Effects of lithium carbonate on cytokine production in patients affected by breast cancer

R.A. MEREINDO1, G. MANCUSO1, E. TOMASELLO1, D. GAZZARA1, V. CUSUMANO1, S. CHILLEMI2, P. SPADANO3, M. MESITI2

1 Institute of Microbiology
2 Institute of Oncology, Medical School, University of Messina
3 IST Genoa, Satellite Unit of Messina - Italy

ABSTRACT: It has been reported that lithium salt compounds influence hematoipoiesis, which is known to be regulated by a number of cytokines, including tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6). Since lithium can induce TNF production in human monocytes, we wished to determine if lithium carbonate treatment of neutropenic patients affected by breast cancer results in increased cytokine production. Serum levels of TNFα, IL-1 and IL-6 were measured before and at 7 and 180 days after treatment with lithium carbonate. Results indicate that this therapy produced TNFα and IL-6, but not IL-1α, elevations in patients affected by unmetastasized breast cancer. Conversely, TNFα, but not IL-6, elevations were detected in metastatic patients. Studies are under way to investigate the mechanisms by which lithium salts affect cytokine production in monocytes from cancer patients. (J Biol Regul Homeost Agents, 1994; 8: 88-91)

KEY WORDS: Lithium carbonate, Breast cancer, Cytokines, Tumor Necrosis Factor, Interleukin-1, Interleukin-6

INTRODUCTION

The administration of lithium to psychiatric patients and normal volunteers has been associated with increased granulocyte production (1, 2). In vitro and in vivo investigations suggest that lithium influences the hematoipoietic system either by a direct action on pluripotent stem cells (3), or by indirectly increasing the production of growth factors (4). For example, tumor necrosis factor alpha (TNFα) stimulates endothelial cells to produce granulocyte-macrophage colony-stimulating factor (GM-CSF), which, in turn, promotes the differentiation of hematopoietic progenitor cells to granulocytes and macrophages (5, 6). Lithium can stimulate cultured monocytes and macrophages for increased TNF production (7).

Interleukin-1 (IL-1) and interleukin-6 (IL-6) are also multifunctional cytokines involved in the regulation of the immune response and inflammation as well as hematoipoiesis. The functions of these cytokines are closely related as emphasized by observations that IL-1 and TNF are potent inducers of IL-6, and IL-6 inversely regulates TNF expression (8).

Since the anti-tumor capabilities of TNF have been well documented, we were interested to determine if lithium carbonate treatment affects serum levels of TNF, IL-6 and IL-1 in cancer patients. Serum cytokine levels were measured before and after lithium treatment in neutropenic patients affected by unmetastasized and metastasized breast cancer.

MATERIALS AND METHODS

Patients

Serum samples of three groups of age-matched, postmenopausal female donors (10 per group) were examined: patients affected by unmetastasized breast cancer (BCa/M0); patients affected by metastasized breast cancer (BCa/M1) and healthy adults (HD) controls (Tab. 1). All the tumors were diagnosed as “Infiltrating Ductal Carcinoma” by an experienced pathologist using the breast cancer classification of the Union International Contre le Cancer (UICC) (9).

Both BCa/M0 and BCa/M1 patients received a one-day i.v. treatment with a “CMF” combination of antiesthetic drugs consisting in cyclophosphamide, 600 mg/m²; methotrexate, 40 mg/m²; fluorouracil, 600 mg/m². The same treatment was repeated once 8 days later. After resting for 21 days, patients were tested for white blood cell (WBC) counts. If their WBC levels were > than 4000/mm³, they were treated again with the same CMF regimen. Otherwise they were tested again after a 7 day cycle of lithium carbonate (900 mg/die) and treated thereafter with CMF. Each patient received 5-6 administrations of CMF and at least 2 cycles of lithium carbonate treatment.

