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Short communication

In vivo and in vitro clomipramine treatment decreases the migration of macrophages in the rat

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Abstract

We studied the effect of in vitro and in vivo treatment with the tricyclic antidepressant clomipramine on spontaneous mobility and on *N*-formyl-L-methionil-L-leucyl-L-phenylalanine (fMLP)-induced chemotaxis of rat peritoneal macrophages. When added in vitro clomipramine was able to diminish (from 10^{-4} to 10^{-7} M) both spontaneous and stimulated migration of macrophages. A similar effect was observed after the in vivo administration of the drug. In fact, both spontaneous and fMLP-induced mobility of peritoneal macrophages in vitro were significantly reduced after the subcutaneous injection of 20 and 40 mg/kg of clomipramine in comparison to the chemotaxis of macrophages obtained from saline-treated animals. These results give further evidence that psychoactive drugs can affect some immune parameters, and could contribute to explain the antiinflammatory action of clomipramine.

Keywords: Antidepressant; Inflammation; Chemotaxis; Macrophage

1. Introduction

In the last years it has been shown that psychoactive drugs, such as benzodiazepine, phenothiazines and tricyclic antidepressants, can interact with the cells of the immune system and modulate different immune responses, e.g. lymphocyte proliferation, natural killer activity, antibody production (Margaretten et al., 1987; Stacey and Craig, 1987; Sacerdote et al., 1993; Taupin et al., 1993; Xiao and Eneroth, 1995).

We previously showed that the tricyclic antidepressant drug clomipramine decreases human neutrophil chemotaxis in vitro (Sacerdote et al., 1994) and exerts antiinflammatory activity in different experimental models of inflammation in the rat (Bianchi et al., 1994, 1995).

It is well known that the recruitment of monocytes and macrophages to sites of inflammation and injury is a crucial step in the development and maintenance of inflammatory processes (Wahl, 1981). For all these reasons, we considered it of interest to evaluate whether either in vitro or in vivo treatment with clomipramine could affect the spontaneous mobility of macrophages and their ability to migrate in response to the classical chemotactic stimulus *N*-formyl-L-methionil-L-leucyl-L-phenylalanine (fMLP) (Schiffmann, 1982). We chose peritoneal macrophages since this macrophage population is readily available and is representative of other populations of the monocyte/macrophage cell line (Unanue, 1989).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g body weight) were housed at 22 ± 2°C with a light-dark cycle of 12:12 h and free access to water and food.

2.2. Collection of peritoneal macrophages

Each animal was decapitated, the abdomen was rinsed with 70% ethanol, the abdominal skin was carefully dissected without opening the peritoneum, and 15 ml of Hanks' solution (Sigma, St. Louis, MO, USA) was injected intraperitoneally. Then the abdomen was massaged and approximately 10 ml of Hanks' solution was removed. Resting macrophages, determined by morphology, were counted in a Neubauer chamber, and were adjusted with the same medium to 300 000 cells/ml. Cellular viability was routinely determined before and after each experiment

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using the test of trypan blue exclusion. The cells derived from a single animal were assessed for chemotaxis.

2.3. Chemotaxis assay

Chemotaxis was measured using a Boyden modified 48-well microchemotaxis chamber, in which the upper and the lower compartments were separated by a nitrocellulose filter (Biomap, Italy), with a pore diameter of 3 μm. Cells (15 000 macrophages/well) were placed in the upper chamber, and aliquots of either Hanks' solution (in order to evaluate spontaneous mobility) or of the chemoattractant fMLP (in order to evaluate chemotaxis) were placed in the lower chamber. The chambers were incubated for 3 h at 37°C, in an atmosphere of 5% CO_2, and then the migrated cells that adhered to the distal part of the filters were fixed and stained. Migrated cells were quantitated by counting three fields in triplicate with an optical image analyser (Sacerdote et al., 1993; De La Fuente et al., 1994).

2.4. In vitro effect of clomipramine

When the effect of clomipramine was determined in vitro, the drug at concentrations ranging from 10^{-4} to 10^{-10} M was added to the lower chemotaxis chamber without or together with a fixed concentration of fMLP (10^{-8} M).

In a different experiment, macrophages obtained from untreated rats were preincubated for 30 min at 37°C, in an atmosphere of 5% CO_2, in Hanks' solution (600 000 cells/2 ml) in the presence or absence of clomipramine at concentrations ranging from 10^{-4} to 10^{-10} M. At the end of preincubation the chemotactic activity of the cells was evaluated in the Boyden chamber in the presence of 10^{-8} M fMLP.

