In Vitro Effect of Lithium on Carbamazepine-Induced Inhibition of Murine and Human Bone Marrow-Derived Granulocyte-Macrophage, Erythroid, and Megakaryocyte Progenitor Stem Cells (42837)

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Abstract. **Lithium (Li) is a known agent capable of producing leukocytosis, first observed in manic depressive patients receiving Li as therapy; however, a certain percentage of cases are nonresponsive to Li therapy. These patients are responsive to carbamazepine (CBZ); however, severe hematopoietic side effects have been associated with CBZ treatment such as leukopenia. We report here the results of dose-response studies (0.1–100 μg/ml) that demonstrate CBZ treatment inhibits both murine and human bone marrow-derived granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and megakaryocyte progenitor (CFU-Meg) cells. The addition of Li prior to and simultaneously with CBZ to marrow cultures was effective in reversing the CBZ-induced toxicity only in the presence of an optimal Li dose (1.0 m*M*) known to stimulate bone marrow function. However, when the addition of Li was delayed 24 hr to CBZ-treated cultures no protective effect was observed for any marrow progenitor. Thus, the time of Li-CBZ exposure is critical to observe the protective effect of Li. These results demonstrate that the leukopenia associated with CBZ treatment may be due to the abiity of CBZ to inhibit marrow progenitor cells and suggest Li may be an effective agent to reverse the marrow toxic effects of CBZ.** [P.S.E.B.M. 1989, Vol 190]

The monovalent cation lithium (Li) has been used successfully in the treatment of manic depressive illness for many years (1, 2).It has become well established that a side effect, first noted in these manic-depressed patients, was the appearance of a peripheral blood leukocytosis (3, 4). This leukocytosis effect has led to the use of Li in a wide variety of hematopoietic conditions associated with either depressed or inadequate granulopoiesis, such as Felty's syndrome (5), canine cyclic neutropenia (6), a congenital neutropenia (7), idiopathic granulocytopenia (8), cancer chemotherapy-induced granulocytopenia (9), neuroleptic induced granulocytopenia (10), and aplastic anemia (11).

The capacity of Li to restore hematopoietic bone marrow function following the exposure of toxic agents that either inhibit or depress granulopoiesis has been investigated. Li effectively stimulates the reconstitution of bone marrow hematopoiesis by increasing the number of hematopoietic progenitor cells, regardless of whether the hematopoietic perturbation is a chemotherapeutic drug (12–16) or radiation (17, 18). Although reports fail to demonstrate a role for Li in certain hematopoietic conditions (19–21), these results may be the consequence of inadequate Li dosing. Nevertheless, Li-induced hematopoietic effects are of extreme interest and warrant further study. The hematologic effects of Li and its use in a variety of hematologic conditions have been the subject of recent reviews (22–25).

Although lithium is used in the treatment of recurrent affective disorders to reduce the frequency and severity of manic depressive illness, certain drawbacks do exist, such as its low therapeutic index and the tendency to induce side and toxic effects in some patients. For these reasons, alternative substances have been investigated for their ability to provide the same degree of therapeutic effectiveness with less toxicity.

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Recent studies have indicated that carbamazepine (CBZ), an imipramine-like anticonvulsant, may also be an extremely effective agent in the treatment of a variety of psychiatric conditions (26–29). More recent studies have indicated that for a select group of manic patients, the combined modality of Li-CBZ may be more effective than either treatment alone. Patients receiving CBZ have an earlier time course response compared with patients receiving Li, when their response is often delayed until after several weeks of treatment (30, 31). It has been suggested that this shortened latency of antidepressive episodes occurs when adjuvant Li treatments are used is a reflection of Li potentiation (32).

Although CBZ has gained a growing respectability as an extremely effective treatment for a wide variety of psychiatric conditions, it unfortunately can produce side effects such as leukopenia and aplastic anemia (33). It appears that leukopenia is associated with approximately 2–10% of treated cases (34, 35); therefore, close clinical hematologic monitoring of treated patients is indicated. The mechanism of CBZ-induced suppression of hematopoiesis is currently ill-defined.

