The Effect of Lithium Carbonate on Lymphocyte, Granulocyte, and Platelet Function

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Lithium affects an increase in granulocyte counts in humans and has been promoted in the treatment of granulocytopenia and as adjuvant cancer chemotherapy to reduce the incidence of bacterial infections. In this study, eight healthy volunteers were studied by means of a panel of quantitative and cellular function tests before and after a seven-day course of lithium carbonate. Granulocyte, lymphocyte, and platelet function was assessed by the test panel. This panel included T cell and B cell enumeration, lymphocyte transformation to a number of mitogens and antigens, phagocytic and bactericidal activities of peripheral leukocytes, nitroblue tetrazolium (NBT) reduction, chemotaxis, chemiluminescence, platelet aggregation studies, and Ivy bleeding time. There was a significant reduction in bactericidal capacity ($p < 0.005$) and a significant reduction in lymphocyte response to PPD ($p < 0.01$). Although lithium increases the granulocyte count, the reduction in bactericidal capacity of granulocytes may reduce the beneficial effect of the granulocytosis. Prospective clinical studies are indicated to assess the clinical efficacy of the drug.

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LITHIUM HAS BEEN PROMOTED for the treatment of granulocytopenia and as an adjuvant for cancer chemotherapy.[11,12,25,26,28,33,49,51] Although lithium has been shown to cause granulocytosis in humans,[37,38,43,47] there is little information about the function of the granulocytes, nor is there much about the function of lymphocytes and platelets. Since the rationale behind the use of lithium is to decrease the incidence of infection in patients who have granulocytopenia, the functional capabilities of granulocytes, lymphocytes, and platelets were measured to determine whether there was a beneficial or detrimental effect.

Materials and Methods

Study Subjects

Healthy volunteers were obtained from the employee population of the institution. After an informed consent was obtained, a sample of venous blood was drawn for a battery of granulocyte, lymphocyte, and platelet function tests. *In vivo* granluocyte function was measured by a quantitative skin window technique and the subjects placed on 300 mg of lithium carbonate (Lithotabs™) orally 3 times a day. (Lithium was supplied by Dr. Ben Greenwell of Rowell Laboratories, Inc., Baudette, Minnesota.) After seven days of therapy, the entire battery of functional tests was repeated. The results were compared statistically to the pretreatment values by Student's *t*-test. In addition, each patient was given a battery of tests designed to monitor cognitive or personality changes. These tests were administered and analyzed by Dr. Edwin Hoeper of the Marshfield Clinic and will be the subject of a separate report.

Lymphocyte Function Tests

The lymphocyte system was assessed by methods previously outlined by this laboratory.[29] Briefly, lymphocyte subpopulations were identified by surface markers after separation on a density gradient Ficoll-Hypaque column centrifuged at 400 × *g*. Sheep red blood cell rosetting (E-RFC) was used to identify T cells, while complement receptors (EAC-RFC) and the presence of surface immunoglobulins were used to identify B cells. Percentages (mean ± S.D.) from a normal population were as follows: E-RFC = 55–75%; EAC-RFC = 10–34%; IgG = 5–20%; IgM = 3–15%; and IgA = 0–4%. Values for all patients were within normal ranges at

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the beginning of this study. Lymphocyte function was assessed *in vitro* by a lymphocyte transformation assay.[29] Lymphocyte transformation was assessed as the degree of stimulation of ^{3}H-thymidine (1 μCi/5 $\times$ 10^5 lymphocytes) incorporation when added to a standardized dilution of preservative-free heparinized whole blood. Each subject was tested for reactivity to phytohemagglutinin (PHA), concanavalin A (Con-A), pokeweed mitogen (PWM), streptokinase-streptodornase (SK-SD), mumps, candidin, histoplasmin, and tuberculin PPD. The results were expressed as the stimulation index (SI) or ratio of counts in the cultures with mitogen to the counts in control cultures. Greater than two-fold stimulation by antigen was considered a positive test.

The skin of each subject was tested with PPD, candidin, and SK-SD before and after the lithium treatment by means of an intradermal injection of 0.05 ml of each antigen. The results were expressed as the diameter of induration at 48 hours. A test was considered positive if the area was greater than 5 $\times$ 5 mm.