In the majority of patients, measurements of serum TNF, IL-1 and IL-6 were done using three drawings obtained before and at 7 and 180 days after treatment with lithium carbonate. We were able to obtain 2
TABLE I - BREAST CANCER PATIENTS

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>* Type of neoplasia</th>
<th>** Stage (TNM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCa/M0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt-1</td>
<td>58</td>
<td>I.D.C.</td>
<td>T1 N0 M0</td>
</tr>
<tr>
<td>Pt-2</td>
<td>63</td>
<td>I.D.C.</td>
<td>T1 N1 M0</td>
</tr>
<tr>
<td>Pt-3</td>
<td>56</td>
<td>I.D.C.</td>
<td>T1 N0 M0</td>
</tr>
<tr>
<td>Pt-4</td>
<td>69</td>
<td>I.D.C.</td>
<td>T2 N0 M0</td>
</tr>
<tr>
<td>Pt-5</td>
<td>65</td>
<td>I.D.C.</td>
<td>T2 N1 M0</td>
</tr>
<tr>
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<td>72</td>
<td>I.D.C.</td>
<td>T1 N0 M0</td>
</tr>
<tr>
<td>Pt-7</td>
<td>74</td>
<td>I.D.C.</td>
<td>T2 N1 M0</td>
</tr>
<tr>
<td>Pt-8</td>
<td>67</td>
<td>I.D.C.</td>
<td>T1 N1 M0</td>
</tr>
<tr>
<td>Pt-9</td>
<td>68</td>
<td>I.D.C.</td>
<td>T2 N0 M0</td>
</tr>
<tr>
<td>Pt-10</td>
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<td>I.D.C.</td>
<td>T1 N1 M0</td>
</tr>
<tr>
<td>BCa/M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt-11</td>
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<td>I.D.C.</td>
<td>T2 N1 M1</td>
</tr>
<tr>
<td>Pt-12</td>
<td>60</td>
<td>I.D.C.</td>
<td>T1 N1 M1</td>
</tr>
<tr>
<td>Pt-13</td>
<td>68</td>
<td>I.D.C.</td>
<td>T2 N0 M1</td>
</tr>
<tr>
<td>Pt-14</td>
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<td>T3 N1 M1</td>
</tr>
<tr>
<td>Pt-15</td>
<td>71</td>
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</tr>
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<td>Pt-20</td>
<td>61</td>
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<td>T2 N2 M1</td>
</tr>
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</table>

BCa/M0: Unmetastasized breast cancer patients
BCa/M1: Metastasized breast cancer patients
I.D.C.: Infiltrating Ductal Carcinoma
**TNM: classification based on UICC (Union International Contre le Cancer)

Fig. 1 - Serum TNFα levels in patients with unmetastasized (BCa/M0) and metastasized (BCa/M1) breast cancer at various times after the beginning of lithium carbonate therapy.

Measurement of cytokines

Serum levels of all cytokines were measured using ELISA kits (TNFα ELISA Kit, Cytokit-6 and Cytokit-1α) distributed by Omnia Res (Milan-Italy).

Data expression and statistical analysis

Serum cytokine concentrations are expressed as means ± standard deviations. To calculate mean values, results below the detection levels were arbitrarily assigned a theoretical value of half the lower limits of detection of the assays. Differences in serum cytokine levels were analysed by one way analysis of variance (ANOVA) and the Student-Newman-Keuls test. Differences were considered significant with p values < 0.05.

RESULTS

Figures 1 and 2 show serum TNFα and IL-6 levels respectively in BCa/M0 and BCa/M1 patients before and at 7 and 180 days after the beginning lithium carbonate therapy. Basal TNFα levels were significantly (p < 0.05) higher in BCa/M1 than in BCa/M0 patients.
Lithium carbonate and cytokine production

Fig. 2 - Serum IL-6 levels in patients with unmetastasized (BCa/M0) and metastasized (BCa/M1) breast cancer at various times after the beginning of lithium carbonate therapy.

