Lithium Carbonate Enhancement Of Granulopoiesis In The Beagle Dog

Kelly Greene
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LITHIUM CARBONATE ENHANCEMENT OF GRANULOPOIESIS IN THE BEAGLE DOG

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology at Carroll College, Helena, Montana.

Kelly Greene
March 22, 1983
This thesis for Honors recognition has been approved for the Department of Biology.

Dr. Tom E. Fritz
Dr. John Christenson
Dr. James J. Manion

March 22, 1983
TABLE OF CONTENTS

LIST OF TABLES.................. .iii
LIST OF ILLUSTRATIONS .......... iv
ACKNOWLEDGEMENTS.............. v
ABSTRACT...................... vi
INTRODUCTION AND LITERATURE REVIEW................. 1
MATERIALS AND METHODS........ 4
    Experiment 1 .......................... 4
        Animals ................................ 4
        Hematology............................. 4
        Collection of Marrow Samples ........ 4
        Hemopoietic Stem Cell Analysis .... 5
        Serum Lithium Levels................... 6
    Experiment 2 ......................... 8
RESULTS.......................... 9
    Experiment 1 ......................... 9
    Experiment 2 ......................... 16
DISCUSSION AND CONCLUSION ...... 20
LITERATURE CITED................ 23
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Hematological data of beagle dogs receiving lithium carbonate.</td>
<td>14</td>
</tr>
<tr>
<td>II. Statistical analysis of circulating neutrophils (PMN's) in lithium-treated beagle dogs using a Two-way anova table.</td>
<td>15</td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Absolute number of circulating neutrophils (PMN's) x 10^-3 in control vs. lithium-treated beagle dogs</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>Absolute number of marrow granulocyte-committed colony forming units (CFU-c) in control vs. lithium-treated beagle dogs</td>
<td>11</td>
</tr>
<tr>
<td>3.</td>
<td>Absolute number of white blood cells x 10^-3 in control vs. lithium-treated beagle dogs</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>Number of circulating platelets x 10^-3 in control vs. lithium-treated beagle dogs</td>
<td>13</td>
</tr>
<tr>
<td>5.</td>
<td>Absolute number of circulating neutrophils (PMN's) x 10^-3 in irradiated control dog vs. lithium-treated irradiated dogs</td>
<td>18</td>
</tr>
<tr>
<td>6.</td>
<td>Number of circulating platelets x 10^-3 in irradiated control dog vs. lithium-treated, irradiated dogs</td>
<td>19</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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ABSTRACT

The effects of lithium carbonate on granulopoiesis in the beagle dog and the protection offered these dogs from radiation-induced marrow injury were investigated. Comparisons were made between control and lithium-treated dogs concerning marrow granulocyte-committed colony forming units (CFU-c) and peripheral blood counts. The results did not support previous studies and demonstrated no enhancement of granulopoiesis in the lithium-treated dogs.
INTRODUCTION AND LITERATURE REVIEW

Lithium carbonate has recently come into increasing use in the treatment of patients with acute mania and recurrent manic-depressive episodes. A side effect of this lithium therapy has been an association with a reversible increase in the number of circulating neutrophils (PMN's) (3,19,25). There is evidence that lithium increases granulocyte production rather than merely shifting granulocyte distribution (27,32).

In addition to the enhanced granulopoiesis, some studies have shown a significant increase in the number of circulating platelets in patients receiving lithium (3,24).

The mechanism of the granulopoietic effect is not fully understood. The regulator system for granulocyte production is extremely complex -- and it is possible lithium may have multiple effects. Several animal studies have suggested that lithium may have an effect on the pluripotential hematopoietic stem cell (9). The fact that the granulocyte and thrombocyte counts are elevated in these patients, whereas the erythrocyte and reticulocyte counts remain normal suggests that lithium either exerts a stimulator effect on granulocyte and thrombocyte
precursors in the bone marrow or preferentially influences cells in the stem cell compartment to differentiate to precursors of granulopoiesis and thrombopoiesis (3).

Lithium also has an established effect on increasing granulocyte production by stimulating formation of colony stimulating activity (CSA) (8,9,11,14,16,17,19,23,31,33). CSA has been characterized as a hormone-like glycoprotein produced by monocytes, macrophages and activated T cells. It is required for in vitro (and perhaps in vivo) proliferation and differentiation of granulocyte precursors (9-11,14,19,23,26,31,33). Richman et al, (3) suggest that the stimulatory effects of lithium on marrow granulocyte-committed colony forming units (CFU-c) are most effective when CSA is suboptimal. This result may be explained in part by the recent data showing the dual role of the macrophage/monocyte in regulation of granulopoiesis (25). When CSA levels are low, macrophages produce CSA. When CSA levels are high, macrophages produce prostaglandins which inhibit the action of CSA. Lithium may act at some point in this regulatory pathway (23).