Granulocyte Function Tests

The functional integrity of the granulocyte system was assessed by a battery of tests including: nitroblue tetrazolium (NBT) reduction; chemotaxis in response to bacteria derived factor(s) and zymosan-induced C5a; phagocytic and bactericidal capabilities, and chemiluminescence. These granulocytes were separated by sedimentation at 1 $\times$ *g* for 45 minutes at 37°C with 10% dextran (Dextran 250®, Pharmacia Fine Chemicals, Piscataway, New Jersey). The cell rich plasma, which contained an average of 66% PMN and 26% lymphocytes, was then layered on a Ficoll-Hypaque gradient and centrifuged for 10 minutes at 400 $\times$ *g*. The sedimented cells were 95% pure granulocytes. The granulocytes were washed in Hank's balanced salt solution (HBSS) and resuspended to appropriate concentrations.

NBT Reduction

The model utilized for NBT reduction was that described by Park *et al.*[39] Venous blood was collected with 10 U of heparin/ml. The blood was incubated with 0.2% NBT in normal saline at 37°C for 15 minutes. Blood smears are prepared, stained with Wright's stain, and a 200-cell differential was made noting the number of cells which have the reduced formazan deposits within the cytoplasm. These cells are easily recognized as PMNs with large, irregular, dark amorphous masses within the confines of the cytoplasm. Healthy individuals routinely showed values indicating that less than 20% of the cells in the unstimulated cultures contained the reduced formazan.

Chemotaxis

The chemotactic response was determined as outlined in detail by Ward.[52] The chemotactic chambers (Bellco Glass, Vineland, New Jersey) were prepared using 5 micron porosity SMWP 02500 Millipore filters. The top chambers were loaded with 1.5 $\times$ 10^6 granulocytes diluted in RPMI 1640. The bottom chamber contained the chemotactic factor. Chemotactic factors used included a bacterial culture filtrate of E. coli[52] and zymosan activated normal human serum (NHS). Zymosan was added to NHS at 10 mg/ml for 1 hour at 37°C. After centrifugation to remove the zymosan, the activated serum was used as a chemotactin at a concentration of 10% in RPMI 1640. The chambers were incubated at 37°C for 3 hours. The filters were carefully removed from the chambers, fixed in absolute propanol, stained with Wright's stain, and finally cleared in xylene. The filters were placed on conventional glass slides with mounting media and observed at $\times$25 magnification. A minimum of 10 fields ($\times$25) was viewed and the number of cells migrating completely through the filter was counted. The results were expressed as the ratio of the average number of cells per $\times$25 field in the chamber with the chemotactin compared to the average number of cells per $\times$25 field in the chambers without chemotactin.

Phagocytic and Bactericidal Activities

The ability of the granulocytes to ingest and kill bacteria was studied by the method outlined by Weir.[54] An 18-hour culture (mid-log phase growth) of *Staphylococcus aureus*, strain 502A, (ATCC, Bethesda, Maryland) was incubated with 1 $\times$ 10^7 granulocytes in RPMI 1640 with 10% NHS and incubated in a 37°C water bath in a ratio of bacteria to granulocytes of 1:1. For phagocytic indices, aliquots of the bacteria-cell mixture were sampled at 30, 60, and 120 minutes. The aliquots of cells were centrifuged at 400 $\times$ *g* and the number of bacteria remaining in the supernatant was measured by viable plate counts. Bactericidal abilities of these granulocytes were determined by mixing cultures of *S. aureus* and granulocytes in a ratio of 1:1 for 15 minutes at 37°C in RPMI 1640. Excess bacteria were removed by washing three times with HBSS. Aliquots were then taken at 30, 60, 90, and 120 minutes. The number of bacteria contained within the granulocytes was determined by viable plate counts after lysing the washed granulocytes with distilled water. The results of these two assays were expressed as the phagocytic index (PI) and bactericidal index (KI) and are defined as the log difference of the number of bacteria at 0 minutes and at 120 minutes. A normal linear regression line can be plotted between these points.