The results reported here were designed to determine the effect of CBZ treatment on bone marrow function using cultures of granulocyte-macrophage (CFU-GM), burst-forming erythroid (BFU-E), and megakaryocyte (CFU-Meg) progenitor cells from normal mouse and human bone marrow. Studies were conducted to (i) determine whether the addition of Li to CBZ-treated marrow cells would reverse or ameliorate the level of CBZ-induced hematopoietic suppression or (ii) determine the extent of CBZ toxicity to marrow cells when added 24 hr after marrow cells treated with Li or when the addition of Li was delayed 24 hr after CBZ-treated marrow cells.

Materials and Methods

Murine Bone Marrow. C3H/HeJ male mice, 8–10 weeks of age (The Jackson Laboratory, Bar Harbor, ME), were used as a source of murine bone marrow. They were housed in plastic cages and fed Purina lab chow and water *ad libitum*. Following sacrifice by cervical dislocation, femurs were removed and nucleated cells harvested under sterile conditions. Single-cell bone marrow suspensions were prepared in Iscove's modified Dulbecco's medium (GIBCO, Grand Island, NY) after repeated passage through a 25-gauge needle. Bone marrow cell suspensions were centrifuged at 2000 rpm at 4°C for 10 min followed by resuspension in Iscove's medium. A cell count was determined using a Coulter Counter Model ZBI (Coulter Electronics, Hialeah, FL).

Human Bone Marrow. Human bone marrow cells were obtained from 10 normal marrow donors harvested for the Bone Marrow Transplant Program at the University of Kentucky Medical Center, Lexington, KY. Approval was obtained from the Human Investigation Review Board (protocol 86-00329), University of Kentucky Medical Center. Briefly, unseparated marrow cells suspended in Iscove's medium supplemented with 10% fetal bovine serum (Hyclone) were obtained by centrifugation of whole bone marrow at 1500 rpm for 15 min. Low density marrow cells (i.e., <1.077 g/ml) were collected following separation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ) at 1500 rpm for 30 min. Cells at the interface were collected and washed three times, and then resuspended in Iscove's medium containing 10% heat-inactivated (56°C, 30 min) fetal bovine serum (Hyclone).

Preparation of Nonadherent Bone Marrow Fractions. Both murine and human bone marrow samples were further separated into nonadherent and adherent populations following incubation on plastic tissue culture dishes (Falcon 3003; Becton-Dickinson, Oxnard, CA) for 90 min at 37°C at 5% CO_2. The nonadherent cell fraction was collected by gently swirling the dishes and slowly removing the suspended cells. This nonadherent progenitor cell-enriched fraction was examined for cell viability using the trypan blue exclusion procedure and then used as the source of bone marrow for the following studies.

Carbamazepine, Lithium, and Bone Marrow Cells. To determine the effect of CBZ on bone marrow cells, either murine- or human-derived nonadherent marrow cells were plated at a concentration of 10^5 cells/ml in the presence or absence of CBZ (Tegretol, lot 1T061332, concentration range 0.1–100 μg/ml; Ciba-Geigy, Ardsley, NY). The initial CBZ concentration prepared was 100 μg/ml in 95% ethanol diluted 1/2 in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (Hyclone). The stock working solution of CBZ was 50 μg/ml, with all further dilutions prepared with Iscove's medium. The total volume of CBZ added to obtain the final concentration per milliliter of culture was 10 μl. Control plates contained the ethanol diluent in Iscove's medium. To further examine the ability of CBZ to influence bone marrow colony formation, additional marrow cells were also cultured in the presence of Li (ultrapure, lithium chloride; Alpha Products, Danvers, MA) at three concentrations based upon previous data demonstrating the ability of Li to increase CFU-GM and CFU-Meg colony formation; i.e., a suboptimal dose, 0.5 m*M*; an optimal dose, 1.0 m*M*, and a toxic dose, 5.0 m*M* (54). Suitable control groups consisted of only added Li or phosphate-buffered saline. To further determine the combined effect of CBZ and Li on bone marrow cells, additional marrow cell cultures were plated in the presence of either CBZ or Li alone. After 24 hr of incubation at 5% CO_2 and 37°C, cultures were removed and Li (0.5, 1.0, or 5.0 m*M*) was added to marrow cultures that had received CBZ 24 hr earlier or CBZ (0.1, 0.5, 1, 5, and 10 μg/ml) was added to marrow cultures that had received Li 24 hr earlier. The culture plates were returned to the