It has been suggested that the stimulatory effects of lithium are most pronounced in patients receiving cytotoxic therapy for malignant tumors. Under such therapy the drugs have the undesirable effect of producing myelosuppression and significant thrombocytopenia (Richman - personal communications). In studies done by Richman,
patients with ovarian carcinoma received lithium during alternate cycles of chemotherapy (23). It was determined that lithium significantly decreased chemotherapy-induced suppression of leucocytes, granulocytes and platelets. The effect on platelets was significant only when thrombocytopenia was produced (24).

These results suggest several potential clinical applications. It has been proposed that lithium be used in reversing the neutropenia associated with chemotherapy and radiotherapy, thereby decreasing the chances for infection and permitting longer and more intensive therapy. Lithium may be particularly helpful in patients with poor marrow tolerance who would otherwise receive reduced chemotherapy doses. Since dose is one of the most important determinants of response to chemotherapy, lithium could possibly increase dose tolerance and thus the chance of responding to chemotherapy in these patients (5,24).

The goal of this investigation was to determine the effects of lithium on marrow granulocyte-committed colony forming units and peripheral blood counts in the beagle dog and the protection offered these dogs from radiation-induced marrow injury.
MATERIALS AND METHODS

Experiment 1

**Animals:** Eight normal and healthy outbred beagles of both sexes, approximately 3-year-old, were selected from the closed Argonne National Laboratory colony (21). Five of the eight dogs were used in previous lithium studies; however, as shown by the two pre-exposure serum lithium levels, all dogs were at normal lithium levels prior to the start of the experiment (6). Six dogs were assigned to the experimental group and the remaining two served as controls. All dogs were handled in an identical fashion, caged in an anteroom adjoining the radiation facility. The animals received water ad libitum and were given 150 g of standard dog food twice a day with each dosage of lithium; food was withheld overnight.

**Hematology:** Complete blood counts were performed bi-weekly. All analyses (cell counting, sizing and calculations) were performed electronically (Coulter Counter Model S) except for the platelet counts which were done either by phase microscopy or electronically (Ultra-Flo 100, Clay Adams).

**Collection of Marrow Samples:** Marrow samples were
collected from the dogs once a week. The technique was performed under general anesthesia. The iliac crest cells were withdrawn with pediatric spinal needles into syringes containing 1-2 ml of EDTA. Approximately 15 ml of bone marrow were collected.

**Hemopoietic Stem Cell Analysis:** Marrow granulocyte-committed colony forming units (CFU-c) were assayed according to the soft agar cloning technique (26). The marrow was washed once in Hank's wash and was then passed through a glass wool column. Mononuclear enriched cell fractions were obtained by a single ficol-gradient centrifugation step. Nucleated cell counts were made using the Coulter S and diluted to 2x10^6 cells. Marrow cells were plated out in concentrations of 1x10^5 and 2x10^5 nucleated cells/ml in 1 ml of complete cloning medium (TC 199 media supplemented with 0.3% agar) (18). The cells were cultured in 35 mm plastic tissue culture dishes containing a one-ml feeder layer (TC 199 media, 10^6 peripheral blood cells obtained from a control dog, 10% serum from a lethally irradiated dog, 15% - 0.3% agar) (18). All specimens were cultured in quadruplicate and incubated at 37 C in a fully humidified 5% CO_2 atmosphere. Colonies were counted using an inverted tissue culture microscope and defined as aggregates of greater than 50 cells after 7 days of incubation.

**Lithium Administration:** Baseline peripheral blood
studies were obtained on three separate occasions and marrow CFU-c counts were obtained on two separate occasions on each dog prior to lithium administration. The minimum and maximum therapeutic lithium levels in beagle dogs range from 0.3 mEq/l to 2.0 mEq/l -- although values between 0.5 and 1.5 mEq/l are considered adequate (1). Above 1.6 mEq/l side effects such as discomfort, ataxia and thirst have been reported to appear, followed by nausea, vomiting, abdominal pain and diarrhea. Levels above 3.5 mEq/l are considered as lethal (22). The dogs received the lithium in the form of lithium carbonate enclosed in gelatin capsules. The control dogs received empty gelatin capsules. Initially, all experimental dogs received 150 mg of lithium carbonate twice daily for a total of 300 mg. After 2 wk of receiving lithium carbonate and bi-weekly monitoring of serum lithium levels, the dosages of dogs #3811, 3840 and 3843 were raised to 600 mg a day in attempts to achieve therapeutic levels. Dog #3845 had its dosage raised to 450 mg and dogs #3841 and 3873 remained at 300 mg a day. On day 30, dogs #3811 and 3843 had their dosages reduced to 450 mg a day for the remainder of the experiment.

