Effect of Lithium on the Myelosuppressive and Chemotherapeutic Activities of Vinblastine

Carol E. Cass, A. Robert Turner, Milada Selner, M. Joan Allalunis, and Teresita H. Tan

INTRODUCTION

Lithium carbonate p.o. induces a reversible neutrophilia in patients being treated for affective disorders (18). Lithium salts have been shown to increase production of a granulopoietin, or colony-stimulating activity, in mice and humans (5, 7, 22). As well, lithium appears to stimulate growth of the murine pluripotential hematopoietic stem cell (8). Recently, several investigators have reported that the administration of lithium carbonate to patients being treated with cytotoxic chemotherapy for cancer attenuates the neutropenia produced by these drugs (4, 6, 19). The reduction of neutropenia appears to reduce infective complications (9, 23). Lithium carbonate has thus been advocated as adjunctive therapy in patients receiving myelotoxic therapy. However, the effects of lithium on the efficacy of chemotherapy have not been established.

The current work was undertaken to determine if lithium has antitumor activity as a single agent and if it has any effect on the cytotoxic activity of the myelosuppressive agent vinblastine. A recent report of lithium antagonism of the effects of vinblastine on isolated microtubules (1) suggested that lithium therapy, when used to protect against severe neutropenia, might counteract the antitumor activity of vinblastine.

MATERIALS AND METHODS

Experimental tumors were the L1210 leukemia and the P388 leukemia maintained in ascites form. The L1210 and P388 leukemias were obtained, respectively, from D. W. R. Laster, Jr., Southern Research Institute, Birmingham, Ala., and G. A. LePage of this laboratory. The mice used, C57BL × DBA/2 (hereafter called BD2F1), were obtained from the Health Sciences Animal Breeding Unit, University of Alberta, Edmonton, Alberta, Canada. In chemotherapeutic studies, mice of the same sex and weight (±0.5 g) were assigned to treatment groups, each member of which received identical doses (i.p.) of vinblastine sulfate and/or lithium chloride dissolved in phosphate-buffered saline (134 mM NaCl, 1.84 mM CaCl2, 4 mM KCl, and 9.6 mM sodium phosphate buffer, pH 7.4) or phosphate-buffered saline alone; for all injections, volumes were 0.1 ml/g body weight. Drug treatments were initiated 24 hr after i.p. implantation of 106 tumor cells. The effects of drugs on the survival of normal mice were determined in parallel experiments using tumor-free animals. Serum lithium levels were determined by flame photometry (Model 450; Corning Scientific Instruments, Medfield, Mass.) of serum prepared from venous blood collected at specified times after i.p. injection of lithium chloride (160 mg/kg). Peripheral WBC were determined on a Model ZBI Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) from blood obtained by retroorbital puncture. Wright’s stained smears were used to do a 200-cell differential.

Two murine cell lines, leukemia L1210/C2 and neuroblastoma Neuro-2A, were used; their origins have been described previously (2, 3). Both cell lines were initiated from Mycoplasma-free frozen stock cultures and maintained at 37° in a humidified atmosphere of 5% CO2 in air in growth medium free of antibiotics. L1210/C2 cells were grown in suspension culture, with dilutions at 2- to 3-day intervals to 5 x 10⁴ cells/ml, in Fischer’s medium supplemented with 10% horse serum. Monolayer cultures of Neuro-2A cells were maintained in 25-cm² plastic culture flasks in Eagle’s minimum essential medium supplemented with 1-glutamine, nonessential amino acids, and 10% fetal calf serum; cultures were transferred at weekly intervals using 0.05% trypsin and 0.02% EDTA in 0.9% NaCl solution.

Experiments to determine drug effects on proliferation of L1210/C2 cells were initiated by mixing equal volumes of growth medium containing logarithmically proliferating cells at 2 x 10⁵ cells/ml with growth medium containing additives at twice the concentration to be tested. The resulting 20-ml cultures, prepared in duplicate, were incubated with antibiotics (penicillin G, 100 units/ml; streptomycin, 100 μg/ml; gentamycin, 50 μg/ml) at 37° in loosely capped bottles in 5% CO2 in air; at 24-hr intervals, cell concentrations were determined with an electronic particle counter.

REFERENCES

1. Supported by the National Cancer Institute of Canada.

2. To whom requests for reprints should be addressed.

Received June 9, 1980; accepted November 14, 1980.