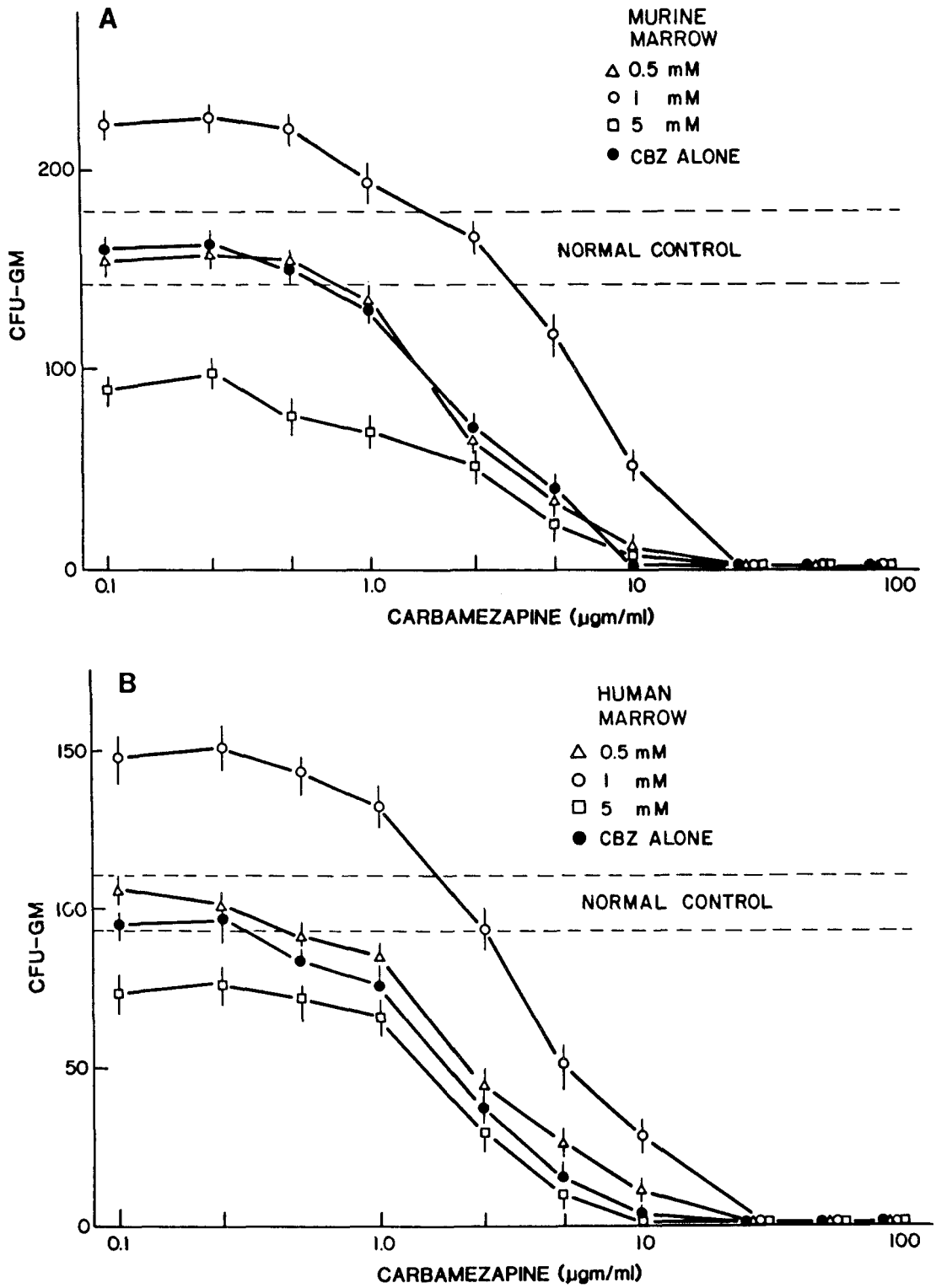

Figure 1. A, Dose-response effect of added CBZ and lithium chloride (LiCl) (0.5, 1.0, and 5.0 m*M*) on murine bone marrow CFU-GM colony formation (±SEM from triplicate studies). B, Dose-response effect of added CBZ and LiCl (0.5, 1.0, and 5.0 m*M*) on human bone marrow CFU-GM colony formation (±SEM from triplicate studies).

incubator for the remainder of their incubation period until they were removed and scored for CFU-GM.

Assay for CFU-GM. The method for cloning CFU-GM was reported previously from this laboratory for murine (34) and human (35) bone marrow. Briefly, nonadherent bone marrow cells were cultured at 10^5 cells/ml using 10% pokeweed mitogen spleen cell-conditioned medium as the source of granulocyte-macrophage colony-stimulating factor for murine cultures and pokeweed mitogen-stimulated human peripheral

blood-derived mononuclear cells for human CFU-GM. Cultures were incubated for 7–14 days in the presence of 5% CO_2 in air at 37°C. Colonies containing 50 or more cells were scored as CFU-GM using a dissecting microscope.

Assay for BFU-E. Femoral bone marrow cells were cultured in methylcellulose as previously reported from this laboratory for both murine and human tissues (36). Briefly, 10^5/ml nonadherent marrow cells were cultured using erythropoietin lot 806; Amgen, Thousand Oaks, CA, 2 units /ml for murine cultures and 1 unit/ml for human cultures. Cultures were incubated for 9–14 days in the presence of 5% CO_2 in air at 37°C. Colonies containing 50 or more hemoglobinized cells were scored as BFU-E using a Nikon Diaphot tissue culture inverted microscope (Nikon, Tokyo, Japan).