**Serum Lithium Levels:** Serum lithium levels were determined by flame photometry, using the atomic emission spectrophotometer. In flame photometry, the metal is excited from the energy imparted to it thermally by
the flame. As it returns to the ground state it emits radiation at a characteristic wavelength. This radiation is then isolated by a monochromator and subsequently its intensity is directly proportional to the concentration of the element present.

A standard curve for lithium was prepared at concentrations of 5, 10, 15 and 20 ml lithium in a control-dog serum base. A hollow cathode was used as the light source. A wavelength of 690.9 nm was utilized -- the most sensitive emission wavelength for lithium. An air-acetylene, oxidizing flame was used with a slit width of approximately 160 mm and a bandpass of 0.5 nm.

Lithium is unique among drugs in that blood level determinations are clinically applicable and can be rapidly and accurately obtained. This is possible, in part, because lithium is neither metabolized nor protein bound. Blood levels are, of course, only an approximation of drug concentration in the central nervous system (12). In order to minimize a major variable that affects serum levels, it is important to standardize the timing of the sample. Following a dose of lithium carbonate, blood levels rise to a peak at 2-4 hr and absorption is complete in 6-8 hr and samples should be obtained well beyond this period at a time when the change in level is more gradual. An interval of 12 hr after the last dose has arbitrarily been accepted as the optimal
interval (12). Due to laboratory schedules, the time interval for blood drawing for this experiment was approximately 16 hr.

Experiment 2

Three of the original eight dogs were chosen for this experiment. Two of the dogs had been receiving 300 mg of lithium carbonate daily for 21 days and were at therapeutic levels. The third dog served as a control dog with no previous exposure to lithium. All three dogs were exposed for 20 min in a 60cobalt gamma-ray field and received a midline, bilateral exposure of 200 rads. Six complete hemograms were obtained for each dog over a period of 15 days. Two marrow CFU-c analyses were also obtained during this period.
RESULTS

Experiment 1

The lithium carbonate treatment of these dogs did not result in any significant increase of peripheral blood PMN's or marrow CFU-c. The mean data of all eight dogs (control vs. treated) are shown in Table I and are depicted graphically in Fig. 1-4.

When analyzed by the Two-way anova with unequal but proportional subclass numbers test (Sokal and Rolfe, 1966) there was no significant difference in PMN, CFU-c or circulating platelet values between the control group and the experimental group. (F=0.01 for all tests) The anova table for the PMN values is shown in Table II.

When analyzed by the non-parametric Friedman two-way analysis of variance by ranks test (Siegel, 1956) the PMN, CFU-c and circulating platelets data were all found to be significant. (p=0.02 for all tests)

Using the two-way anova test compensates for the unequal sizes in the control group and experimental group allowing for proportionality of subclasses. The treated mean squares are tested over the error variance for significance (29). The Friedman analysis of variance used by Rossof in his study with mongrel dogs (26) does
Fig. 1

Absolute number of circulating neutrophils (PMN's) $\times 10^{-3}$ in control vs lithium-treated beagle dogs

Values represent mean of six lithium treated dogs.

Values represent mean of two control dogs.
Fig. 2

Absolute number of marrow granulocyte-committed colony forming units (CFU-c) in control vs. lithium-treated beagle dogs

--- Values represent mean of six lithium treated dogs.
-- Values represent mean of two control dogs.
Fig. 3

Absolute number of white blood cells $\times 10^{-3}$ in control vs. lithium-treated beagle dogs

---Values represent mean of six lithium-treated dogs.

---Values represent mean of two control dogs.
Fig. 4

Number of circulating platelets $\times 10^{-3}$ in control vs. lithium-treated beagle dogs

Values represent mean of six lithium-treated dogs.
Values represent mean of two control dogs.
Table 1.