1000

CANCER RESEARCH VOL. 41

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1981 American Association for Cancer Research.
Viability of L1210/C2 cells after exposure to drugs was determined by a modification of the soft-agar technique described previously (2). Cell suspensions (5 ml/culture tube) prepared in cloning medium containing 0.13% Noble agar were incubated at 37°C in a humidified atmosphere at 5% CO2 in air. After 10 days, the agar was poured into Petri dishes for counting with a dissecting microscope at low magnification. The cloning medium consisted of Fischer’s medium supplemented with gentamicin (50 μg/ml), 10% horse serum, and 10% conditioned medium. The latter consisted of cell-free medium collected by centrifugation of 48-hr cultures followed by filtration through 0.22-μm nitrocellulose filters.

Tumorigenicity of L1210/C2 cells after drug exposure was determined as described previously (2) by i.p. implantation of variously treated cells contained in 0.25 ml growth medium into groups of 6 female BDF1 mice.

The effect of drugs on proliferation of Neuro-2A cells was determined as follows. Six-day logarithmically growing cultures were trypsinized and pooled, and cells were suspended in growth medium containing antibiotics. Replicate cultures were prepared with an automatic pipetting device for inoculation of 25-sq cm plastic flasks (2 × 105 cells/flask; 4 ml/flask). Cultures were loosely capped and incubated at 37°C in 5% CO2 in air. Twenty-four hr after plating, drug exposures were initiated by complete replacement of culture fluids. At timed intervals, variously treated cultures were trypsinized (3 flasks/condition) for determination of cell numbers.

The effect of drug treatment on neurite formation was determined as described previously (3) by examination of cellular morphology when neuroblastoma cells were subjected to serum deprivation. At various times after beginning drug treatment, cultures prepared as described above were washed once in serum-free medium with or without the appropriate drug. The proportion of cells that exhibited neurites (nerve-like processes extending a cell body length or more) in serum-free medium was determined by examination of at least 400 cells/condition with an inverted phase-contrast microscope equipped with an ocular counting grid.

Cell culture reagents and tissue culture plasticware were obtained from Grand Island Biological Co., Calgary, Alberta, Canada. Vinblastine sulfate (VELBE) is a product of Eli Lilly and Co., Ltd., Toronto, Ontario, Canada, and lithium chloride was purchased from Fisher Scientific Co., Ltd., Edmonton, Alberta, Canada.

RESULTS

Chart 1 shows the serum concentration of lithium ion following i.p. injections of lithium chloride at the dose used in most of the toxicity and chemotherapy experiments reported below. Five min after injection of 160 mg/kg, serum lithium was 6.2 ± 1.0 mm (S.D.). The serum level fell in 2 phases. The first phase had a half-life of approximately 30 min. By 60 min after injection, the serum lithium level was 1.9 ± 0.3 mm. The second phase of disappearance had an apparent half-life of 2 hr, and, by 7 hr after injection, the serum lithium concentration was 0.13 ± 0.09 mm. A small amount of lithium was detectable in 3 of 7 animals at 24 hr.

The effects of both sequential and simultaneous administration of lithium chloride and vinblastine on normal and leukemic mice were examined. The sequential schedule, with daily doses of lithium chloride given after a single dose of vinblastine, resembled the schedule used in our clinical study of lithium after myelosuppressive chemotherapy of patients with testicular cancer (21). The simultaneous schedule was tested to determine if coadministration of the 2 agents would be more effective than sequential administration.

Results in which the 2 agents were administered sequentially to mice bearing the L1210 or P388 leukemias are summarized in Table 1.3 Not shown are results from similar experiments with normal mice since no deaths from toxicity occurred during the 60-day period after treatment. The survival times of tumor-bearing mice treated with vinblastine alone or with vinblastine followed by lithium chloride were similar, indicating that lithium treatment did not enhance or reduce the activity of vinblastine against either the L1210 or the P388 leukemia. The deaths of vinblastine-treated animals (Groups C and D) which occurred against either the L1210 or the P388 leukemia. The deaths of vinblastine-treated animals (Groups C and D) which occurred before Day 6 (Experiment 1) or Day 10 (Experiment 2) were attributed to the greater vulnerability of leukemic mice, relative to normal mice, to the toxic effects of vinblastine.