Chemiluminescence

The ability of granulocytes to generate photons of visible light after stimulation was measured by a modification of the method of Allen *et al.*[1] Polymorphonuclear leukocytes were separated as described and resuspended to 1 × 10^7 PMN/ml in HBSS. The cells were incubated in a 37°C water bath with 1 mg of zymosan previously opsonized with NHS. Opsonization was performed by incubating NHS with 1 mg/ml zymosan at 37°C for 1 hour. Chemiluminescence was measured in a Packard Tri-Carb scintillation counter with coincidence turned off for 0.2 minutes at 1% gain. Consecutive duplicate counts were obtained and the samples were immediately returned to the 37°C water bath. Repeat counts were made every 10 minutes for 2 hours. The results were expressed as the cpm/10^7 granulocytes at the peak of the curve, which occurred at 20 minutes in all cultures. Cells from healthy individuals tested in this manner yielded a mean ± standard error of 7711 ± 528 counts/min.

In Vivo Granulocyte Migration

The capability of granulocytes to migrate to a site of inflammation was measured by the technique outlined by Senn and Jungi.[45] A plastic chamber was placed over an area of abraded skin and filled with fresh autologous serum. The chambers were glued in place with collodion. The contents of the chambers were collected after 24 hours. The number of cells, type, and viability were determined routinely. The following parameters were calculated on the basis of cell counts: total leukocyte mobilization (TLM) = cumulative number of leukocytes/cm²/24 hr; leukocyte mobilization rate (LMR) = average leukocytes/cm²/24 hr; and blood granulocyte clearance (BGC) = TLM/circulating neutrophils/ml of blood. Total white blood cell counts were performed with a Coulter Model "S" and a 200 white cell differential count was done. Values obtained in a normal population are TLM = 89.5 ± 8.7 (mean ± S.E.), LMR = 3.7 ± 0.3, and BGC = 31.8 ± 4.5 (n = 14).

Platelet Function Tests

The effect of lithium treatment on platelet function was assessed by Ivy bleeding times and by platelet aggregation tests as previously described.[23] Platelet rich plasma was obtained by centrifugation at 1,000 × *g* for 7.5 minutes. The aggregation tests were performed with 1.0 μg/ml of epinephrine, a solution of collagen prepared as described by Day and Holmsen,[13] and 20 μM, 2 μM, and 1 μM of ADP. The percent aggregation was determined from the aggregometer tracings at the point of maximal aggregation within 3 minutes from the time the aggregating agent was added. The tracings were also examined for any qualitative change, such as abolition of the primary or secondary waves of aggregation, or in the slope of the curves.

TABLE 1. Granulocyte Function (*In Vivo*)

	Pre-Lithium	Post-Lithium	p
WBC	5606 ± 359*	7575 ± 430	<.001
Granulocytes	2987 ± 202	4791 ± 331	<.001
LMR	2.65 ± 0.45	2.20 ± 0.54	NS
TLM	64.0 ± 10.9	52.5 ± 13.1	NS
BGC	18.6 ± 3.8	11.8 ± 2.9	NS

* Mean ± S.E.M. N.S. = not statistically significant; LMR = leukocyte mobilization rate; TLM = total leukocyte mobilization; BGC = blood granulocyte clearance; WBC = total white cell count; Granulocytes = the total number of segmented polymorphonuclear leukocytes.

Lithium Concentration

The plasma and erythrocyte lithium concentration was measured by atomic absorption as described.[31]

Results

Granulocyte Function

There was a significant increase in the total white blood cell count and absolute granulocyte count but no change in the LMR, TLM, or BGC (Table 1). There was no greater than 1% band (or more immature) forms either before or after lithium treatment. Although there was a consistent trend in the skin window tests toward decreased migration with lithium, this was not statistically significant. There was no significant change in NBT reduction, phagocytosis, chemiluminescence, or chemotaxis, although there was a trend toward improved chemotaxis after lithium (Table 2 and Fig. 1). There was a significant reduction in bactericidal ability of the granulocytes after lithium therapy ($p < 0.005$). The pretreatment values for each of these tests were not significantly different from control values obtained on healthy individuals in our laboratories.

