Effects of lithium carbonate on carboplatin-induced thrombocytopenia in dogs

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Objective—To describe the effects of lithium carbonate on thrombopoiesis in clinically normal dogs and in dogs treated with carboplatin.

Animals—18 young adult sexually intact female Beagles.

Procedures—Dogs were assigned to each of 3 treatment groups (6 dogs/group). Group 1 received 150 mg of lithium carbonate (14 to 16 mg/kg), PO, every 12 hours on days 1 through 21. Group 2 received carboplatin (300 mg/m², IV) on day 0 and cephalexin (30 mg/kg, PO, q 12 h) on days 14 through 21. Group 3 received lithium, carboplatin, and cephalexin at the aforementioned doses and schedules. Plasma lithium and blood platelet concentrations were measured on days 0, 2, 4, 7, 9, 11, 14, 16, 18, and 21. Number of megakaryocytes in bone marrow specimens and the percentage of large unstained cells and CD34+ mononuclear cells in bone marrow aspirates were determined on days 0, 7, 14, and 21 by manual enumeration, automated hematologic analysis, and flow cytometric immunophenotyping, respectively.

Results—Plasma lithium concentrations ranged from 0.12 to 2.41 mmol/L. All dogs given lithium achieved a concentration within the target interval of 0.5 to 1.5 mmol/L by days 4 to 7. Thrombopoiesis was increased in dogs receiving lithium alone. All dogs given carboplatin developed mild thrombocytopenia. There were no differences between group 2 and group 3 throughout the study.

Conclusions and Clinical Relevance—Lithium stimulated thrombopoiesis in clinically normal dogs. Lithium administration at the doses and schedules used, with concurrent administration of cephalexin, did not prevent thrombocytopenia induced by carboplatin. (Am J Vet Res 2010;71:555–563)
kines are being developed for this purpose. The only such drug currently available is recombinant human interleukin-11. This drug stimulates platelet production in clinically normal and myelosuppressed dogs, but in the authors’ opinion, the drug is too expensive for routine use in veterinary medicine. Furthermore, although immunologic reactions limiting therapeutic benefit have not been detected in dogs, this is probably because studies involving daily drug administration were restricted to ≤14 days. Immunologic reactions are likely to be seen with prolonged or repeated use, as has been reported with the use of other human hematopoietic growth factors in dogs.

Lithium carbonate, a psychomodulating drug used to treat manic-depressive illness in humans, can increase neutrophil and platelet concentrations. This effect is probably a result of direct stimulation of hematopoietic cells and enhanced activity of hematopoietic colony-stimulating factors, but the exact mechanisms of action are not fully understood. This observation prompted investigations into the use of lithium as a treatment for chemotherapy-induced neutropenia in the 1970s and 1980s. Investigations on the use of lithium ceased as attention shifted to the granulopoietic cytokines, such as granulocyte colony-stimulating factor and GM colony-stimulating factor. Some interest in the use of lithium as a thrombopoietic agent continued, in part because of the slower development of more specific thrombopoietic agents. There has been a renewed interest in the use of lithium because, in contrast to hematopoietic cytokines, it is inexpensive and may be given orally.

Lithium has been used with some success as a hematopoietic stimulant in dogs with congenital cyclic hematopoiesis, idiopathic megakaryocytic hypoplasia, chemotherapy-induced myelosuppression, and estrogen-induced myelosuppression. This effect could potentially be exploited as a cost-effective approach for use against thrombocytopenia associated with chemotherapy.

The purposes of the study reported here were to evaluate the use of lithium as a thrombopoietic stimulant in clinically normal dogs and in dogs treated with carboplatin. Although the thrombocytopenia induced by carboplatin is not often clinically relevant, carboplatin was selected and used as a proof-of-principle agent to determine whether lithium could ameliorate thrombocytopenia induced by chemotherapeutic drugs.