Assay for CFU-Meg. The technique for culturing the murine megakaryocyte colony progenitor stem cell was described previously from this laboratory (37, 38). Murine bone marrow (10^5/ml) was cultured in 1-ml aliquots in 35 m*M* Costar plates (Costar). All cultures were incubated in 95% air and 5% CO_2 in a fully humidified incubator at 37°C and scored on Day 7. For murine cultures, murine spleen cell-conditioned medium (100 μl) was used to induce optimal murine colony formation. Murine CFU-Meg were identified by the presence of their acetylcholinesterase activity as previously described (39).

Human CFU-Meg were cloned in a methylcellulose culture system optimized for the generation of cloned megakaryocyte progenitors as described by Pallavicini *et al.* (40) for murine megakaryocyte progenitors in agar. Briefly, light density prepared human mononuclear cells were cultured in 0.8% methylcellulose in Iscove's modified Dulbecco's medium (GIBCO). The final concentration of heat-inactivated human AB serum was 20%. The source of CFU-Meg stimulating activity was supplied by pokeweed mitogen-stimulated human peripheral blood mononuclear cell-conditioned medium and was used at 10%. Cultures were maintained at 37°C in a humidified atmosphere of 10% CO_2 in air for 14 days. Colonies containing groups of three or more megakaryocytes were scored as a human CFU-Meg. For light microscopic examination and confirmation, individual colonies were plucked and placed on glass slides for staining with Harris hematoxylin for megakaryocyte identification.