TABLE 2. Granulocyte Function (*In Vitro*)

	Pre-Lithium	Post-Lithium	p
Chemotaxis			
BF	2.74 ± 0.29*	4.98 ± 1.87	NS
Zymosan	3.14 ± 1.21	7.78 ± 1.65	NS
Phagocytic Index	1.18 ± 0.10	0.98 ± 0.07	NS
Bactericidal Index	0.72 ± 0.06	0.47 ± 0.03	<0.005
NBT Reduction (U)	3.33 ± 0.36	2.17 ± 0.92	NS
NBT Reduction (S)	40.5 ± 3.22	36.9 ± 3.98	NS
Chemiluminescence	172.0 ± 18.5†	167.2 ± 8.2	NS

* Mean ± S.E.M.

† cpm × 10^{-3} at peak response.

NS = not statistically significant; (U) = unstimulated; (S) = stimulated with LPS.

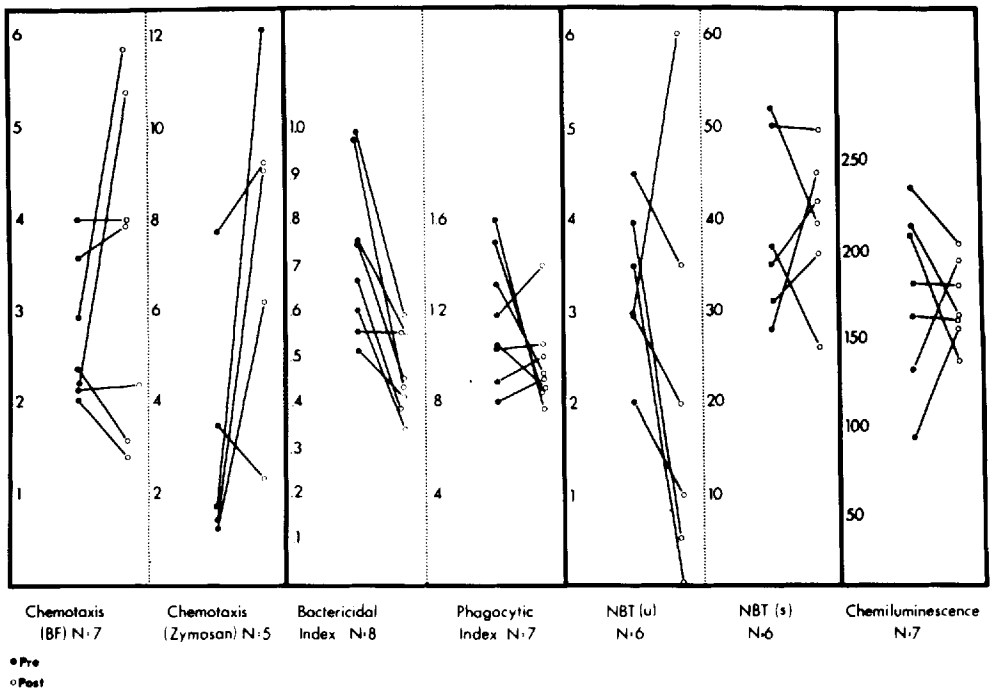

FIG. 1. *In vitro* granulocyte function. The values obtained on the individual subjects (n) are presented for each of the granulocyte function tests. Values are compared for each individual before and after the course of lithium carbonate.

Lymphocyte Function

There was no change in the absolute lymphocyte count or in the absolute number of T and B cells after lithium (Table 3 and Fig. 2). There was a consistent, but statistically not significant, reduction in lymphocyte response to PHA in seven of eight subjects and with Con-A in six of eight subjects (Table 4). Four of eight subjects had an improved response to PWM and four of eight had a reduced response following lithium. The response to the recall antigens tested was not affected by lithium except PPD. There was a significant and consistent decreased response in four subjects who were initially positive ($p < 0.01$).

No subject had a positive PPD skin test before or after lithium. All subjects except two had positive skin test reactions to SK-SD before lithium. One subject developed a severe hypersensitivity reaction on retesting with SK-SD immediately after the lithium requiring local application of steroids. This individual's response to *in vitro* lymphocyte transformation was moderately reduced compared to pre-lithium levels (the stimulation index with SK-SD of 65.9 fell to 42.0 post-lithium).

TABLE 3. Lymphocyte Function (*In Vivo*)

	Pre-Lithium	Post-Lithium	p
Lymphocyte	2029 ± 275*	2013 ± 280	NS
T cells	1206 ± 158	1213 ± 187	NS
B cells	275 ± 35	250 ± 37	NS

* Mean ± S.E.M.
NS = not statistically significant.