2.5. In vivo clomipramine treatment

Clomipramine was administered subcutaneously (s.c.) at the doses of 10, 20 and 40 mg/kg. Control animals were injected s.c. with the same volume of saline. The animals were killed 2 h after treatment. This experimental protocol was chosen on the basis of results obtained in previous studies (Bianchi et al., 1994, 1995). Each experimental group consisted of 6 rats.

Peritoneal macrophages obtained from saline- or clomipramine-treated rats were tested in vitro for their ability to migrate toward three concentrations of fMLP (10^{-8}, 10^{-9}, 10^{-10} M).

2.6. Drugs

The chemotactic peptide fMLP (Sigma) was stored as a stock solution of 10^{-2} M in dimethyl sulfoxide (DMSO) at −80°C and diluted in the chemotaxis buffer just prior to assay. The tricyclic antidepressant clomipramine (Ciba-Geigy, Origgio, Italy) was prepared fresh for each experiment in the chemotaxis buffer at concentrations ranging from 10^{-4} to 10^{-10} M.

2.7. Statistical analysis

The statistical analysis of results was obtained by analysis of variance followed by Bonferroni's *t*-test for multiple comparisons.

3. Results

In a first experiment we evaluated the ability of different concentrations of fMLP to induce chemotaxis of peri-

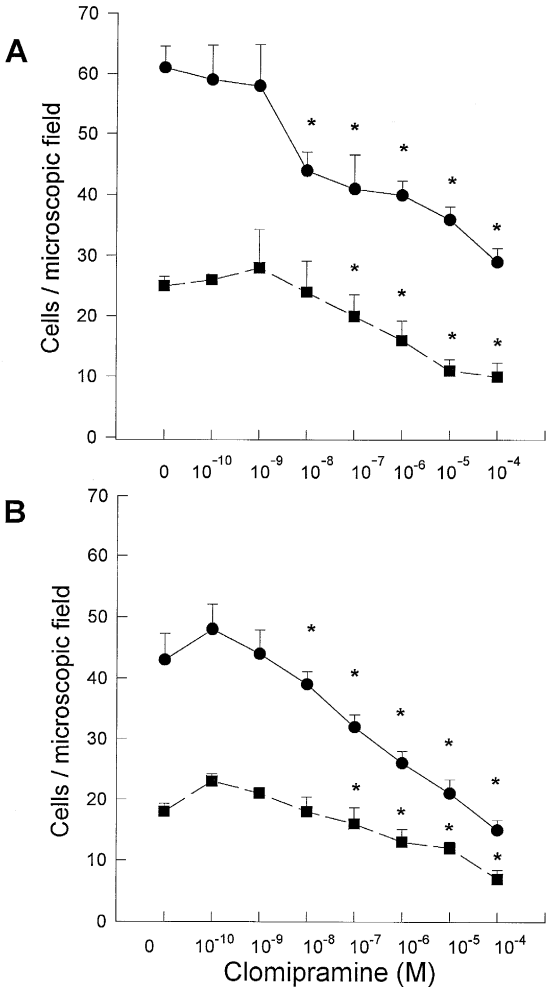

Fig. 1. (A) Effect of clomipramine, added in vitro, on spontaneous mobility (■) and on fMLP-induced chemotaxis (●) of rat peritoneal macrophages. fMLP was used at the concentration of 10^{-8} M. (B) Effect of 30 min preincubation with clomipramine, at the indicated concentrations on spontaneous mobility (■) and on fMLP-induced chemotaxis (●) of rat peritoneal macrophages. fMLP was used at the concentration of 10^{-8} M. The results are the means ± S.D. of 6 experiments. * $P < 0.01$ vs. control values (0 clomipramine).

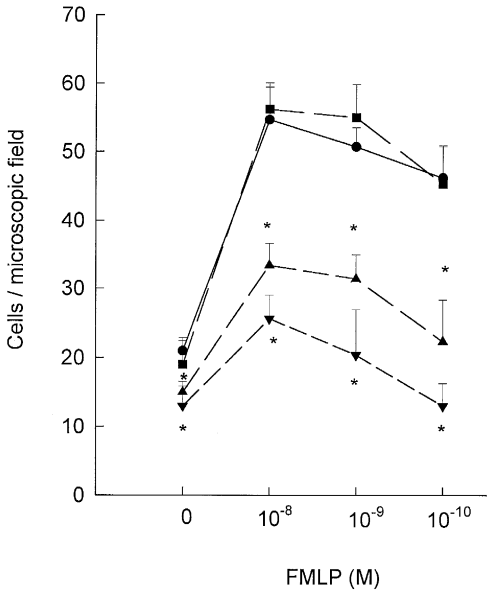

Fig. 2. Effect of the in vivo administration of clomipramine on spontaneous mobility (0 fMLP) and on fMLP-induced chemotaxis of peritoneal macrophages. (■) Saline; (●) clomipramine 10 mg/kg; (▲) clomipramine 20 mg/kg; (▼) clomipramine 40 mg/kg. The results are the means ± S.D for 6 animals. * $P < 0.01$ vs. saline.