Hematological data of beagle dogs receiving lithium carbonate

<table>
<thead>
<tr>
<th>Day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Px</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>12</th>
<th>14</th>
<th>19</th>
<th>21</th>
<th>26</th>
<th>28</th>
<th>33</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-c&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0-*</td>
<td>42°</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42</td>
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<td></td>
<td></td>
<td></td>
<td>-0-*</td>
<td>8°</td>
</tr>
<tr>
<td>PMN/ul</td>
<td>4917</td>
<td>5760</td>
<td>5805</td>
<td>6200</td>
<td>5652</td>
<td>5229</td>
<td>5305</td>
<td>5623</td>
<td>4689</td>
<td>4962</td>
<td>7212</td>
<td>6399°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6136</td>
<td>5863</td>
<td>7928</td>
<td>6376</td>
<td>5704</td>
<td>5317</td>
<td>6167</td>
<td>5156</td>
<td>5928</td>
<td>5600</td>
<td>7105</td>
<td>6177°</td>
<td></td>
</tr>
<tr>
<td>WBC/ul&lt;sup&gt;1&lt;/sup&gt;</td>
<td>9.7</td>
<td>8.3</td>
<td>9.0</td>
<td>9.0</td>
<td>9.9</td>
<td>8.7</td>
<td>8.5</td>
<td>8.8</td>
<td>8.7</td>
<td>7.6</td>
<td>6.9</td>
<td>8.3</td>
<td>8.4°</td>
</tr>
<tr>
<td>x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>9.0</td>
<td>8.8</td>
<td>9.0</td>
<td>11.4</td>
<td>9.2</td>
<td>8.4</td>
<td>7.8</td>
<td>8.4</td>
<td>8.5</td>
<td>7.8</td>
<td>8.0</td>
<td>6.7</td>
<td>6.4°</td>
</tr>
<tr>
<td>Plts</td>
<td>390</td>
<td>438</td>
<td>428</td>
<td>396</td>
<td>402</td>
<td>404</td>
<td>413</td>
<td>414</td>
<td>40C</td>
<td>355</td>
<td>35E</td>
<td>389</td>
<td>341°</td>
</tr>
<tr>
<td>x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>364</td>
<td>343</td>
<td>352</td>
<td>353</td>
<td>354</td>
<td>388</td>
<td>307</td>
<td>387</td>
<td>35E</td>
<td>302</td>
<td>28C</td>
<td>307</td>
<td>305°</td>
</tr>
<tr>
<td>Li</td>
<td>.004</td>
<td>.51</td>
<td>.54</td>
<td>.60</td>
<td>.39</td>
<td>.44</td>
<td>.84</td>
<td>.41</td>
<td>.67</td>
<td>.77°</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mEq/1</td>
<td>.001</td>
<td>.0007</td>
<td>.0007</td>
<td>.0007</td>
<td>.0007</td>
<td>.0007</td>
<td>.002</td>
<td>0.0</td>
<td>.002</td>
<td>.0015°</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Day after lithium carbonate treatment

<sup>b</sup>Number of marrow granulocyte-committed colony forming units

°Mean of six lithium-treated dogs

°°Mean of two control dogs

*Contaminated cultures
Table II.

Statistical analysis of circulating neutrophils (PMN's) in lithium-treated beagle dogs using a Two-way anova table

<table>
<thead>
<tr>
<th>ANOVA TABLE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroups</td>
<td>23</td>
<td>3.97 x 10^7</td>
<td>1.726 x 10^6</td>
<td></td>
</tr>
<tr>
<td>B rows (days)</td>
<td>11</td>
<td>2.75 x 10^7</td>
<td>2.5 x 10^6</td>
<td>1.3256 ns*</td>
</tr>
<tr>
<td>A columns (treats)</td>
<td>1</td>
<td>3.6 x 10^6</td>
<td>3.6 x 10^6</td>
<td>1.9089 ns*</td>
</tr>
<tr>
<td>A x B interaction</td>
<td>11</td>
<td>8.6 x 10^6</td>
<td>7.8182 x 10^5</td>
<td>.414 ns*</td>
</tr>
<tr>
<td>w/in subgroups (error)</td>
<td>71</td>
<td>1.339 x 10^8</td>
<td>1.8859 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


*not significant
not take into account any type of control. Rossof could only conclude from his analysis that there was significant differences in PMN and CFU-c values between days. One cannot conclude that the post-treatment values were higher than the baseline values. As shown by my data, this same significant difference exists between days in control dogs receiving no lithium carbonate (Carnes - personal communications).

During the lithium treatment period the serum lithium levels in the individual dogs ranged from 0.3 mEq/l to 1.9 mEq/l. The mean value of the six dogs however, was within therapeutic range by Day 2 of the experiment and remained within this range for the entire 35 days of the experiment.