Although the survival data of Table 1 suggested no value in lithium subsequent to vinblastine therapy, examination of the effects of the sequential schedule on peripheral WBC suggested otherwise. Animals were given i.p. implants of 105 L1210 cells and treated with drug as outlined in Table 1, and peripheral WBC and differentials were performed 1, 3, 5, 7, and 9 days after tumor injection. The changes in polymorphonuclear counts are demonstrated in Chart 2. Lithium treatment alone had no effect on the number of granulocytes in peripheral blood. Although animals treated with vinblastine alone and with lithium plus vinblastine had similar nadirs of polymorphonuclear cell counts between Days 3 and 5, those animals that were treated with both agents exhibited a greater recovery of granulocyte counts. The increase observed on Day 9 in the latter group was well above that seen in the other treatment groups, suggesting that the rebound of granulocyte count was enhanced by lithium.

The effects of simultaneous administration of lithium chloride

3 Although a report in the literature (20) suggests that the P388 leukemia is more responsive to vinblastine therapy than is the L1210 leukemia, the McEachern Laboratory lines were similarly affected by vinblastine treatment. Accordingly, the L1210 leukemia line, which can be propagated in vitro, was used for detailed studies.
C. E. Cass et al.

Table 1
Effects of sequential administration of vinblastine and lithium chloride to leukemic mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Vinblastine (mg/kg)</th>
<th>LiCl (mg/kg)</th>
<th>Survival time (days)</th>
<th>Median survival time (days)</th>
<th>Range (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>6.4 ± 0.2b</td>
<td>6</td>
<td>6–8</td>
</tr>
<tr>
<td>B</td>
<td>0 +</td>
<td>0</td>
<td>7.4 ± 2.4</td>
<td>7</td>
<td>6–10</td>
</tr>
<tr>
<td>C</td>
<td>+ 0</td>
<td>0</td>
<td>9.1 ± 3.9</td>
<td>9</td>
<td>4–12</td>
</tr>
<tr>
<td>D</td>
<td>+ +</td>
<td>0</td>
<td>9.4 ± 2.3</td>
<td>10</td>
<td>10–12</td>
</tr>
</tbody>
</table>

| Experiment 2c   |                    |              |                      |                            |              |
| A               | 0                  | 0            | 11.0 ± 1.7           | 10                         | 10–14        |
| B               | 0 +                | 0            | 10.5 ± 0.8           | 10                         | 10–12        |
| C               | + 0                | 0            | 13.0 ± 7.2           | 17                         | 4–24         |
| D               | + +                | 0            | 13.3 ± 7.8           | 16                         | 8–20         |

a Twenty-five mice/treatment group.
b Mean ± S.D.
c Twelve mice/treatment group.

and vinblastine on survival of normal and leukemic mice are presented, respectively, in Tables 2 and 3. Lithium treatment did not change survival of normal mice treated with moderately toxic levels of vinblastine (Table 2). When leukemic mice were treated with the 2-drug combination (Table 3), the animals which received vinblastine plus the higher lithium doses (180 or 160 mg/kg) exhibited survival times only slightly greater than those of animals which received vinblastine alone; the animals which received lithium doses one-tenth lower (Experiment 2) exhibited survival times similar to those of mice treated with vinblastine alone. Treatment of leukemic mice with lithium alone was without effect.

The results of the preceding studies indicated that, while lithium treatment of leukemic mice did ameliorate somewhat the myelosuppressive effects of vinblastine, the antitumor activity of vinblastine was not significantly altered. Studies of the combined effects of lithium and vinblastine on cultured cells (see below) also indicated that lithium did not counteract the cytotoxic effects of vinblastine.

Because lithium has been reported to affect a variety of physiological and cellular processes (15), its activity, alone and in combination with vinblastine, was examined in cultured L1210 leukemia and murine neuroblastoma cells. The objective was to determine if lithium, at pharmacological levels, has