toneal rat macrophages. As expected, fMLP was able to induce significant chemotaxis at the concentrations of 10^{-10}, 10^{-9} and 10^{-8} M, with a peak activity at 10^{-8} M (data not shown). This concentration was therefore chosen in order to evaluate the in vitro effect of clomipramine on macrophage chemotaxis. Fig. 1, panel A, shows that clomipramine, when added in vitro to the chemotaxis chamber in a wide range of concentrations, was able to significantly reduce both the spontaneous mobility of macrophages and the chemotaxis induced by fMLP. The effect on spontaneous mobility was significant at concentrations between 10^{-4} and 10^{-7} M, and on chemotaxis between 10^{-4} and 10^{-8} M. Similar results were observed when macrophages had been pre-incubated for 30 min in the presence of clomipramine (Fig. 1, panel B).

These effects of the drug were not due to a toxic effect on the cells, since the trypan blue exclusion test demonstrated that the cells were viable (data not shown).

Fig. 2 shows the effect of in vivo acute treatment with three doses of clomipramine on macrophage spontaneous mobility and on chemotaxis stimulated by three concentrations of fMLP.

Clomipramine dose dependently decreased the activity of macrophages: spontaneous mobility and chemotaxis of cells obtained from treated animals (20 and 40 mg/kg of clomipramine) were significantly reduced in comparison to those of macrophages obtained from control animals.

4. Discussion

The ability to migrate towards chemoattractant substances, such as fMLP, is a first and crucial event in macrophage physiology, and is essential in the immune response of the host to inflammatory stimuli (Schiffmann, 1982; Wahl, 1981).

The data of the present study indicate that the tricyclic antidepressant drug clomipramine interacts with rat macrophages and down-regulates their functions. In fact, both in vitro and in vivo administration of clomipramine decreased the spontaneous mobility and the chemotaxis of macrophages. We previously showed that clomipramine in vitro was able to block human neutrophil chemotaxis over a range of concentrations similar to those observed in this study for macrophages (Sacerdote et al., 1994). In this perspective, the in vitro effect of clomipramine on chemotaxis extends to macrophages an effect which has already been observed for another cell population involved in the inflammatory responses, i.e., granulocytes. The ability of clomipramine to decrease leukocyte mobility has been attributed mainly to its tricyclic chemical structure, since other non-tricyclic antidepressant drugs, i.e., fluoxetine and fluvoxamine, do not modify cell migration (Sacerdote et al., 1994). Since tricyclic antidepressant binding sites are present on immune cells (Audus and Gordon, 1982), clomipramine is likely to exert its effect by binding to these receptors.

Most interesting, however, is the observation that clomipramine blocked peritoneal macrophage chemotaxis also after in vivo administration. To our knowledge, this is one of a few papers showing an effect of in vivo administration of a tricyclic antidepressant drug on immune responses, and the first concerning cells of the monocyte-macrophage line. The peritoneal macrophage population has been shown to be affected by different physiological or pathological conditions, such as age or exposure of the animal to stressful conditions (De la Fuente et al., 1993; Forner et al., 1994), and therefore it represents a good target for in vivo drug treatment.

Also when administered in vivo, clomipramine seems able to directly interact with macrophages, decreasing their ability to respond to chemotactic stimuli.

The effects of clomipramine on the chemotaxis of immune cells can be involved in the reported antiinflammatory activity of this drug in different experimental models of inflammation. Clomipramine, in fact, induces antiinflammatory activity and decreases macrophage chemotaxis at the same doses and with a superimposable time course (Bianchi et al., 1994, 1995). By decreasing the responses of macrophages, the antidepressant drug can decrease the recruitment of these cells to sites of inflammation, thereby decreasing the release of proinflammatory mediators and the development of inflammatory phenomena.

Finally, another aspect of our results needs to be discussed. It has been described that patients with affective disorders could have an impaired immune function (Denman, 1986), and the suggestion that depressive states may increase an individual's susceptibility to infection and even tumour diseases has emerged (Khansari et al., 1990).

Therefore, the decrease of some immune functions induced by clomipramine could worsen a pre-existing immunosuppression.

In conclusion, our data, with the demonstration that clomipramine decreased both in vivo and in vitro macrophage migration, contribute to clarification of the pharmacological profile of this antidepressant drug.

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