EFFECTS OF SHORT-TERM LITHIUM TREATMENT ON PERIPHERAL BLOOD LYMPHOCYTES AND GRANULOCYTES IN HEALTHY VOLUNTEERS

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Lithium carbonate has been widely used in the treatment of manic-depressive psychosis. Since leukocytosis and neutrophilia frequently occur during prolonged treatment, several authors have investigated the possible use of lithium in neutropenic disorders.

The mechanism of lithium action is still unknown, although Tisman et al. have postulated that the neutrophilia is due to an increase in the total blood granulocyte pool rather than to a secondary pool mobilization.

The observation that lithium enhances colony stimulating factor (CSF) production by adherent cells suggests a direct action of the drug on the bone marrow. Lithium has been shown to enhance several functions of human peripheral blood lymphocytes (PBLs) and granulocytes (PMNs), probably by altering intracellular cyclic nucleotide levels. There is, however, no general agreement on this hypothesis. It was decided, therefore, to study some lymphocyte and granulocyte functions during short-term lithium treatment of healthy volunteers (900 mg/day per os for five days).

For this purpose, the ability of PBLs to form E-rosettes and of PMNs to phagocytize and kill Candida albicans were investigated before, during and

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EFFECTS OF LITHIUM TREATMENT ON LYMPHOCYTES AND GRANULOCYTES after lithium treatment. In order to obtain an indirect evaluation of lithium interference with the adenylate cyclase/cyclic AMP (cAMP) system, we investigated the modification, during the treatment, of the theophylline-induced inhibition of these cellular functions (theophylline is known to increase cellular levels of cAMP). Moreover, the time pattern of the lithium-induced bone marrow stimulation was studied by correlating the blood levels of the drug with the peripheral PMN count and variations in Arneth’s index.

MATERIALS AND METHODS

Cell preparation - Six healthy adult volunteers (aged 20 to 30) who gave their informed consent were included in this study. Venous blood was drawn before, during (1st and 5th day) and after (10th day, i.e. the 240th h from the start of drug administration) short-term lithium treatment (900 mg per os the first day, 300 mg every 8 h for the following 4 days).

Peripheral blood lymphocytes and granulocytes were separated by flotation at 400 g on Lymphoprep (density 1.077 g/ml) according to BOYUM. Viability, determined by trypan blue exclusion, was usually more than 95%. E-rosette assay was performed according to AIUTI et al. The same test was repeated after incubation of the cells with 10⁻⁴ M theophylline-ethylenediamine (hereafter, theophylline) in Hanks’ balanced salt solution (HBSS) for 120 min at 37 °C in a 5% CO₂ atmosphere. This incubation time and concentration were chosen because in preliminary experiments they maximized the inhibition without any loss of cell viability. Phagocytosis and killing of Candida albicans by PMNs were assessed according to LEHRER and CLINE.

The percentage of stained, nonviable Candida albicans was determined on at least 300 cells from each tube. The candidacidal activity of phagocytes was calculated by subtracting the percentage of stained cells in the control tubes from that found in tubes where Candida albicans was incubated with PMNs. The same test was repeated after incubation of the cells with 10⁻⁴ M theophylline, as previously described.

Lithium blood levels were evaluated by the usual flame spectrophotometry technique.

The number of PMNs and the variations in Arneth’s index were assessed by a count in a Burker chamber and by a differential count after May-Grünwald-Giemsa staining.

Cyclic 3',5'-adenosine monophosphate determination was performed according to GOODWIN et al. Every lymphocytic fraction was resuspended at a final concentration of 10⁶ cells/ml in Tris-ethylenediamine-tetraacetate (EDTA) buffer, pH 7.2. Samples preincubated with theophylline were previously washed once at 200 g for 10 min at 4 °C. All the tubes were frozen for 5 min in liquid nitrogen, then put in boiling water for 5 min. Cellular waste was removed by centrifugation at 2,000 g for 20 min at 4 °C. Fifty μl of the supernatant were withdrawn for cAMP determination using the cyclic AMP assay kit.

Intracellular levels of cAMP were determined for PBL and PMN granulocytes from blood taken from three of the six volunteers before lithium treatment, on the 2nd day after the first dose and on the 2nd day after stopping the treatment.