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Vinblastine (mg/kg)</th>
<th>LiCl (mg/kg)</th>
<th>Survival time (days)</th>
<th>Median survival time (days)</th>
<th>Range (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.2 ± 0.6b</td>
<td>7.0</td>
<td>6–8</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>10.8 ± 0.5</td>
<td>11.0</td>
<td>9–12</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>11.2 ± 0.6</td>
<td>11.0</td>
<td>10–12</td>
<td></td>
</tr>
<tr>
<td>Experiment 2c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>6.5 ± 0.3</td>
<td>6.5</td>
<td>6–7</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>18</td>
<td>6.8 ± 0.7</td>
<td>7.0</td>
<td>6–8</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>18</td>
<td>10.2 ± 0.6</td>
<td>10.5</td>
<td>9–11</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>11.2 ± 0.2</td>
<td>11.0</td>
<td>11–12</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
<td>10.5 ± 0.5</td>
<td>10.5</td>
<td>10–11</td>
<td></td>
</tr>
<tr>
<td>Experiment 3c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>6.3 ± 0.3</td>
<td>6.0</td>
<td>6–7</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>180</td>
<td>6.2 ± 0.2</td>
<td>6.0</td>
<td>6–7</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>0</td>
<td>9.8 ± 3.0</td>
<td>10.0</td>
<td>6–12</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>180</td>
<td>11.8 ± 0.4</td>
<td>10.0</td>
<td>9–11</td>
<td></td>
</tr>
<tr>
<td>Experiment 4c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>6.5 ± 1.1</td>
<td>6.0</td>
<td>5–8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>160</td>
<td>6.5 ± 1.0</td>
<td>6.0</td>
<td>6–8</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>0</td>
<td>10.0 ± 1.0</td>
<td>10.5</td>
<td>5–12</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>160</td>
<td>11.2 ± 2.2</td>
<td>10.5</td>
<td>8–13</td>
<td></td>
</tr>
</tbody>
</table>

a Injections i.p., every 24 hr for 5 days.
b Mean ± S.D.
c Injections i.p., every 4 hr for 5 days.
d Both animals died on Day 8.

d}
cytostatic or cytotoxic activity as a single agent and to determine whether it counteracts or enhances the activity of vinblastine against cultured cells.

Results from studies of antiproliferative activity against cultured L1210 leukemia cells are illustrated in Chart 3. Exposure to pharmacological levels of lithium had relatively little effect on proliferation rates (Chart 3A), and exposure to lithium (1 mM) together with vinblastine was no more inhibitory than was exposure to vinblastine alone, exhibiting IC50's, respectively, of 3.4 and 3.5 nM.

Concentrations of vinblastine 3 to 4 orders of magnitude higher than the growth-inhibitory levels described in Chart 3 were required to achieve significant reduction of reproductive viability of L1210 cells (Tables 4 and 5; Chart 4). For example, after a 4-hr exposure to 50 μM vinblastine, viability was reduced by about 80% in the cloning assay (Table 4) and by more than 90% in the tumorigenicity assay (Table 5). Increasing the length of exposure to vinblastine (Chart 4) increased the cell kill significantly; <1, 3, and 4 log kills, respectively, were obtained after exposures of 4, 24, and 48 hr to 5 μM vinblastine (48-hr data not shown). Although vinblastine exposures of long duration were much more effective in reducing viability, shorter pulse exposures (e.g., 4 hr) were used in viability experiments because pharmacokinetic studies in humans (12) suggest that short exposures more closely resemble the situation during clinical use of vinblastine. Also, our measurements of serum levels of lithium in mice (Chart 1) had indicated that in vivo exposures to significant levels of lithium would be of limited duration.

The effect of lithium on viability was slight. Exposure of L1210 cells to 1 mM lithium for 4 hr reduced viability in the cloning assay by about 10% (Table 4). The combined effects of lithium and vinblastine on viability appeared to be additive. Results from the tumorigenicity assays (Table 5) also indicated that lithium may contribute somewhat to toxicity during exposure to both agents.

Lithium has been reported to protect isolated microtubules from the depolymerizing effects of vinblastine and colchicine (1), suggesting that it might antagonize vinblastine effects against microtubules in intact cells. Neurite formation by neuroblastoma cells is a vinblastine-sensitive process dependent on polymerization of microtubules and has been used as a biological measure of drug effects on the assembly of microtubules (3, 10, 16–18).

We first examined the effect of vinblastine alone and in combination with 1 mM lithium on proliferation of neuroblastoma cells in monolayer culture (Chart 5). Continuous exposure to concentrations of lithium ranging from 0.01 to 5 mM had no effect, and at 10 mM there was only a slight effect on proliferation (data not shown). The IC50 for neuroblastoma cells treated with vinblastine alone was 15 nM. Addition of an otherwise ineffective level of lithium (1 mM) to culture fluids reduced the vinblastine IC50 to 10 nM, indicating that lithium enhanced the antiproliferative activity of vinblastine only slightly.