Statistical Analysis of the Data. Statistical analysis of the data was determined using Student's *t* test. The statistics were performed on the raw data, comparing control vs experimental groups from three separate trials with $P < 0.05$ used as the level of significance.

Results

The effect of CBZ on the number of granulocyte-macrophage progenitors from normal murine and human bone marrow is shown in Figure 1. CBZ inhibited both murine and human CFU-GM formation in a dose-dependent fashion with 50% inhibition observed at approximately 2.5 μg/ml ($P < 0.01$). Concentrations greater than 10 μg/ml were completely inhibitory. This observation was also observed for the effect of CBZ on megakaryocyte colony progenitor cells (Fig. 1). CBZ again induced a dose-dependent reduction in CFU-Meg with 50% inhibition observed at 2.5 μg/ml ($P < 0.01$). As was observed for CFU-GM, concentrations greater than 10 μg/ml were completely inhibitory. The effect of CBZ on early erythroid progenitors (BFU-E) is shown in Table I. Exposure of either murine or human marrow cells to CBZ produced a dose-response reduction in BFU-E. BFU-E cultures plated in the presence of Li plus CBZ showed no statistical difference when compared with the CBZ controls, indicating that Li did not protect erythroid progenitors and in fact in the presence of high Li levels (5.0 m*M*) the CBZ effect was exacerbated in the presence of Li. This response however is not surprising since Li has been demonstrated to reduce the number of erythroid progenitors when

Table I. Effect of CBZ in the presence or absence of Lithium Chloride on Murine and Human Marrow-Derived BFU-E Colony Formation[a]

	Murine	% Control	Human	% Control
Control[b]	36.5 ± 7.8	100	45.4 ± 5.6	100
CBZ (μg)				
0.1	41.5 ± 5.1	100	48.4 ± 4.5	100
0.5	42.0 ± 3.2	100	46.0 ± 5.5	100
1	25.0 ± 3.5	68	28.5 ± 4.5	62
5	8.2 ± 1.8	22	9.5 ± 3.5	20
10	3.2 ± 0.9	8	3.5 ± 0.5	7
LiCl (m*M*)				
0.1	40.2 ± 5.0	100	44.5 ± 5.5	100
CBZ				
0.1	45.0 ± 5.1	100	46.5 ± 2.5	100
0.5	48.0 ± 7.0	100	43.5 ± 4.0	100
1	29.0 ± 2.9	72	25.8 ± 3.2	57
5	6.5 ± 1.2	16	7.6 ± 2.4	17
10	2.7 ± 0.5	6	2.5 ± 1.0	5
LiCl (m*M*)	28.0 ± 2.1	100	32.0 ± 2.8	100
CBZ (μg)				
0.1	26.0 ± 4.6	100	30.5 ± 2.5	100
0.5	29.0 ± 3.5	100	31.5 ± 3.5	100
1	14.5 ± 2.6	51	15.0 ± 2.8	46
5	1.5 ± 1	5	1.5 ± 0.5	4
10	0.7 ± 0.5	2	0.5 ± 0.1	1
LiCl (m*M*)				
5	22.7 ± 5.1	100	25.5 ± 1.5	100
CBZ (μg)				
0.1	25.5 ± 2.0	100	26.0 ± 2.0	100
0.5	21.5 ± 2.3	100	23.5 ± 2.5	100
1	7.7 ± 3.2	33	5.5 ± 0.5	21
5	0	0	0	0
10	0	0	0	0

[a] Values represent data from triplicate experiments ± SEM.
[b] Phosphate-buffered saline added as control with CBZ diluent as described in Materials and Methods.

cultured from murine (37) or human (41) hematopoietic tissues. CBZ concentrations greater than 10 μg/ml were completely inhibitory (data not given).

