ARTIGO ORIGINAL

# Paroxetine and bupropion have no *in vitro* effects on lynphocyte proliferation and viability

*Paroxetina e bupropiona não apresentam efeito na viabilidade nem na proliferação de linfócitos in vitro*

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## RESUMO

**Objetivo:** Os estudos iniciais com antidepressivos tricíclicos demonstraram que estes prejudicam a atividade do sistema imune. Estudos mais recentes sugerem que os inibidores seletivos da recaptação de serotonina poderiam apresentar efeitos imunológicos estimulantes. No presente estudo, exploramos os efeitos imunológicos *in vitro* de dois antidepressivos usados na prática clínica, paroxetina (inibidor seletivo da recaptação de serotonina) e bupropiona (inibidor da recaptação da noradrenalina e dopamina). **Método:** Obtiveram-se amostras de sangue periférico de 16 voluntários saudáveis e as célu-las mononucleares do sangue periférico foram isoladas e cultivadas *in vitro*. Avaliamos os efeitos de bupropiona e da paroxetina em termos de viabilidade das células, como também a habilidade para suprimir a proliferação de linfócitos induzida por fitoemaglutinina. **Resultados:** Nenhum efeito significativo foi produzido por ambos os antidepressivos na viabilidade das células nem na proliferação de células T. **Conclusões:** Esses resultados podem ser de valiosa informação para a prática clínica quando essas drogas são administradas. Esses resultados indicam um efeito mais favorável desses psicofármacos quando comparados aos efeitos imunológicos relacionados ao uso de antidepressivos tricíclicos ou lítio.

**Palavras-chaves**
Neuroimunomodulação, antidepressivos, linfócitos, proliferação de células.

## ABSTRACT

***Objective:*** *Initial studies with tricyclic antidepressants demonstrated that they jeopardize the immune system activity. Recent studies suggested that selective serotonin reuptake inhibitors would have stimulating immunological effects. Here, we explored the* in vitro *immunological effects of two antidepressants used in clinical practice, paroxetine (selective serotonin reuptake inhibitor) and bupropion (norepinephrine and dopamine reuptake inhibitor).* ***Method:*** *Peripheral blood samples were obtained from 16 healthy volunteers and the peripheral blood mononuclear cells were isolated and cultured* in vitro*. We evaluated the effects of bupropion and paroxetine on cell*

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**Key-words**
Psychoneuroimmunology, antidepressive agents, lymphocytes, cell proliferation.

*viability as well as the ability to suppress phytohemagglutinin-induced lymphocyte proliferation.* ***Results:*** *Both antidepressants produced neither significant effect on cell viability nor on T-cell proliferation.* ***Conclusions:*** *This could be of valuable information for the clinical practice when these drugs are administered. These results indicate a more favorable effect of such psychopharmacological drugs when compared to reported immunological effects associated with tryciclic antidepressants.*

## INTRODUCTION

Mood disorders are major public health problems with mood fluctuations occurring from time to time (Bakish, 2001). The unipolar depression has a high prevalence in the general population (approximately 5%) (Sadock and Sadock, 2005), increasing patients's risk of suicide and being associated with other diseases like cancer and cardiovascular diseases. It is also considered one of the main causes of morbidity and mortality in the western countries (World Health Organization, 2006).

One of the main features observed in clinical practice is the relationship between depressive episodes and changes in the immune system. Such relationship was later corroborated by many researches. There are two aspects to consider here: immune responses appear to be blunted in cases of severe depression (Zorrilla *et al.*, 1996) or cytokine treatments can cause symptoms of depression in patients with no previous history of mental disorder (Pollak and Yirmiya, 2002; Musselman *et al.*, 2001).

Similarly, the serotonin transporters (implicated in the pathogenesis of mood disorders) that are present in the central nervous system (CNS) may also be found in cells of the immune system. The study of the cerebral distribution of serotonin transporters (5HT) (Barker and Blakely, 1995) showed high concentration areas of them in the amygdala, thalamus, hypothalamus, substantia nigra, hippocampus, locus ceruleus and raphei nuclei – a high-density structure of serotonergic neurons (Owens and Nemeroff, 1994). The 5HT transporters are thoroughly distributed in several peripheral areas like platelets, placenta, lung, mast cells and lymphocytes. Although the exact physiological mechanism of 5HT transporters in such cells is unknown, it seems that in the lymphocytes they have positive immunomodulatory properties (Lima and Urbina, 2002). Therefore, the "serotonin deficit" in the CNS, observed in cases of severe depression, could be associated with dampened immune responses.

