ARTIGO ORIGINAL

Selective serotonin-reuptake inhibitor and norepinephrine dopamine reuptake inhibitor antidepressants do not affect natural killer cell activity *in vitro*

Antidepressivos inibidores seletivos de recaptação da serotonina e inibidores da recaptação de noradrenalina e dopamina não afetam a atividade celular natural killer in vitro

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RESUMO

Objetivo: Avaliar o efeito citotóxico de dois antidepressivos comumente utilizados na prática, a paroxetina e a bupropriona. Além disso, buscou-se avaliar a atividade *natural killer* (ANK) após a incubação dos linfócitos com esses fármacos. **Métodos:** Sangue venoso de 15 participantes foi coletado e as células mononucleares (PBMCs) foram separadas e incubadas por 24h com (ou sem = grupo-controle) concentrações de paroxetina e bupropiona em 30, 100 e 1.000 ng/ml. Após a incubação, a quantidade das células mortas foi contada utilizando-se o método *trypan blue*. Posteriormente foi avaliada a ANK por meio do ensaio clássico de liberação do Cr^{51}. **Conclusões:** Ocorreu morte celular de PBMCs proporcionais às doses dos fármacos, no entanto, a ANK não foi afetada, mesmo com a redução do número de células efetoras.

Palavras-chave
Atividade *natural killer*, antidepressivos, sistema imune, psicoimunologia, psicofarmacologia, toxicidade medicamentosa.

ABSTRACT

Objective: *This study aims to evaluate the citotoxic activity of two commonly used anti-depressants: paroxetine and bupropion. We also evaluated the in vitro natural killer activity (NKA) after incubating the blood samples with the antidepressants.* ***Methods:*** *Peripheral blood samples from 15 healthy volunteers were collected and the mononuclear cells (PBMCs) were isolated and incubated for 24h with (or without = control cells) paroxetine and bupropion, in concentrations of 30, 100 and 1000 ng/ml. After the incubation period in both groups, the amount of dead cells was calculated using trypam blue technique. NKA was evaluated using the classic ^{51}Cr release assay.* ***Conclusions:*** *PBMCs dead cells occurred in both groups and in proportion to all pharmacological concentrations. Nevertheless, the NKA was not affected, even with the reduction in the number of effective cells.*

Keywords
Natural killer activity, antidepressants, immune system, psychoimmunology, psychopharmacology, drug toxicity.

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INTRODUCTION

The relation between depressive episodes and changes in the IS is clear in clinical practice. There are two aspects to be considered here: immune responses are blunted in cases of severe depression[1] or cytokine treatments can cause symptoms of depression in patients with no previous history of mental disorders[2].

A study of the cerebral distribution of serotonin transporters (5HT)[3], also found in IS cells, showed high concentration of 5HT in areas of the amygdala, thalamus, hypothalamus, substantia nigra, hippocampus, locus ceruleus and raphei nuclei – structure of serotonergic neurons[4]. It seems that in lymphocytes 5-HT it has immunomodulatory properties[5]. Some authors suggested that the immunomodulatory role of SSRI is originated from the direct action on lymphoid cells.

Paroxetine, for instance, is one well known and widely used drug for the treatment of major depression, anxiety disorder and post-traumatic stress disorder[6]. Some studies have demonstrated stimulating effects on peripheral leukocytes[7], whereas others found no significant changes[8]. Other studies that investigated the role of medications other than SSRIs, usually involved antidepressants with some kind of serotonergic activity such as venlafaxine and mirtazapine[8,9]. Bupropion is an antidepressant drug with a unique mechanism of action: it has noradrenergic and dopaminergic (NDRI) activity with no clinically significant effects on serotonin reuptake[10]. Except for some controversial discussion about the role of bupropion in the pathogenesis of medical conditions with immunological features (e.g. erythema multiform)[11,12], no investigations have been made to elucidate the immunological effects of bupropion in healthy individuals.

Tricyclic antidepressants (TCA) like imipramine, that act upon both the noradrenergic and the serotonergic systems jeopardize the immune system, and that, conversely, ISSR like paroxetine improve it, raised an important question. In order to contribute to answer this question we decided to evaluate separately the effect in the IS of a drug with only serotonergic activity (paroxetine) and a drug with only noradrenergic/dopaminergic activity (bupropion)[13]. As we had the results regarding the activity of both drugs on lymphocyte proliferation[15] we decided to examine their citotoxicity and NKA action.

METHOD

Subjects

Fifteen healthy young adults (20-40 yrs; 6 females), university students were selected for subsequent analysis. A psychiatric and a physical examination was performed. Individuals suffering from mental disorder or any disease (as well as history of), and those individuals in current use of medication, except for oral contraceptives, were excluded.

Procedures *in vitro*

Incubation with mononuclear cells

Peripheral blood 20 ml samples were collected from 15 normal volunteer and mononuclear cells were isolated by centrifugation over a Ficoll-Hypaque gradient (Sigma, St. Louis, MO USA). Peripheral blood mononuclear cells (PBMC) were ressuspended at a concentration of $5x10^6$ cells/ml in RPMI-1640 culture medium (Sigma), supplemented with penicillin 1%, streptomycin 1%, with 10% fetal calf serum (FCS – Gibco). Paroxetine and bupropion were dissolved in distilled water and diluted to concentrations of 25, 100 and 1000 ng/ml in RPMI with 10% FCS. Mononuclear cells were incubated for 24h at 37º and 5% CO_2, in culture flasks, with the different concentrations of paroxetine or bupropion. After the incubation period, the amount of dead cells, incubated or not with the drugs, was calculated using trypan blue. The cell suspension resulting was adjusted to a final concentration of 5×10^6/ml viable cells. Control cells were incubated under identical conditions without drugs. After the incubation period the cells that survived were washed, resuspended in RPMI with 10% FCS, serially diluted in a microtiter plate, and assessed for cytotoxic activity using the ^{51}Cr release assay as described below.