**Materials and Methods**

**Animals**—Eighteen healthy young adult sexually intact female Beagles were enrolled in the study. Body weight of each dog was between 9.5 and 11 kg. Dogs were fed a standard commercial diet twice daily; no other food was offered because dietary sodium content may affect serum lithium concentrations. All procedures met guidelines established by the Canadian Council on Animal Care. The study was approved by the Animal Care Committee at the University of Guelph and was performed in accordance with the Animals for Research Act (Ontario, 1980).

**Experimental design**—Dogs were assigned by use of a computerized random number generator to each of 3 treatment groups (6 dogs/group). Number of dogs in each treatment group was based on a mean ± SD reference platelet count of 266 ± 73 × 10^9 cells/L and a predicted difference of 150 × 10^9 cells/L (from preliminary data on 2 dogs) on a 2-tailed test for the difference in platelet counts between days 0 and 21 of group 1 and between nadir platelet counts of groups 2 and 3.

Group 1 received lithium alone, group 2 received carboplatin and an antimicrobial, and group 3 received a combination of lithium and carboplatin as well as an antimicrobial. Dogs of group 1 received 150 mg of lithium carbonate (14 to 16 mg/kg) PO every 12 hours on days 1 through 21, with the intention of achieving a concentration within the target therapeutic interval of 0.5 to 1.5 mmol/L. Dogs of group 2 received carboplatin (300 mg/m^2, IV) during a 15-minute period on day 0. Dogs of group 3 received lithium and carboplatin at the same doses and times as for groups 1 and 2. The first dog treated with carboplatin in group 2 had a neutrophil count of 0.01 × 10^9 cells/L on day 14 and had signs consistent with sepsis. This dog received supportive treatment with IV administration of fluids and antimicrobials. Therefore, to prevent development of sepsis in the other dogs treated with carboplatin, all subsequent dogs from groups 2 and 3 were treated prophylactically with cephalexin (30 mg/kg, PO, q 12 h) from day 14 through 21, regardless of their neutrophil counts. This decision was made to avoid illness and stimulation of thrombopoiesis by sepsis. Cephalexin was chosen over a fluoroquinolone because ciprofloxacin may act as a bone marrow stimulant.

Samples for CBCs, bone marrow analyses, serum lithium concentrations, serum biochemical analyses, and urinalyses were obtained from every dog on day 0 before any experimental procedures or treatments were initiated. Thereafter, serum lithium concentration and a CBC were determined on days 2, 4, 7, 9, 11, 14, 16, 18, and 21, and samples for SUN and serum creatinine concentrations, alanine aminotransferase and alkaline phosphatase activities, bone marrow analyses, and urine specific gravity were obtained on days 7, 14, and 21.

**Collection of bone marrow samples**—Dogs were premedicated with acepromazine maleate (0.03 mg/kg, SC) and hydromorphone (0.05 mg/kg, SC) and subsequently anesthetized with propofol (4 to 6 mg/kg, IV, given to effect). Biopsy sites were rotated among several anatomic locations (left humerus on day 0, left ilium on day 7, right humerus on day 14, and right ilium on day 21). A core biopsy specimen was obtained by use of a Jamshidi needle, and the specimen was placed in a fixative solution. The same Jamshidi needle then was used to aspirate 3 mL of bone marrow into a 10-mL syringe that contained 0.2 mL of 1% EDTA. The core biopsy specimens were embedded in paraffin on the day after collection, but sectioning and staining with H&E were performed on batches of specimens.

**Variables analyzed**—Samples for measurement of serum lithium concentrations were obtained immediately before the morning dose of lithium was administered. Serum samples were stored at 4°C for a maximum of 6 days (per manufacturer’s instructions) before submission to a laboratory for measurement of
lithium concentration by use of a direct ion-selective electrode method.\(^{10}\)

Samples for CBC and serum biochemical analysis were submitted to a laboratory\(^8\) and analyzed with automated hematoLogic\(^-\) and biochemical analyzers\(^5\) respectively. Urine specific gravity was measured with a refractometer.\(^4\)

Megakaryocytes in bone marrow specimens were counted at a magnification of 100× in 10 nonoverlapping fields, and the mean number of megakaryocytes per field was calculated. The percentage of LUCs in bone marrow aspirates was measured with an automated hematologic analyzer\(^9\) within 4 hours after sample collection.

