

The patient continued to feel well and was followed at weekly intervals. Six weeks following VLB therapy the liver and spleen again became larger, and blast cells reappeared in the peripheral blood. A second course of VLB therapy in the same dosage caused a drop in hemoglobin and in white cells. The subsequent rise in the white cell count was accompanied by the relative increase of erythroid and myeloid elements. Blast cells were not seen at this time.

Of the patients with malignant lymphoma one had Hodgkin's disease, three lymphosarcoma, and one giant follicular lymphoma. Following treatment a change in physical signs was observed in four patients. In one case these changes were striking.

On 3 consecutive days the patient received VLB, 0.15 mg/kg. On the 4th day he felt definite improvement, and it continued to improve. The white cell count was at its lowest (830 per cu. mm.) on the 21st day.

With the larger doses of VLB the main systemic effects noted were nausea, starting 7-8 hours following treatment and usually lasting through the following morning. On one occasion the patient had diarrhea. Thrombophlebitis occurred in the antecubital vein of the left arm and was undoubtedly related to administration of VLB.

One month after beginning treatment he weighed 165 lbs., and his general clinical condition was much improved. All areas of disease showed marked regression. Nodes were no longer palpable on the right side of the neck, and those on the left side had almost disappeared. Crusting was present in previously ulcerated areas with weeping, healing dermis underneath. Hemoglobin was 8.8 gm. per cent; W.B.C., 1,250 per cu. mm.; platelets, 220,000 per cu. mm.

One week later there was a suggestion of exacerbation of his disease, and he received four additional doses of VLB (0.2 mg/kg). Slight regression of disease was noted. One month later he was re-admitted with further extension of disease and with what was interpreted as a motor and sensory neuropathy. VLB therapy had no beneficial effect, and he died 10 weeks following initial treatment with VLB.

Several features of interest were noted in the remaining group of patients with solid tumors.

**Male, age 56 years, Case No. 11, lymphosarcoma.**—In January, 1959, this patient developed a swelling on the left side of the neck. Biopsy was diagnosed as lymphosarcoma. He was referred for radiotherapy March, 1959, and had a good response. Within 1 month, however, tumor masses had recurred in the treated areas and had extended to involve both sides of neck, chest wall, and superior mediastinum. Further radiation proved ineffective, and he was admitted to hospital May 8th for chemotherapy. Nitrogen mustard, nitrogen mustard combined with urethan, endoxan (N,N-bis[β-chloroethyl]-N',O-propylene phosphoric acid ester diamide) and prednisone given over the next 3 weeks failed to halt the progressive course of his disease. Figure 1 shows the patient just prior to treatment with VLB. At this time he had a "tight feeling in neck," moderate stridor and dysphagia, shooting pains in right arm referable to brachial plexus involvement, and fullness of the abdomen. Free fluid could be demonstrated in the abdomen, and x-ray of the chest showed mediastinal involvement. Weight was 181 lb.; hemoglobin, 13.3 gm. per cent; W.B.C., 4,500 per cu. mm.; platelets, 176,000 per cu. mm.

On 3 consecutive days the patient received VLB, 0.15 mg/kg. On the 4th day he felt definite improvement, and it was noted that the tense, blue appearance of his skin was less and that areas of disease had diminished in size and extent. On days 7, 11, 14, 15, and 18 VLB was repeated in dosage of 0.18 mg/kg.
of disease to the supraclavicular area. Again there was no response to radiotherapy, and she was admitted July 30, 1959, for VLB therapy.

The patient was well nourished and of good color. Supraclavicular nodes were palpable. The whole chest wall was involved by disease, the skin being bluish-red in color, thickened, and indurated. Disease extended into the neck region, over the deltoid, and posteriorly to the posterior axillary line. An ulcer 4 cm. in diameter was present over the manubrium. Its base was necrotic and appeared to involve sternum. The left arm and hand were edematous (Fig. 3). Hemoglobin was 14 gm. per cent; W.B.C., 7,000 per cu. mm., normal differential count; platelets, 240,000 per cu. mm.; chest x-ray normal.

On 4 consecutive days the patient received intravenous injections of VLB, 0.15 mg/kg. Nausea and vomiting were noted after the first injection. For 4 days she was bothered by a burning sensation in the area of disease. By the 5th day erythema had decreased, the ulcer was cleaner, and its base covered with granulation tissue.

At this time the patient developed an odd behavior pattern unaccompanied by abnormal neurological signs. She seemed unable to understand spoken questions and walked along the corridor unsteadily. She was not emotionally upset and gave indication that she understood she was behaving unusually and expressing herself poorly. The only intellectual deficit noted was slowness in arithmetic. She was depressed for the next few days.

Five days after starting treatment she complained of "searing pains in all my bones." W.B.C., 4,000 per cu. mm.; platelets normal. The marrow at this time was markedly hypoplastic with absence of granulocytes, but megakaryocytes were plentiful. Three days later, W.B.C. count was 400 per cu. mm. with absence of granulocytes; platelets unaffected. The ulcer on chest wall was epithelializing rapidly and erythema of chest wall subsiding.