The effect of lithium on CBZ-induced marrow progenitor cell inhibition was investigated. The addition of Li ameliorated the reduction in both murine and human CFU-GM and CFU-Meg colony formation (Fig. 2) ($P < 0.01$). However, Li concentrations that are known to be either suboptimal (i.e., 0.5 m*M*) or toxic (i.e., 5 m*M*) for their effects on hematopoiesis either were noneffective (0.5 m*M* dose) or were even more toxic (5 m*M*). Li, at the optimal dose effective for the

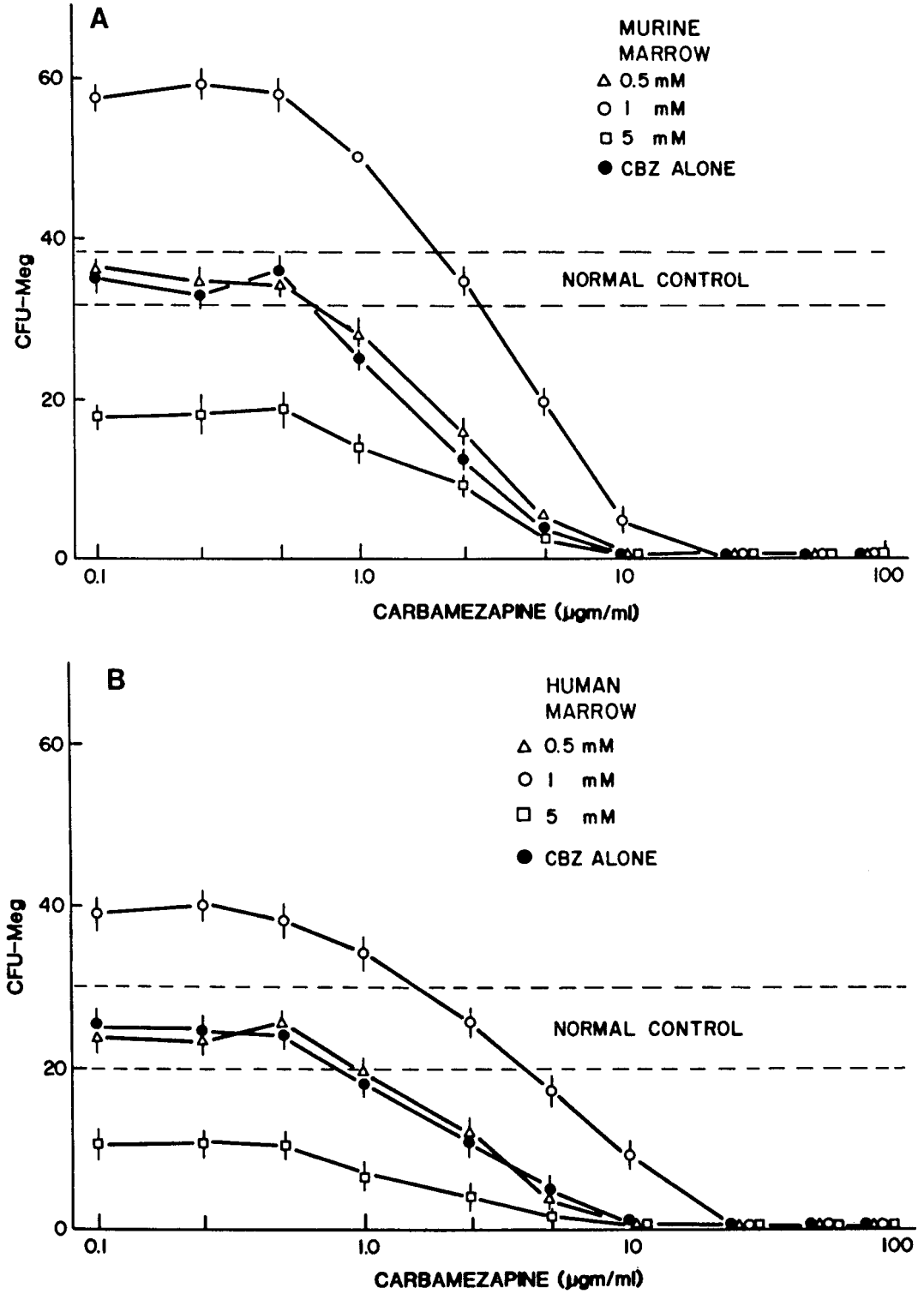

Figure 2. A, Dose-response effect of added CBZ and LiCl (0.5, 1.0, and 5.0 m*M*) on murine bone marrow CFU-Meg colony formation (±SEM from triplicate studies). B, Dose-response effect of added CBZ and LiCl (0.5, 1.0, and 5.0 m*M*) on human bone marrow CFU-Meg colony formation (±SEM from triplicate studies).

maximum effect on hematopoiesis (1.0 m*M*) was capable of reversing the toxic effect of CBZ. To examine further the ability of Li to influence the effect of CBZ on marrow-derived progenitor cells (CFU-GM), experiments were performed that delayed either the addition of Li 24 hr following marrow cell exposure to CBZ or the addition of CBZ to marrow cells first cultured in the presence of Li. As indicated in Table II, Li added 24 hr following CBZ on marrow CFU-GM failed to reverse CBZ-induced toxicity regardless of the Li concentration. These data indicate that the effect of CBZ on marrow progenitors is time dependent. Data listed in Table III demonstrate that when CBZ was added following Li exposure to marrow cells, the toxicity to CFU-GM was significantly reduced. Li provided a degree of protection to marrow progenitor cells when added prior to CBZ.

Discussion

CBZ is an effective agent for a wide variety of psychiatric conditions; however, adverse hematologic side effects have been associated with CBZ use such as leukopenia and aplastic anemia (31–33). The degree and extent of hematologic toxicity of CBZ on bone marrow function has not been well studied to date.