Lithium did not antagonize vinblastine inhibition of neurite formation by neuroblastoma cells. In Table 6 are presented results of several experiments that demonstrated the failure of lithium to modulate the effects of vinblastine on the formation of neurites under conditions of serum deprivation. The experiments of Table 6 were conducted using exposure conditions
C. E. Cass et al.

Table 6

<table>
<thead>
<tr>
<th>Vinblastine (nM)</th>
<th>Without LiCl</th>
<th>With LiCl (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>51</td>
<td>66</td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>300</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

*Four cultures per condition; 400 cells scored per culture.

that did not significantly reduce viability, thus avoiding the possibility that failure to form neurites reflected lethal toxicity rather than reversible inhibition by vinblastine of microtubule assembly.

**DISCUSSION**

Lithium carbonate is under investigation clinically as an adjuvant therapy for patients receiving myelotoxic therapy (4, 6, 9, 22, 23). There being no information about the effect of lithium administration on the efficacy of chemotherapy, the current work was undertaken to determine whether lithium therapy directed toward attenuating myelosuppression contributes to or reduces the antitumor activity of vinblastine. Vinblastine was selected for this study because of its myelosuppressive activity and because a recent report suggested that lithium might antagonize the action of vinblastine (1).

The in vivo experiments used a schedule of vinblastine and lithium similar to that used to attenuate neutropenia in patients being treated for testicular cancer (22). In those patients, a significant reduction in the degree of neutropenia was seen as well as a reduction in febrile complications. The pharmacokinetic data for lithium chloride in the mouse were similar to those reported for the rat (11). The peaking and trailing off of serum lithium concentration after i.p. injection in the mouse differed from the relatively constant serum levels obtained in humans with lithium carbonate administration p.o. (15).

In tumor-bearing mice treated with vinblastine and lithium, no difference could be detected in the degree of neutropenia produced in mice treated with vinblastine alone or with vinblastine plus lithium. However, there was a marked increase in the rebound of polymorphonuclear cells in mice treated with both agents. This rebound leukocytosis may have been the result of enhanced production of colony-stimulating activity (14, 21) or increased proliferation of pluripotential stem cells (8). The increased leukocytosis may have contributed to the slight prolongation of survival noted.

Lithium was ineffective in single-agent chemotherapy against the L1210 or the P388 leukemia. Lithium therapy may have contributed slightly to an increase in survival of mice bearing the L1210 or P388 leukemia and treated with vinblastine. Two schedules of administration of vinblastine were used, a single dose and 4 (or 5) daily doses beginning 24 hr after implantation of tumor. Administration of multiple daily doses of vinblastine was more effective in prolonging survival times of leukemic mice than administration of a single dose. Addition of lithium therapy did not significantly alter the results obtained with the marginal single-dose schedule and enhanced only slightly the results obtained with the more effective multiple-dose schedule of vinblastine administration.

The cytotoxic activity of lithium at levels comparable to those observed in the serum of lithium-treated mice was slight. Pulse exposure of L1210 cells to lithium reduced colony-forming
ability by less than 10% and did not detectably alter tumorigenicity. The failure of in vitro exposure to lithium to significantly reduce reproductive viability was consistent with its lack of therapeutic activity in leukemic mice.

Because lithium antagonizes the effects of vinblastine on isolated microtubules (1), the question arose as to whether it would antagonize vinblastine inhibition of a biological process dependent on microtubules. The formation of neurites by cultured neuroblastoma cells, a process dependent on polymerization of microtubules, was used to assess the effects of lithium and vinblastine on the assembly of microtubules. Lithium did not interfere with disruption by vinblastine of microtubules in intact cells, as evidenced by its failure to alter vinblastine inhibition of neurite formation by cultured neuroblastoma cells. In a brief report, Reiser et al. (13) indicated that nonpharmacological levels of lithium chloride (10 mm) protected neuroblastoma cells from colchicine- but not vinblastine-induced neurite retraction.

In conclusion, lithium did not alter the effects of vinblastine against proliferation, viability, tumorigenicity, or microtubule assembly. We suggest that these agents may be used in combination without direct antagonism by lithium of the effects of vinblastine against proliferating cells. The potential benefit to be derived from lithium therapy appears to be a consequence of lithium stimulation of neutrophil production (14, 21, 23), thereby reducing the myelosuppressive activity of anticancer agents such as vinblastine.

REFERENCES


Effect of Lithium on the Myelosuppressive and Chemotherapeutic Activities of Vinblastine


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/3/1000

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.