The percentage of CD34+ BMMCs was determined by use of flow cytometry. Bone marrow aspirates were separated into 2 flow cytometry tubes; 1 was used as a negative control sample, and the other was used for CD34 monoclonal antibody staining. Erythrocytes were lysed in ammonium chloride buffer\(^1\) and then centrifuged (400 × g for 5 minutes); the supernatant was removed and the cell pellet was loosened. The cells in each tube were washed with 1 mL of flow cytometry buffer solution and centrifuged again (400 × g for 5 minutes). The supernatant was removed and the cell pellet was loosened; then, the cells in selected tubes were incubated with 3 μL of a 1:10 dilution of a monoclonal antibody conjugated to phycoerythrin and directed against the canine CD34 molecule.\(^2\) Samples were incubated at 4°C for 20 minutes, washed with 1 mL of flow cytometry buffer, and analyzed on a flow cytometer.\(^3\)

**Statistical analysis—Lithium concentration, platelet count, neutrophil count, mean number of megakaryocytes per field, percentage of LUCs, and percentage of CD34+ BMMCs were analyzed by use of a generalized linear mixed-model.** Because data were repeatedly measured over time, the Akaike information criterion was used to determine an error structure for the autoregression.

The assumptions of the ANOVA for repeated measures were assessed by comprehensive residual analyses. The Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were conducted to assess overall normality. Residuals were plotted against predicted values and explanatory variables to evaluate patterns in the data that suggested outliers, unequal variance, or other problems. Because the group × day effect was significant, a Tukey post hoc test was used to compare groups at each day, and a Dunnett test was used within each group to compare values for each day with baseline values on day 0. An equation was fitted from this mixed model with predicted values and upper and lower confidence intervals.

Logarithmic transformation was applied to the LUC and CD34+ BMMC results to normalize the data. Individual regression models for platelet and neutrophil counts were fitted to data for each of the dogs over time. Parameters from these fitted curves, which included overall mean across time, linear slope, and quadratic slopes, were compared by use of an ANOVA with a post hoc Tukey test. To specifically evaluate whether lithium stimulated thrombopoiesis in dogs treated with lithium alone, results from group 1 were also analyzed with an ANOVA for repeated measures and post hoc Tukey tests to detect significant differences between platelet concentrations measured on different days.

Areas under the lithium concentration curve were calculated as overall mean values multiplied by 21 days, and the difference between groups 1 and 3 was compared with a Satterthwaite \(t\) test. For all analyses, values of \(P < 0.05\) were considered significant.

**Results**

Lithium was undetectable (concentration < 0.1 mmol/L) in all serum samples analyzed on day 0. Serum lithium concentrations then fluctuated cyclically above and below the lower limit of the target interval (0.5 mmol/L) for all dogs, except for 1 dog in group 3 in which the concentration fluctuated above and below the upper limit of the target interval (1.5 mmol/L; Table 1). There was no significant (\(P = 0.273\)) difference in area under the lithium concentration curve for groups 1 and 3. All dogs in group 1 achieved the target lithium concentration on day 7, whereas all dogs in group 3 achieved it on day 4. The overall mean lithium concentrations for groups 1 and 3 were 0.52 and 0.72 mmol/L,