The immunomodulatory role of selective serotonin reuptake inhibitor (SSRI) is suggested by the direct action on lymphoid cells. Several studies had evaluated the role of serotonin and drugs that act as SSRI on the immune system. Paroxetine, for instance, is one of the most well known and widely use drug for the treatment of major depression – as well as for other psychiatric conditions such as anxiety disorders and post traumatic stress disorder (Schatzberg *et al.*, 2004). By acting at the presynaptic nerve terminal as a SSRI, paroxetine shows an important therapeutic effect in depression and anxiety symptoms (Sadock and Sadock, 2005). Paroxetine has produced conflicting immunological effects in cells of patients and healthy subjects. A large number of studies have demonstrated stimulating effects on peripheral leukocytes (Iga *et al.*, 2005; Kim *et al.*, 2004; Frank *et al.*, 1999) whereas others found no significant changes (Denys *et al.*, 2006). However, there are a few studies on the immunological effects in healthy subjects. Furthermore, the immunological effects of antidepressants with no serotonergic action are largely unknown. Some studies that investigated the role of other medications than SSRIs usually involved antidepressants with some kind of serotonergic activity such as venlafaxine and mirtazapine (Denys *et al.*, 2006; Pena *et al.*, 2005).

Bupropion is an antidepressant drug with a unique mechanism of action: it has dopaminergic and noradrenergic activity with no clinically significant effects on serotonin reuptake (Sadock and Sadock, 2005). It has successfully been used for major depression as well as tobacco addiction treatments. The immunological effects of this drug, however, remain largely unclear. Except for some controversial discussion about the role of bupropion in the pathogenesis of medical conditions with immunological features (e.g. erythema multiform; Carrillo-Jimenez *et al.*, 2001; Drago and Rebora, 2002), no investigations have been made to elucidate the immunological effects of bupropion in healthy individuals.

In the present study, we explored the immunomodulatory effects of an antidepressant with a predominant action on the serotonin system (paroxetine) and another medication acting on norepinephrine and dopamine systems (bupropion).

## METHODS

### Subjects

Sixteen healthy young adults (20-40 yrs; 6 females) were selected from PUCRS (Porto Alegre, RS) for subsequent analysis. Individuals suffering from organic or psychiatric diseases, as well as taking medications, except for oral contraceptives,

were not included in the sample. Those individuals were considered healthy based on a clinical evaluation constituted by a physical examination.

The individuals who agreed to participate in the project read and signed an informed consent term. The project was approved by the Research Ethics Committee of PUCRS, reference number 646/05 (July 25, 2005).

### Collection of peripheral blood mononuclear cells (PBMCs)

Twenty ml of peripheral blood were collected by venepuncture in the morning (between 9-10 a.m.) and samples were stored into lithium-heparin tubes prior to analysis. The PBMCs were isolated by centrifugation (900 x G, 30 min) by means of a density gradient (Ficoll-Histopaque, Amersham). After being washed in isotonic solution (Hanks, Sigma), the cell count was performed in a Neubauer camera (100 x) using Trypan Blue (Sigma) and the cell viability was always higher than 95%. The cells were resuspended in complete culture medium (RPMI-1640, supplemented with 0.5% of gentamicin, 1% of glutamine, 1% of hepes, 0.1% of fungizone and with 10% fetal calf serum; all from Sigma) and adjusted for a concentration of $3 \times 10^6$ cells/ml.

### Lymphocyte proliferation/viability assays

PBMCs were cultured in flat bottomed 96-well microplates in a final concentration of $1.5 \times 10^5$ cells/well, in complete culture medium, for 96 hours at 37°C under a 5% $CO_2$ atmosphere. Stimulation was performed by the selective T-cell mitogen phytohemagglutinin (PHA; from Gibco) in triplicates (100 µl/well) to yield an optimal concentration (1%). In non-stimulated cultures (PHA 0), the mitogen was replaced by culture medium (Mossman, 2002; Collaziol *et al.*, 1983). To assess the *in vitro* sensitivity to the tested drugs, bupropion (200 to 6.25 ng/ml) or paroxetine (600 to 1.2 ng/ml), were added in triplicates (50 µl/well) to mitogen-stimulated (PHA 1%) and non-stimulated (PHA 0) cultures. The drug range used here mimicked the therapeutic levels found for bupropion (25 to 100 ng/ml (Cordioli, 2006) or paroxetine (30 to 200 ng/ml) (Denys *et al.*, 2006) during treatment.

The proliferative responses were estimated by a modified colorimetric assay that correlates with the number of viable cells. In the last 4 hours of culture, 100 µl of the supernatant was gently discarded and 40 µl of freshly prepared MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) (Sigma) solution (5 mg/ml in sterile PBS) was added to each well. The cell cultures were incubated for 4 hours, at 37ºC, under a 5% $CO_2$ atmosphere. After 96 hours of culture, the supernatant was completely aspirated and 120 µl of Dimethyl Sulfoxide (DMSO; Sigma) was added to each well in order to dissolve the formazan crystals completely. The optical density (OD) was determined using Biorad ELISA plate reader at a wavelength of 570/655 nm.