Natural killer activity assay

The NKA was assayed in a standard ^{51}Cr release assay, using as target the K562 human erythroleukemia derived cell line. Viability of target cells exceeded 95%, as judged from their ability to exclude trypan blue. Mononuclear effector cells were adjusted to 5×10^6 cells/ml viable cells. K562 target cells were labelled with 150 µCi of $Na^{51}CrO_4$ (IPEN-CENEN, São Paulo, Brazil) for 2 h at 37ºC and 5% de CO_2, washed and adjusted to 5×10^4 cells/ml in RPMI and 10% FCS. Cells were plated (using V-bottom microplates) in triplicate with several effector-target ratios (100:1, 50:1, 25:1, and 12:1) and incubated at 37ºC in 5% CO_2 for 4 hours. Aliquots (100 µl of supernatant) were analysed in a γ-counter and percent of specific lysis was determined according to the formula:

$$\% \text{ of Lysis} = \frac{100 \times \text{Experimental release (cpm)} - \text{Spontaneous release (cpm)}}{\text{Total (cpm)} - \text{Spontaneous release (cpm)}}$$

The mean specific release for the three optimum effector-target cell ratios was utilized as the unit of measurement of NKA.

Statistical analysis

NKA results were analyzed by ANOVA for repeated measures with post hoc analyses. A difference was considered

statistically significant when $p < 0.05$. All statistical analyses were performed with the statistical software SPSS/PC 12 (Chicago, EUA).

Ethics considerations

The individuals who agreed to participate in the project read and signed an informed consent term. The project was approved by the Ethics Committee in Research of the Pontifícia Universidade Católica do Rio Grande do Sul (CEP/PUCRS n. 646/05) (July 25, 2005).

RESULTS

Bupropion

Analyzing the amount of dead cells using the ANOVA for repeated measures, there was a statistical difference related to the concentration of the drug. The number of cells from the control group (mean = 4.90×10^6; SD = 0.52×10^6) was different from the number of cells on the bupropion group in the concentration of 30 ng/ml (mean = 4.15×10^6; SD = 0.46×10^6); 100 ng/ml (mean = 3.60×10^6; SD = 0.35×10^6) and 1000 ng/ml (mean = 2.77×10^6; SD = 0.59×10^6). There was a significant difference in direction of a lower number of cells in all the concentrations at the bupropion group when compared to the control ($p < 0.001$).

Regarding the NKA comparing all concentration levels of bupropion (i.e., 30 ng; 100 ng; 1000 ng/ml), accordingly to the ANOVA for repeated measures analysis there was no difference in the lytic activity between control lymphocytes and lymphocytes pre-incubated with bupropion ($p = 0.260$).

Paroxetine

Regarding the amount of dead cells the ANOVA for repeated measures analysis showed no statistical difference between the number of cells at the control group (mean = 4.82×10^6; SD = 0.48×10^6) and cells incubated with paroxetine at 30 ng/ml (mean = 4.46×10^6; SD = 0.35×10^6). However there was a difference when the control group cell number was compared to the number of cells on the paroxetine group using the concentration of 100 ng/ml (mean = 3.70×10^6; SD = 0.36×10^6) or 1000 ng/ml (mean = 2.43×10^6; SD = 0.47×10^6). There was a significant difference in the direction of a lower number of cells in the concentrations of 100 ng/ml and 1000 ng/ml at paroxetine group when compared to the control ($p < 0.004$).

Comparing the NKA at all concentration levels of paroxetine (ie., 30 ng; 100 ng; 1000 ng/ml), there was no difference in the lytic activity between control lymphocytes and lymphocytes pre-incubated with paroxetine ($p = 0.128$).

DISCUSSION

Despite the fact that pre-incubation with paroxetine and bupropion produced, *in vitro,* a clear toxic effect in PBMC, even when concentrations were higher than therapeutic plasma levels (1000 ng/ml), the ability of the remaining viable cells to kill the eritroleukemic K562 tumor cells was not affected. Because these drugs present serotonergic and noradrenergic action, these findings suggest that their action on these systems are probably not related to NKA inhibition. These results suggest that the blunted NKA and lymphocyte proliferation seen with TCA might be related to their action on other receptors such as the histaminergics or cholinergic ones. Another hypothesis is that such effects result from a direct action of those old medicines molecules over the IS. It is possible that these two new agents do not have such intrinsic property.

Some studies reported that paroxetine had stimulatory effects on peripheral leukocytes[7] whereas others showed no significant changes[9]. One aspect to be considered is that the lack of response in our study could be due to the fact that the cells examined were obtained from healthy individuals, supposedly without alterations in 5HT transporters[14]. It is worth mentioning that even in depressed patients the paroxetine may not have the 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, melancholy and associated clinical co morbidities[1,2]. Furthermore, another study reported for the fist time that bubropion has no effect on lymphocyte proliferation[15] and, in the present study, cells pre-treated with bupropion maintained NK activity. Previous studies observed a tendency for improvement of the immune response with the administration of other double action antidepressants, with at least some serotonin action (mirtazapin)[9].

CONCLUSION

Despite the limitations of this study (small sample and there was no comparison between the results of these ADs with other drugs), paroxetine and buproprion didn't seem to affect the NKA in any of the plasma concentrations, even in doses much higher than the recommended practice. The present findings show that these drugs have a toxic effect for the PBMCs but they seem not to interfere in the NKA. So, they might have a more favorable effect on the immune system, specially when we compare the results with others described in the literature with older agents, such as the tricyclics ADs.

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