(79.8 ± 7.2 versus 28.9 ± 9 pg/ml, respectively). These values were significantly (p < 0.05) higher than those of healthy controls, which were always below the lower limit of detection of the assay (10 pg/ml). Lithium treatment produced significant (p < 0.05) elevations of TNFα levels at 7 days after the beginning of therapy in both BCa/M0 patients (604.1 ± 201.6 pg/ml) and BCa/M1 patients (210.1 ± 60.1 pg/ml). TNFα concentrations returned to basal levels at 180 after the beginning of therapy in both BCa/M0 and BCa/M1 patients (26.3 ± 5.8 and 74.0 ± 4.3 pg/ml, respectively).

Serum levels of IL-6 in HD were always below the limit of detection of the assay (0.1 ng/ml). Figure 2 shows IL-6 concentrations in the same samples shown in Figure 1. Basal IL-6 levels of both BCa/M0 and BCa/M1 patients were higher than those of HD (4.3 ± 2.0 and 4.5 ± 1.4 ng/ml, respectively; p < 0.05).

Concentrations of IL-6 increased significantly above basar values at 7 days after the beginning of treatment in BCa/M0, but not in BCa/M1 patients (Fig. 2). In all serum samples, including those of neoplastic patients IL-1α levels were below the lower limit of detection of the assay, irrespectively of time after treatment or patient group.

DISCUSSION

Our data show that lithium carbonate treatment can affect TNFα and IL-6 but not IL-1α, serum levels in neoplastic breast cancer patients. In the present study, cancer patients showed significantly higher basal serum TNFα and IL-6 levels than those of healthy donors. In addition patients with metastatic cancer showed significantly higher serum TNFα, but not IL-6, basal levels than those without metastasis. These findings are in general agreement with those of Balkwell et al who showed that sera of patients with active cancer have higher TNF-like activity (10). Janicke and Manns also showed that tumor cell membrane constituents can activate human monocytes for in vitro TNF synthesis (11). Thus, it can be hypothesized that factors released from tumor tissue stimulate the production of TNFα in cancer patients. Accordingly, higher TNFα levels are seen in patients with metastatic cancer than in those without metastasis can be accounted for by the greater mass of tumor tissue. However no differences were noted between these groups of patients in IL-1 and IL-6 levels.

The mechanisms underlying these differences are not entirely clear. It is possible that different mechanisms are involved in the regulation of IL-1 and IL-6, as opposed to TNFα, production. Treatment with lithium carbonate for seven days determined transient elevations of TNFα in cancer patients. This is in agreement with studies showing that monocytes from healthy subjects can be stimulated for increased in vitro TNF production by lithium chloride. Since, the transcription of TNFα genes is not inducible by lithium, the increase in TNFα production is likely to be due to the release of preformed stored TNFα from the membrane.
mRNA is thought to be controlled by a short-lived repressor, it was hypothesized in this study that lithium inhibits this repressor or, alternatively, the degradation of TNFα mRNA (7).

However, studies with monocytes from cancer patients have not been performed. Our studies show that lithium therapy can significantly affect in vivo TNFα production. Since TNFα can induce GM-CSF, it is possible that lithium induced granulopoiesis is mediated by these cytokines. Further studies are underway to test this hypothesis. Our data also show that cytokine responses to lithium therapy are, at least in part, dependent on the clinical status. For example, lithium therapy produced IL-6 elevations only in cancer patients without metastasis, but not in those with metastasis. Again the mechanisms underlying these differences are not entirely clear. It is possible that suppressive factors released by tumor tissue are involved in these effects, in addition to the above mentioned stimulatory mediators.

Since, in previous studies, we have found a marked impairment of some functions of mononuclear phagocytic cells in patients affected by neoplasia (12, 13, 14), it may be of interest to study in vitro response of monocytes from cancer patients treated with lithium carbonate in terms of transcription and degradation of TNFα, IL-1 and IL-6 mRNA.

ACKNOWLEDGEMENTS

This work was supported in part by a grant (60%) from the "Ministero dell'Università e della Ricerca Scientifica" of Italy.

Reprint requests to:
Prof.ssa Rosaria Alba Merendino
Istituto di Microbiologia
Piazza XX Settembre
98100 Messina, Italy

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Received: April 7, 1994
Accepted: December 1, 1994