On the 12th day the supraclavicular nodes were no longer palpable, and induration and thickening of skin had disappeared. There was less swelling of the arm. She appeared pale and haggard and had short periods of not seeming able to understand what was going on around her.

Nineteen days following treatment she felt "like my old self." W.B.C. count was 15,000 per cu. mm., with predominance of young cells including occasional myelocytes and myelophages. The marrow showed reactive hyperplasia.

The patient was discharged feeling well, apart from stiffness in right elbow and left shoulder. There was no apparent intellectual deficit. The ulcer on chest wall had completely epithelialized. Erythema of chest wall was slight with no induration or thickening. Hemoglobin was 11.5 gm. per cent; W.B.C., 5,100 per cu. mm. with normal differential. No significant change in platelets was noted during the period of observation.

This remission was maintained for 6 weeks. She has since been placed on a regimen of androgen and thiopTEPA (thiophene thioureasoramide) with negligible response.

Post-mortem examination.—Autopsies were performed on eight patients, including three of acute leukemia in childhood (Case Nos. 2, 3, and 7), two of lymphosarcoma (Case Nos. 11 and 13), and one each of melanocarcinoma (Case No. 16), bronchogenic carcinoma (Case No. 19), and retinoblastoma (Case No. 15). Although the material is limited, it is suggested that degenerative changes in lymphoid tissue and hypoplasia of bone marrow are of much greater degree than is usually seen in untreated cases of these disorders. In all instances the marrow had undergone a profound hypoplasia, and in three of the four lymphoma cases a sharp reduction in the amount of lymphoid infiltration had occurred. In the three leukemia and two lymphosarcoma patients the number of lymphoid cells in the nodes was reduced. Degenerative changes in the individual cells included fragmentation and reduction in the amount of nuclear chromatin and a shrinking and ruggedness of the cytoplasm. Sometimes hyperplasia of the reticuloct cells in the background could be seen, and fragments of nuclear debris were evident in their plump eosinophilic cytoplasm. In contrast, no effect on carcinomatous tissues was evident in the two cases examined.

Where viscera were involved by lymphoma, changes were the same as those seen in lymph nodes. In Case No. 11 with clinical evidence of involvement of the nervous system, there was extensive infiltration of Virchow-Robin spaces by lymphoma cells.

No overt toxic effects attributable to VLB could be found in any organs. No unusual ulceration or hemorrhage was found in the gastrointestinal tract.

DISCUSSION

Because only a small number of patients have been treated and essentially one dosage schedule applied, it is not possible to reach any final conclusions concerning the efficacy of VLB in the treatment of malignant disease. However, it has been clearly demonstrated that the agent has a definite, though brief, effect on malignant disease. Of interest is the observation that the frequently observed decrease in size of tumor masses and involved organs, such as spleen and liver, is seldom accompanied by subjective improvement. There was a suggestion in two cases at least that, while the malignant process was initially sensitive to VLB, it later was unaffected by the same or higher doses of the material. This development of resistance may be the same as that seen with other carinostatic agents. Other agents had been used previously in our cases, and it would seem that cross-tolerance is not a factor of importance.

It remains to be determined whether a better initial effect and longer remissions can be obtained by a different dosage schedule and maintenance therapy. At Dr. Noble's suggestion we are now assessing the efficacy of a total dose of 0.2 mg/kg given in ten injections at hourly intervals.

Of the toxic manifestations of VLB the blood changes are the most striking. The drop in hemoglobin in some patients following administration
seems more rapid than might be accounted for by the simple cessation of red cell production. Further investigation is indicated to determine whether or not VLB has a specific hemolytic effect.

Granulocytopenia occurs rapidly, but recovery is equally rapid and complete. Although the opportunity presented itself, we did not feel justified in treating patient with chronic myelogenous leukemia. The most remarkable feature of VLB is that megakaryocytes and platelets are spared. Thrombocytopenia, even when present before treatment, does not seem to be made worse by VLB.

The mental changes observed in several patients presented a bizarre pattern with which we are unfamiliar. That it occurred in adults and not in children may be a coincidence.

Several observations would seem to indicate that VLB has a mode of action different from those of other agents now used in the chemotherapy of malignant disease. Patients resistant to alkylating agents, metabolic antagonists, and corticosteroids may repond to VLB. The pattern of systemic reactions is unlike that which follows treatment with these other agents, and the relative lack of toxicity for megakaryocytes and platelets is unique.

REFERENCES


Some Biological Effects of Vincaleukoblastine, an Alkaloid in *Vinca rosea* Linn in Patients with Malignant Disease

O. H. Warwick, J. M. M. Darte and T. C. Brown

*Cancer Res* 1960;20:1032.