We report in this study that CBZ treatment of either murine or human bone marrow inhibited the number of granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and megakaryocyte (CFU-Meg) progenitor cells. A concentration of 2.5 μg/ml was effective in reducing the number of colonies by 50% compared with phosphate-buffered saline-treated controls. Concentrations greater than 10 μg/ml totally inhibited colony formation. Therapeutic ranges for CBZ in treating psychotic disorders is with 5–12 μg/ml, which is well within the toxic dose range observed in this study. It therefore can be assumed that the pronounced leukopenia observed with CBZ use clinically is due to CBZ's toxic effect on bone marrow progenitors. Although platelet abnormalities have not been reported, it appears from the data presented here that continued CBZ use has the potential for inducing platelet abnormalities.

The ability of Li to reverse the toxic effect of CBZ

Table II. Effect of Delayed Addition of Lithium Chloride 24 hr following CBZ Exposure to Bone Marrow Cells Cultured for CFU-GM[a]

	Murine	% Control	Human	% Control
Control[b]	162 ± 9.6	100	115 ± 12	100
CBZ (μg)				
0.1	187 ± 14.1	100	111 ± 9	100
0.5	182 ± 7.3	100	105 ± 8	100
1	143 ± 10.2	76	85 ± 6	70
5	51 ± 4.2	27	11 ± 3	9
10	3 ± 0.9	1	3 ± 1	2
LiCl (m*M*)				
0.1	157 ± 8.6	100	105 ± 8	100
CBZ (μg)				
0.1	160 ± 6.0	100	108 ± 5	100
0.5	145 ± 3.0	89	110 ± 11	100
1	127 ± 6.0	78	83 ± 6	72
5	7 ± 2.6	4	8 ± 3	6
10	1 ± 0.9	0.6	2 ± 1	1
LiCl (m*M*)				
1	177 ± 7	100	118 ± 15	100
CBZ (μg)				
0.1	173 ± 4	100	108 ± 10.5	100
0.5	150 ± 7	84	105 ± 7.5	100
1	138 ± 11	77	84 ± 6.5	71
5	6 ± 1	3	5 ± 1	4
10	3 ± 1	1	2 ± 1	1
LiCl (m*M*)				
5	120 ± 7	100	75 ± 8.5	100
CBZ (μg)				
0.1	118 ± 8	100	79 ± 9	100
0.5	97 ± 5.9	80	69 ± 5.5	100
1	78 ± 6.8	65	46 ± 6	61
5	5 ± 2	4	4 ± 2	5
10	0.5 ± 0.2	0.4	1.5 ± 0.5	2

[a] Values represent data from triplicate experiments ± SEM.
[b] Phosphate-buffered saline added as control with CBZ diluent as described in Materials and Methods.