<p>| Table 1—Serum lithium concentrations in 6 dogs treated with lithium carbonate (14 to 16 mg/kg, PO, q 12 h) on days 1 through 21 (group 1) and 6 dogs treated with carboplatin (300 mg/m², IV) on day 0, lithium carbonate on days 1 through 21, and cephalixin (50 mg/kg, PO, q 12 h) on days 14 through 21 (group 3). |
|---|---|---|---|---|---|---|---|---|---|</p>
<table>
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<th>Group</th>
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<td>0.37 ± 0.14</td>
<td>0.97 ± 0.13†</td>
<td>0.45 ± 0.33§</td>
<td>0.23 ± 0.09</td>
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<td></td>
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<td>2</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>3</td>
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<tr>
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<td>Mean ± SD</td>
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<td>1.02 ± 0.35§§</td>
<td>1.06 ± 0.66¶¶</td>
<td>0.75 ± 0.61¶¶</td>
<td>0.54 ± 0.33*</td>
<td>0.73 ± 0.50*</td>
<td>0.72 ± 0.31¶</td>
<td>0.54 ± 0.51†</td>
<td>0.82 ± 0.88‡‡</td>
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<td>No. of dogs within target interval¶</td>
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<td>0</td>
<td>6</td>
<td>4</td>
<td>3</td>
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<td>3</td>
<td>5</td>
<td>3</td>
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*†,‡,§,¶,‖ Variable differs significantly (* \(P < 0.006\); † \(P < 0.001\); ‡ \(P = 0.023\); § \(P = 0.034\); ¶ \(P = 0.012\) from the value on day 0. ††Target therapeutic interval for lithium concentration was 0.5 to 1.5 mmol/L. | 0.27,‖ Value differs significantly (‖ \(P < 0.001\); †‖ \(P = 0.008\); ††‖ \(P = 0.003\) from the value on day 0. *§§Value differs significantly (§§ \(P = 0.010\)) from the mean value of group 1 on the same day. †¶ Includes 1 dog that had a lithium concentration greater than the upper limit of the target interval.
respectively. Of 108 determinations of lithium concentration, 55 (51%) were > 0.5 mmol/L (lower limit of the target interval) and 79 (73%) were > 0.3 mmol/L (minimum concentration at which hematopoietic stimulation may be detected in peripheral blood cell counts). No adverse reactions were identified in dogs receiving lithium. The SUN and serum creatinine concentrations and alanine aminotransferase and alkaline phosphatase activities remained within reference intervals. No abnormalities were detected in results of urinalyses performed on day 0. Urine specific gravity randomly fluctuated from 1.010 to 1.060 throughout the study.

Platelet counts were within the reference interval (117 X 10^9 cells/L to 418 X 10^9 cells/L) on day 0, with no significant differences among groups. Mean platelet count for group 1 increased over time and was fit to a linear equation (Figure 1). Platelet counts on day 21 were greater than platelet counts on day 0 in all group 1 dogs, and platelet counts were above the upper limit of the reference interval in 4 dogs of group 1 on day 21. Platelet counts in groups 2 and 3 decreased after carboplatin administration and were fit to quadratic equations (Figures 2 and 3). Nadir values were detected on day 11; mean ± SD nadir values were 137 ± 26 X 10^9 cells/L for group 2 and 101 ± 26 X 10^9 cells/L for group 3. There were no significant differences between groups 2 and 3 with respect to mean daily platelet counts or overall mean platelet counts. Mean platelet counts on day 11 differed significantly (P < 0.001) between groups 1 and 2 and between groups 1 and 3. Platelet counts for individual dogs reached values above the upper limit of the reference interval during recovery from myelosuppression in 4 dogs of group 2 and 3 dogs of group 3. Regression lines for all groups were compared (Figure 4). There were no significant differences between groups
2 and 3, but the slopes of groups 2 and 3 differed from that of group 1.

Neutrophil counts were within the reference interval (2.9 × 10⁹ cells/L to 10.6 × 10⁹ cells/L) on day 0, with no significant differences among groups. Neutrophil counts were fit to quadratic equations (Figure 5). Neutrophil counts for all dogs in group 1 remained within the reference interval but had a cyclic pattern with peak values significantly greater than values for day 0 on days 4 (P = 0.014), 9 (P < 0.001), and 18 (P < 0.001). Neutrophil counts for groups 2 and 3 decreased after carboplatin administration, with biphase mean nadir values that differed significantly from values for day 0 on days 7 (group 2, P = 0.022; group 3, P = 0.003) and 14 (P < 0.001 for both groups). Four dogs in group 2 and 5 dogs in group 3 had neutrophil counts < 1.0 × 10⁹ cells/L at the second nadir on day 14.