Another subject had a positive skin test with SK-SD and reactions of 3 × 3 mm with PPD and 6 × 6 mm with *Candida* before lithium and no reaction after lithium. The same individual had a reduction of *in vitro* lymphocyte transformation to all antigens and mitogens tested except mumps. The trend of the lymphocyte response to specific antigens, although statistically significant only with PPD, seemed to be either no change or a decreased response (Fig. 2). Very few increased responses post-lithium were observed.

Platelet Function

There was no significant qualitative or quantitative change in platelet aggregation and no change in the bleeding times.

Lithium Concentration

There was no correlation between the intraerythrocytic or plasma lithium levels and the degree of granulocytosis or bactericidal defect of the granulocytes.

Discussion

There have been no controlled clinical studies assessing the incidence of infection in patients with granulocytopenia treated with lithium. Previous studies have shown a significant increase in granulocyte mass and an absolute granulocytosis with lithium which is confirmed in our study.[11,12,26,28,33,37,38,43,47,49] The phagocytic function of granulocytes in humans treated with lithium

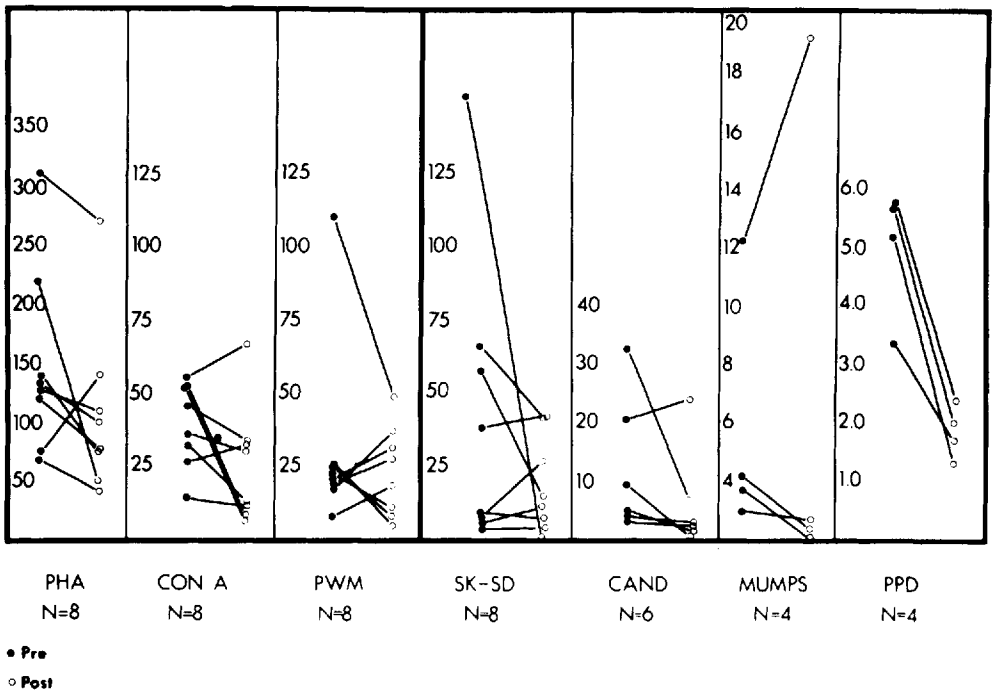

FIG. 2. *In vitro* lymphocyte function. The values obtained on the individual subjects (n) are presented for lymphocyte blastogenic response to the mitogens and recall antigens. Values are compared for each individual before and after the course of lithium carbonate. Only those individuals demonstrating a positive response to an antigen are shown for each antigen.

has been previously described as normal which is also confirmed in this study.[42,50]

Schapira *et al.*[44] described a patient with Felty's syndrome treated with lithium carbonate who had normal NBT reduction and bactericidal activity, imparied chemotaxis, and fever before lithium. Five weeks later all studies were normal and the patient was afebrile. We found no impairment of chemotaxis and a suggestion of improved chemotaxis which may have been significant if more individuals were studied. There was, however, a significant decrease in bactericidal capacity, and no abnormality of oxidative metabolism as measured by NBT reduction and chemiluminescence was found. There was no significant change in the migration of granulocytes into skin window chambers. Rothstein *et al.*[43] found no impairment of migration into skin window chambers utilizing a different technique and perhaps an increased migration. Nine of 10 of their subjects were also taking other psychotropic drugs. The effects of granulocyte migration of these drugs is unknown. The net therapeutic effect of lithium on granulocytes is uncertain because the beneficial increase in granulocyte numbers may be significantly impaired by the decrease in bactericidal activity. More studies of chemotaxis and migration of granulocytes into tissues would be of interest.