Table III. Effect of Delayed Addition of CBZ 24 hr following Lithium Chloride Exposure to Bone Marrow Cells Cultured for CFU-GM[a]

	Murine	% Control	Human	% Control
Control[b]	186 ± 9.8	100	121 ± 8	100
CBZ (μg)				
0.1	187 ± 14.1	100	111 ± 9	100
0.5	182 ± 7.3	100	105 ± 8	100
1	143 ± 10.2	76	85 ± 6	70
5	51 ± 4.2	27	11 ± 3	9
10	3 ± 0.9	1	3 ± 1	2
LiCl (m*M*)				
0.1	190 ± 14.9	100	110 ± 6	100
CBZ (μg)				
0.1	201 ± 13.8	100	112 ± 8	100
0.5	191 ± 9.2	100	106 ± 5	100
1	187 ± 9.8	100	90 ± 5	94
5	47 ± 3.6	24	15 ± 3	13
10	3 ± 0.5	1	3 ± 1	2
LiCl (m*M*)				
1	358 ± 12.4	100	232 ± 11.5	100
CBZ (μg)				
0.1	349 ± 10.8	100	228 ± 19.5	100
0.5	343 ± 12.8	100	237 ± 13	100
1	358 ± 8.8	100	221 ± 11.5	100
5	158 ± 4.9	44	115 ± 7.5	49
10	3 ± 1.2	0.8	4 ± 1	0.3
LiCl (m*M*)				
5	95 ± 6.5	100	81 ± 7	100
CBZ (μg)				
0.1	93 ± 8.0	100	77 ± 5	100
0.5	75 ± 7.0	78	58 ± 6	71
1	54 ± 6.5	56	36 ± 9	44
5	35 ± 2.0	36	23 ± 5	28
10	0	0	0	0

[a] Values represent data from triplicate experiments ± SEM.
[b] Phosphate-buffered saline added as control with CBZ diluent as described in Materials and Methods.

on marrow function may be the result of Li's ability to be an effective stimulator of various aspects of hematopoiesis, specifically granulopoiesis and megakaryocytopoiesis. The ability of Li to reverse CBZ-induced bone marrow toxicity, however, was only observed when a known hematopoietic stimulating concentration of Li was used, i.e., 1 m*M*. In the presence of CBZ concentrations less than 10 μg/ml, Li was capable of stimulating the remaining progenitor population not affected by CBZ. In the presence of CBZ concentrations (>10 μg/ml) that were effective in completely inhibiting marrow stem cell function, Li was ineffective in reversing the CBZ-induced toxicity. However, Li was capable of providing a degree of protection to marrow progenitors exposed to CBZ if marrow was preincubated with Li before exposure to CBZ. Li was incapable of reversing CBZ-induced marrow progenitor toxicity if the cells were first exposed to CBZ regardless of the Li concentration.

Since Li has the capability of producing leukocytosis, it could be argued that Li should be effective in reversing the leukopenia associated with CBZ, if both agents were used clinically. In fact Li has been reported to be an effective agent to counteract the leukopenia associated with CBZ treatment (42). Therefore, Li offers the potential protection from CBZ-induced marrow toxicity as well as providing for additional psychotropic effects. Since psychopathologically Li and CBZ appear to be capable of acting synergistically, it could be argued that Li is capable of minimizing the potential CBZ-induced marrow toxicity in psychiatric patients.

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