Mean number of megakaryocytes per field and percentage of LUCs did not change significantly over time in group 1, whereas in groups 2 and 3, values were significantly lower on day 7 but recovered to values similar to those for day 0 on day 14 (Table 2). The percentage of CD34+ BMMCs had a biphasic increase in group 1 on days 7 and 21, whereas the percentage decreased in groups 2 and 3 on day 7 and then increased on day 21. There were no significant associations between the mean number of megakaryocytes per field, percentage of LUCs or CD34+ BMMCs, and platelet or neutrophil counts.

**Discussion**

In the study reported here, we investigated the use of lithium as an agent to stimulate hematopoiesis and ameliorate myelosuppression, in particular thrombopoietic suppression, in dogs treated with carboplatin. There was a modest effect of hematopoietic stimulation when lithium was given to the clinically normal dogs of group 1. Mean platelet counts for dogs of group 1 were significantly increased on days 16 through 21, compared with counts for day 0, as determined by use of an ANOVA. Values for individual dogs were above the reference interval in 4 of 6 dogs. The platelet count was not significantly increased when evaluated by use of regression analysis. However, the regression line reflected changes from days 0 through 21, which thus included days that did not have a significant increase as determined by use of the ANOVA. It is possible that early during the treatment period, sufficient time had

Table 2—Mean ± SD values for mean number of megakaryocytes per field in bone marrow specimens and percentage of LUCs and CD34+ BMMCs in bone marrow aspirates obtained from dogs in groups 1, 2, and 3 (n = 6 dogs/group).

<table>
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<th>Variable</th>
<th>Group*</th>
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<td>1</td>
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<tr>
<td>Mean No. of</td>
<td></td>
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<tr>
<td>megakaryocytes/field</td>
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<tr>
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<td>3.9 ± 1.9</td>
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<td>4.7 ± 1.5</td>
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<tr>
<td>LUCs (%)</td>
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<td>0.031 ± 0.007</td>
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<td>0.025 ± 0.005</td>
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<td>0.032 ± 0.008</td>
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<td>0.030 ± 0.009</td>
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<td>CD34+ BMMCs (%)</td>
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<tr>
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<td>0.988 ± 0.896</td>
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<tr>
<td></td>
<td>2.100 ± 1.340‡</td>
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<td>1.070 ± 1.020‡</td>
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<td>4.055 ± 3.210±</td>
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</table>

*Dogs in group 1 received lithium carbonate (14 to 16 mg/kg, PO, q 12 h) on days 1 through 21, dogs in group 2 received carboplatin (300 mg/m², IV) on day 0 and cephalexin (30 mg/kg, PO, q 12 h) on days 14 through 21, and dogs in group 3 received carboplatin on day 0, lithium carbonate on days 1 through 21, and cephalexin on days 14 through 21. §Value differs significantly from the value for day 0 (P = 0.003) and from the value for group 1 (P = 0.011). ¶Value differs significantly from the value for day 0 (P = 0.003) and from the value for group 1 (P = 0.016). **Value differs significantly from the value for day 0 (P = 0.005) and from the value for group 1 (P = 0.021). †Value differs significantly from the value for day 0 (P = 0.004) and from the value for group 1 (P = 0.016). ‡Value differs significantly from the value for day 0 and does not differ significantly (P = 0.062) from the value for group 1. ††Value differs significantly (P < 0.001) from the value for day 0.
not yet elapsed for lithium to substantially stimulate hematopoiesis. The lack of a significant effect as determined by use of the regression analysis may also have reflected insufficient power attributable to the number of dogs assessed or variation in the responses among dogs. The inconsistent effect of lithium on thrombopoiesis in dogs in our study also has been established in humans with normal bone marrow function. Increases in platelet counts were not detected in some studies\(^{5,6}\) in humans, and in other studies\(^{17,18}\) in which platelet counts did increase, the increase was not clinically relevant, and platelet counts were not consistently above the upper limits of the reference intervals.