Experiment 2

The lithium carbonate treatment of these dogs offered no apparent protection from radiation-induced marrow injury. Although the mean baseline PMN's, WBC's and circulating platelets were higher in the treated dogs than in the control dog, they did not remain higher during the experiment. Prompt reduction of PMN's and circulating platelets followed radiation in all three dogs with the nadirs for PMN's reached at the 7th post-radiation day in dogs #3774 and 3841, and the 12th post-radiation day in dog #3873. All three dogs had
begun a slight recovery of PMN's by the end of the experiment. The data on PMN's and circulating platelets are depicted graphically in Fig. 5 and 6.
Fig. 5

Absolute number of circulating neutrophils (PMN's) x $10^{-3}$ in radiated control dog vs. lithium-treated radiated dogs

---

- • Lithium-treated dogs.
- ○ Control dog
Fig. 6

Number of circulating platelets $\times 10^{-3}$ in irradiated control dog vs. lithium-treated irradiated dogs

---Lithium-treated dogs.
---Control dog.
DISCUSSION

In the mongrel dog, others have suggested that administration of lithium results in an increase of peripheral blood PMN's and CFU-c (9,26). My results do not support this conclusion.

It is difficult to establish a "normal" CFU-c value due to the large variability between dogs. The "normal" values in one control dog ranged from 28 to 39 colonies whereas in the other control dog the "normal" ranged from 42 to 60 colonies. Aspirates were performed on the dogs once a week to prevent depletion of the marrow. This did not allow for a great deal of data to be obtained in the limited time available. Contamination of cultures also complicated this aspect of the research. Analysis of the data obtained showed no significant increase in CFU-c in lithium treated dogs. These results are confirmed in studies done by Richman in 1983 (10) and in those done by Hammond and Dale in 1982 (24).

Complete blood counts were obtained bi-weekly with no increase noted in either PMN's or platelets. These results might be explained by Richman's suggestion that these dogs were not experiencing any type of neutropenia or thrombocytopenia and therefore any possible effects
of lithium would not be noticeable (Richman - personal communications). These effects should appear however in dogs sustaining marrow injury due to radiation. My results from Experiment 2 do not support this hypothesis. Unfortunately, I was not able to continue the experiment to the point at which all dogs had reached a complete marrow recovery. At termination of the experiment, both the PMN's and circulating platelets were still suppressed.

The mean baseline serum lithium level remained within the therapeutic range, however, there were large fluctuations in the individual dogs throughout the experiment and dosages were adjusted accordingly. In comparison to humans, the individual tolerance level in dogs seems to be much greater than that of humans. The average dosage in human lithium treatment is approximately 900 mg daily. Although there are slight fluctuations, dosage adjustment, if any, is slight (Richman - personal communications).

Although lithium administration may indeed prove to be useful in preventing chemotherapy or radiation-induced myelosuppression, its general use may in fact be quite limited. Other studies have shown many complications in connection to lithium toxicity (26). Gastrointestinal symptoms attributed to lithium include nausea, vomiting, and tremors. These side effects are quite common during the course of lithium therapy and tend
to be more frequent in the initial phases and tend to be both mild and reversible. Lithium appears to be well tolerated by the cardiovascular system with benign, reversible T-wave changes the most common change in ECG noted. The effects of lithium on water metabolism and electrolyte balance are complex. Lithium, a monovalent cation, alters the metabolism of both sodium and potassium. It also has certain similarities to calcium and magnesium and can alter their metabolism by ion substitution. Lithium produces short-term sodium and potassium (and water) diuresis. In addition, the most common side effect is the reduction of renal concentrating capacity which gets worse over the years (13). Over 95% of an ingested dose of lithium is excreted from the body by the kidney. Renal clearance is normally about 15-22cc/min and this may be reduced during lithium intoxication to values of 5 cc/min and less (12).

In my study with mongrel dogs I observed none of the above-mentioned side effects and all dogs were able to remain on lithium for the entire 35 days of the experiment.

In conclusion, from the contrasting results available in lithium treatments, it is apparent that additional controlled trials will be needed to define the overall benefits vs. risks of lithium administration. Adding a drug with many intrinsic side effects to chemotherapy which already has significant toxicity may not prove beneficial for every patient.


3. Carnes, Bruce. Personal communications, Statistician at Argonne National Laboratory, Division of Biology.


6. Fritz, T.E. (Personal Communication.)