There was no significant change in the number of lymphocytes and T and B cells in this study, although others have found a significant lymphopenia in patients treated with lithium.[5] There was a significant reduction of lymphocyte transformation with PPD ($p < 0.001$). Cell mediated immunity as assayed by the skin tests employed in this study was generally unchanged. One individual was capable of having a severe hypersensitivity reaction and another subject had suppression of skin test reactivity and impaired lymphocyte transformation following lithium. Furthermore, there was a consistent trend, although not statistically significant, towards decreased lymphocyte response utilizing PHA, Con-A and PWM following exposure to lithium. Shenkman *et al.*[46] found enhanced host resistance to tumors in mice pretreated with lithium. They also found an increased number of T cells and increased PHA response with lymphocytes which were treated with lithium *in vitro*. These results were not confirmed by the present *in vivo* and *in vitro* studies. The net therapeutic effect of lithium on lymphocyte function is uncertain.

TABLE 4. Lymphocyte Function (*In Vitro*)

	Pre-Lithium	Post-Lithium	p	n
PHA	149.6 ± 28.6*	107.9 ± 25.9	NS	8
Con-A	38.9 ± 5.0	23.9 ± 7.6	NS	8
PWM	30.4 ± 11.6	22.5 ± 5.5	NS	8
SK-SD	38.4 ± 14.9	18.3 ± 5.8	NS	8
Candida	12.2 ± 4.8	6.3 ± 3.5	NS	6
Mumps	5.7 ± 2.2	5.8 ± 4.4	NS	4
PPD	5.0 ± 0.6	1.8 ± 0.2	<0.01	4

* Mean ± S.E.M.

n = number of tests analyzed. The response to specific antigens (SK-SD, Candida, mumps, and PPD) was analyzed only if the initial stimulation index was greater than 2; NS = not statistically significant.

Platelet function as assayed by platelet aggregation and Ivy bleeding times was found to be unchanged. Others have shown inhibition of aggregation when platelets were briefiy exposed to lithium and stimulation of aggregation after more prolonged incubation of platelets with lithium.[24,27,32] With the dose of lithium used in this study, there appears to be no clinically significant alteration in hemostasis.

Glucocorticoids have been shown to produce a granulocytosis in humans.[6] The granulocytosis associated with glucocorticoids is accompanied by a reduction in oxidative metbolism,[36] bactericidal capacity,[36] impaired egress into tissues,[6,40] no effect on neutrophil turnover,[2] and decreased granulocyte adherence.[35] Glucocorticoids also impair cell mediated immunity[18,53,55] which makes them less than ideal in the treatment of granulocytopenia and as an adjuvant to chemotherapy. The granulocytosis associated with lithium is similar to the "steroid effect" in the reduction in bactericidal capacity and impaired lymphocyte function, but differs in all other parameters of granulocyte function and kinetics studied.

The mechanism by which lithium impairs killing capacity is unclear. In many tissues lithium decreases adenylate cyclase activity and lowers cAMP.[14-16,19,22,48] Since decreased levels of cAMP are usually associated with an improvement in granulocyte function,[3,7-10,17,30,41,56] it is possible that intracellular competition between lithium and cations distal to cAMP is responsible for the defect.[20,21] Measurement of cyclic nucleotide levels in granulocytes exposed to lithium and other studies of granulocyte function might clarify the mechanism of the "lithium effect."

Because of the significant reduction in granulocyte function and the incidence of physical and emotional side effects with lithium,[4,34] controlled clinical trials are indicated to assess the incidence of infection and other untoward side effects before the drug is widely used to treat either granulocytopenia or as an adjuvant to cancer chemotherapy.

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