To investigate the effect of lithium on thrombopoiesis at an early stage of hematopoiesis, the mean number of megakaryocytes per field was enumerated in histologic sections. Although platelet concentrations increased in dogs of group 1, no significant changes were observed in the mean number of megakaryocytes per field. This suggests that megakaryocyte enumeration is an insensitive test of mild thrombopoietic stimulation. This may be, in part, because megakaryocyte enumeration is probably less precise than is platelet enumeration, such that mild changes in megakaryocyte numbers were not detectable. Megakaryocyte numbers were not measured in other studies of lithium and thrombopoiesis; however, stimulation of CFU-megakaryocyte by lithium has been reported for in vitro\(^{25,27,28}\) and in vivo\(^{26,27,28}\) studies in mice. Initially, CFU-megakaryocyte assays were attempted in our study but were abandoned because of technical difficulties. Another test of thrombopoietic activity that may have been more sensitive than megakaryocyte enumeration is measurement of megakaryocyte ploidy, which was increased in a study\(^{29}\) of interleukin-11 in dogs, but the magnitude of thrombopoietic response in that study was greater than the magnitude of response for the study reported here.

Hematopoietic stimulation in group 1 dogs was also reflected by increased neutrophil counts on days 4, 9, and 18, compared with counts on day 0, but the effect was less than the effect observed for platelets. These findings were similar to those in another report\(^ {30}\) in which lithium also increased the numbers of CFU-GM. Assays for CFU-GM were not performed in the present study, but the percentage of LUCs in bone marrow aspirates was evaluated. This population of cells consists of mononuclear cells identified by use of laser cytometry–based hematologic analyzers.\(^ {46}\) In clinically normal animals, LUCs probably represent mostly myeloperoxidase-negative myeloid cells, and their concentration correlates with granulocyte and monocyte progenitor cells.\(^ {49}\) In our study, the percentage of LUCs did not vary over time for group 1. However, an increase in LUCs may not have been detected because the cells mature quickly and they were only evaluated once each week.

To evaluate early hematopoiesis, percentage of CD34+ BMMCs in bone marrow aspirates was measured. The CD34 antigen is a cellular marker of primitive progenitor cells that have the ability to generate the various blood lineages in large numbers for a prolonged period.\(^ {30}\) In contrast to the mean number of megakaryocytes per field and percentage of LUCs, there was an increase in CD34+ BMMCs in group 1 over time, which indicated that lithium has a stimulating effect on early bone marrow progenitor cells. This is consistent with the observation that lithium stimulates neutrophil, monocyte, and platelet production in dogs with cyclic hematopoiesis\(^ {26,27,47}\); increases CFU-spleen production in mice\(^ {48}\); and increases peripheral blood CD34+ cells in humans.\(^ {31}\) The apparent cyclic increase in CD34+ cells may reflect an influence of lithium on a change in equilibrium between production and differentiation.

To investigate whether the hematopoietic stimulation detected for group 1 dogs would be sufficient to protect dogs against chemotherapy-induced myelosuppression, carboplatin was given to dogs in groups 2 and 3 at a dose of 300 mg/m\(^2\). Carboplatin was chosen because this drug causes acute thrombocytopenia, more so than other commonly used chemotherapeutic agents in veterinary medicine, but is otherwise tolerated relatively well.\(^ {26,27,38}\) A dose of 300 mg/m\(^2\) was chosen because it is the standard recommended therapeutic dose for tumor-bearing dogs, and myelosuppression is commonly observed at this dose.\(^ {26,27,38}\) In another study\(^ {38}\) conducted to evaluate a thrombopoietic agent, investigators used carboplatin at a dose of 350 mg/m\(^2\), and granulocyte colony-stimulating factor was given to prevent profound neutropenia. The lower dose of carboplatin used in the study reported here, without concurrent administration of granulocyte colony-stimulating factor, probably caused sufficient myelosuppression for our investigation of thrombopoiesis and also allowed us to investigate amelioration of neutropenia by lithium. Platelet counts of dogs in group 2 decreased after administration of carboplatin and reached a nadir of 137 ± 38 × 10\(^9\) cells/L on day 11. Following the nadir, a rebound was observed, with thrombocytosis developing in 4 of 6 dogs. These results are comparable to the reported\(^ {48}\) effects of carboplatin on thrombopoiesis in clinically normal dogs. Group 2 dogs also had evidence of myelosuppression for all other measures of different levels of hematopoiesis. The decrease in mean number of megakaryocytes per field in bone marrow sections on day 7 confirmed that carboplatin suppressed thrombopoiesis. The decrease in CD34+ BMMCs on day 7 is consistent with similar observations in humans.\(^ {52}\)