### Statistical analysis

All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. The results of the lymphoproliferative responses were analyzed by ANOVA. Multiple comparisons among levels were checked with Bonferroni post hoc test. A difference was considered statistically significant when $p < 0.05$. Data are expressed as mean ± SE in all figures and tables. A statistical software (SPSS 11.5, Chicago, USA) was used for the analyses.

## RESULTS

In this study, we assessed the role of two antidepressants (paroxetine and bupropion) in modulating human cell proliferation/viability. It was observed that paroxetine did not change viability of unstimulated PBMCs (figure 1A), $F(10, 150) = 0.24$, $p = 0.99$. Paroxetine was also unable to modulate mitogen-induced T-cell proliferation (figure 1B), $F(10, 150) = 0.83$, $p = 0.60$.

Accordingly, bupropion did not change viability of unstimulated PBMCs (figure 2A), $F(6, 90) = 1.16$, $p = 0.34$. Bupropion was also unable to modulate mitogen-induced T-cell proliferation (figure 2B), $F(6, 90) = 0.82$, $p = 0.56$.

## DISCUSSION

Although severe depression is clearly associated with several immunological changes, it still remains to be established to what extent these changes could be related to psychopharmacological treatments. Little is known regarding the effect of antidepressant drugs in healthy individuals. The present study evaluated the immunological in vitro effects of paroxetine and bupropion considering a wide drug range established by previous data on serum therapeutic levels (Denys *et al.*, 2006; Cordioli, 2006). We demonstrated here that paroxetine and bupropion have no effects of cell viability or mitogen-induced T-cell proliferation.

Previous studies that examined the effects of antidepressant therapy on immunity yielded conflicting results. A large number of studies reported that paroxetine had stimulating effects on peripheral leukocytes (Iga *et al.*, 2005; Kim *et al.*, 2004; Frank *et al.*, 1999) whereas others showed no significant changes (Denys *et al.*, 2006). These conflicting results could be related to differences in methodology, species studied and clinical status. We speculate that the lack of response in our study was due to the fact that the cells examined were obtained from healthy individuals, supposedly without alterations in the 5HT transporters (Hickie *et al.*, 1990; Cruess *et al.*, 2005). It is worth to be mentioned that even in depressed patients the paroxetine may have no immunological effects if it is not paralleled by clinical

changes. There is also a greater probability of finding changes in lymphocyte 5HT transporters in patients with severe depression, with melancholic characteristics and associated clinical co morbidities (Zorrilla *et al*., 1996; Pollak and Yirmiya, 2002; Musselman *et al*., 2001; Frank *et al*., 1999).

We reported for the fist time that bupropion has no effects on cell viability or lymphocyte proliferation. Previous studies observed a tendency of improvement of the immune response with the administration of other double action antidepressants, with at least some serotonin action (mirtazapin) (Pena *et al*., 2005). Other classes of antidepressants were associated with inhibitory action on the immune system. For instance, tricyclic antidepressants (e.g. nortriptyline) had an inhibitory response on the lymphocyte proliferation and natural killer (NK) cell activity (Audus and Gordon, 1982; Gauer, 1995).

Another aspect that can help understanding the immunological effects to psychopharmacological treatment concerns the sample characteristics. For instance, the subject's immunological status could be important during the assessment of peripheral antidepressant effects. In individuals with lower NK cell activity, as compared to equally depressed patients with normal or high NK cell activity, the effects of the SSRI antidepressants seem to be more evident (Denys *et al*., 2006). These data suggest that the immunological effects of SSRIs are more favorable in subjects who had jeopardized immune responses concomitantly with psychiatric symptoms. Furthermore, inpatients usually suffer from more serious depressive symptoms than outpatients and thus are more likely to offer a better immunological response, under appropriate treatment, than outpatients (Carrillo-Jimenez *et al*., 2001). Similarly, severe depressive episodes, unlike those of moderate degrees, present a better response to the antidepressant treatment.

The deficit of 5HT transporters in patients suffering from an untreated severe depression seems to affect to some extent the function of peripheral lymphocytes. Transporters are stimulated and increase in number during the treatment and a more quantitative than qualitative alteration in lymphocytes is therefore observed, which may improve the immune function (Lima and Urbina, 2002). The increased expression of mRNA 5HT transporters in peripheral leukocytes was related to the physiopathology of depression and decreased disease relapses induced by the antidepressants (Cruess *et al*., 2005).

## CONCLUSIONS

In conclusion, the lack of immunological changes ascribed here to paroxetine and bupropion could be of valuable information for the clinical practice whenever these drugs are administered. Further studies are required to investigate other immune parameters not evaluated here (e.g. cell trafficking, activation markers, cytokine production) as well as to explore the *in vivo* immunological effects of these antidepressants in patients with depression.

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