Although lithium is a hematopoietic stimulant, as indicated by changes in group 1 dogs, it did not ameliorate myelosuppression caused by carboplatin administration in our study. There were no differences between groups 2 (carboplatin only) and 3 (carboplatin and lithium) with respect to lithium concentration or any measure of hematopoiesis. Other studies of myeloprotection have yielded conflicting results. Mice given lithium after irradiation\(^ {39,40}\) or administration of cyclophosphamide\(^ {49,55}\) or vinblastine\(^ {49,55}\) had similar nadir values but accelerated recovery of progenitor and peripheral blood cells, compared with results for control mice. Mice given lithium prior to treatment with vinblastine\(^ {49}\) or cyclophosphamide\(^ {49}\) also had accelerated hematopoietic recovery, but in addition, the nadir values of CFU-GM, CFU-megakaryocyte, and peripheral blood cells were not as severe as the nadir values in control mice. There are fewer data for dogs. In a study\(^ {34}\) of 4 dogs treated with cyclophosphamide, 2 dogs given
lithium for 9 days prior to administration of the chemotherapeutic agent had lower nadir values of CFU-GM and neutrophil counts than did the dogs not treated with lithium. Furthermore, the lithium-treated dogs recovered, whereas the dogs not treated with lithium died of myelosuppression. In contrast, in a study\(^6\) of 24 dogs, 12 dogs were treated with vinblastine and 12 with mechlorethamine. In each chemotherapy group, 6 dogs were also given lithium for 6 days prior to administration of the chemotherapeutic agent. There was no difference between lithium-treated and nontreated dogs with respect to CFU-GM or neutrophil counts. Platelet counts were not reported in either of those aforementioned studies.

In most studies of humans treated with lithium because of myelosuppression, there was amelioration of neutropenia, although this did not always result in reduced sepsis.\(^6,28\) \(^29\) Thrombopoietic effects of lithium were less frequently evaluated and inconsistently evident; however, a greater effect was detected in human patients with more severe thrombocytopenia.\(^30\)\(^-\)\(^63\)

To our knowledge, administration of the combination of lithium and carboplatin in domestic animals has not been reported. No complications of lithium administration were detected in our study. Although the nephrotoxic effects of carboplatin are minimal in comparison with those of cisplatin,\(^6\) we were nonetheless concerned that lithium and carboplatin may have nephrotoxic interactions. However, lithium concentrations of group 3 were similar to those of group 1, SUN and creatinine concentrations remained within their respective reference intervals, and the urine specific gravity of dogs varied from minimally to maximally concentrated; therefore, it is unlikely that the treatment caused nephrotoxic effects. The effects of lithium and carboplatin may differ in tumor-bearing dogs.

This study had several limitations. First, the dose of lithium and schedule for administration must be considered. The lithium dose was chosen to provide a target serum concentration of 0.5 to 1.5 mmol/L.\(^6\) This concentration was achieved in all dogs, although concentrations > 0.5 mmol/L were not sustained by any dog, and a cyclic pattern was evident. Even at this dose, lithium concentrations increased to above the upper limit of the target interval in 1 dog, although signs of toxicosis were not observed. The overall mean lithium concentrations in groups 1 and 3 were above the value expected to result in hematopoietic stimulation,\(^6\) although it is possible that the lithium effect may have been more pronounced had lithium concentrations been sustained within the target interval.

The decision to administer lithium after carboplatin was based on current recommendations for the use of other bone marrow stimulants (such as granulocyte colony-stimulating factor and GM colony-stimulating factor)\(^7\)\(^-\)\(^16\) and results of a study\(^6\) of thrombopoietic protection in dogs. Hematopoietic stimulants are typically given after a cytotoxic treatment because of concerns that induction of bone marrow progenitor cells into cycling and differentiation prior to a cytotoxic treatment will increase their susceptibility to injury and exacerbate myelosuppression.\(^6\) However, it has also been reported\(^14\)\(^-\)\(^16\)\(^,\)\(^38\)\(^-\)\(^35\) that hematopoietic stimulation prior to cytotoxic treatment reduces the severity of myelosuppression. Although lithium did not ameliorate myelosuppression in a previous study\(^6\) when given prior to vinblastine and mechlorethamine, it is possible that lithium would have been more effective in our study if it had been given prior to administration of carboplatin, as was reported for administration of cyclophosphamide.\(^34\)

Carboplatin dose and sample size must also be considered. In that other study,\(^16\) on myeloprotection of carboplatin-induced thrombocytopenia in 6 dogs, the number of dogs was sufficient to enable investigators to detect amelioration of thrombocytopenia via an investigational cytokine. In that study,\(^6\) the mean ± SD nadir of thrombocytopenia in the carboplatin-only group was lower (78 ± 38 × 10\(^3\)cells/L) than in our study (137 ± 38 × 10\(^3\)cells/L), and it is possible that a more profound effect of lithium would have been evident had we achieved a higher degree of severity for thrombocytopenia by administration of a higher dose of carboplatin. On the basis of studies\(^59\)\(^-\)\(^63\) in humans, a mean platelet count < 150 × 10\(^9\) cells/L should have been sufficient to detect an effect for lithium; however, the thrombopoietic responses in dogs may differ from those in humans. The lack of a lithium effect in our study was more likely attributable to a relatively weaker potency of lithium. The calculation of sample size was based on a stronger lithium effect. A weak lithium effect may have been detected had a larger number of dogs been used. However, there was complete lack of a positive effect; indeed, there was a trend toward a negative effect, with group 3 dogs having a mean higher lithium concentration than that of group 1 dogs and a lower platelet nadir than that of group 2 dogs. Therefore, any lithium effect would have been unlikely to be clinically relevant.

A final limitation is that antimicrobials were not given to group 1 dogs. Because the purpose of group 1 was description of the effect of lithium on thrombopoiesis in clinically normal, untreated dogs, it was important to avoid any potential effects of other drugs. In contrast, the purposes of groups 2 and 3 were to investigate the protective effect of lithium on thrombopoiesis after chemotherapy. This was impossible to perform without prevention of sepsis by administration of granulopoietic cytokines or antimicrobials, and the results are more clinically relevant because patients with myelosuppression of this degree would be treated with antimicrobials. Serum lithium concentrations did not differ significantly between groups 1 and 3 after cephalexin treatment, which was started on day 14. Cephalexin did not modify the lithium effect (or lack thereof) on the severity of carboplatin-induced myelosuppression because it was not given until after the platelet and neutrophil nadirs had developed. However, it is possible that cephalexin modified the action of lithium on hematopoietic recovery in group 3.

The study reported here confirmed the finding that lithium is a hematopoietic stimulant in dogs. The effect of lithium was mild, which may have been attributable, in part, to the relatively low serum lithium concentrations. Lithium at the dose and administration schedule used, and in conjunction with an antimicrobial, did not protect against carboplatin-induced mild thrombocytopenia or mild to severe neutropenia in clinically normal dogs.
References

31. Case BC, Hauck ML, Yeager RL, et al. The pharmacokinetics and pharmacodynamics of GW395038, a peptide agonist of


