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A new chapter opens in anti-inflammatory treatments:The antidepressant bupropion lowers production of tumor necrosis factor-alpha and interferon-gamma in mice

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Abstract

In a wide range of human diseases of inflammatory nature like Crohn's disease, pathology is mediated in part by pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF) or interferon-gamma. We show here that a commonly used generic antidepressant bupropion, in wide use worldwide to treat depression in humans for a decade now, profoundly lowers levels of TNF, interferon-gamma, and interleukin-1 beta in vivo, in a mouse lipopolysaccharide (LPS) induced inflammation model. Mice challenged with an otherwise lethal dose of LPS were protected by bupropion and levels of the anti-inflammatory cytokine interleukin-10 were increased. Previous data in rodents and humans indicate antidepressant effects of bupropion are mediated by its weak reuptake inhibition of norepinephrine and dopamine. Concordant with this, TNF suppression by bupropion in our mouse LPS model was largely abrogated by beta-adrenergic or dopamine D1 receptor antagonists but not by a D2 antagonist. TNF synthesis is controlled by an inverse relationship with intracellular cyclic adenosine monophosphate (cAMP) and stimulation of either beta-adrenoreceptors or D1 dopaminergic receptors result in increased cAMP but stimulation of D2 receptors lowers cAMP. We conclude that bupropion may suppress TNF synthesis by mediating increased signaling at beta-adrenoreceptors and D1 receptors, resulting in increased cAMP that inhibits TNF synthesis. Bupropion is well tolerated also in non-psychiatric populations and has less risk with long term use than current anti-inflammatory, immunosuppressive or TNF suppressive treatments such as prednisone, azathioprine, infliximab, or methotrexate. New anti-inflammatory treatments are needed. We believe a new chapter in antiinflammatory, TNF lowering treatment of disease has been opened. Bupropion's use for this in humans should be explored.

Keywords: Bupropion; Crohn's disease; Dopamine; Inflammation; Interferon-gamma; Norepinephrine; Tumor necrosis factor-alpha

1. Introduction

Case reports by the late 1990s indicated that antidepressant monamine oxidase inhibitors (MAOI) such as phenelzine, might induce remission of Crohn's disease [1], and rheumatoid arthritis [2]. The neurochemically

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related but non-MAOI antidepressant bupropion likewise occasionally was seen to result in remission of Crohn's disease [3,4] and today bupropion is increasingly used specifically for induction of remission in Crohn's disease in absence of depression. Tumor necrosis factor-alpha, TNF, is central to development of gut aphthous ulcerations of Crohn's disease and bupropion lowering of circulating TNF was noted in a case of hepatitis B [5]. This lead to suspicions that bupropion was lowering TNF. We report here experimental results in mice that show bupropion did indeed lower TNF in a sepsis model.

Lipopolysaccharide, LPS, from *Escherichia coli* cell wall, binds to Toll-like receptor-4 on monocytes' outer cell membrane and is one of the most potent stimuli known for TNF [6]. Massive cytokine release is part of the circulation collapse and multi-organ failure of sepsis. Such catastrophic cytokine release can be experimentally induced by using LPS alone and this is an established model for cytokine release study. We investigated effects of bupropion on TNF and other inflammatory mediators interferon-gamma (IFN-gamma), interleukin-1 beta; and interleukin-10 (IL-1 beta and IL-10) and nitric oxide (NO).

MAOIs increase extracellular amounts of norepinephrine and dopamine by inhibiting their intracellular catabolic enzyme. Bupropion increases extracellular amounts of norepinephrine and dopamine by inhibiting their transport back into the cell after release [7]. We therefore sought to also examine the role of the common denominators-increased extracellular norepinephrine and dopamine in mediating TNF suppression by bupropion, by administering norepinephrine and dopamine antagonists with bupropion.

2. Materials and methods

2.1. Mice

Male, 6-week-old BALB/c mice were used in LPS-induced shock experiments. Mice were raised and maintained at animal facilities at the Gonçalo Moniz Research Center-FIOCRUZ, whose ethical Review Committee approved this work. Mice were provided rodent diet and water ad libitum. Based on previous experience with this sepsis model, animal numbers in experimental groups were thought to be the minimum necessary. All animals were killed after full ketamine/xylazine anesthesia was obtained.

2.2. Endotoxic shock

One hour after intraperitoneal (i.p.) injection of placebo or bupropion, mice were injected i.p. with a dose of 600 μg of LPS (from *E. coli* serotype 0111:B4, Sigma, St. Louis, MO, USA) in saline. This dose was previously determined as the minimum LD 100. Separate groups of mice received i.p. injection of beta-adrenoceptor blocker propranolol (1 mg/kg), or selective D1 antagonist SCH23390 (25 μg/kg), or selective D2 antagonist sulpiride (25 mg/kg) 1 h prior to injection of bupropion, the numbers in each group given in Table 1. Mice were monitored for 4 days. To determine serum TNF, IL-1 beta and IL-10 levels, mice were killed 90 min after injection of LPS and bled by axillary veinipuncture. Levels of IFN-gamma and NO were assayed using sera of mice killed 4 h after LPS injection. Platelet and leukocyte cell counts were performed using blood collected with EDTA 4 h post LPS challenge, using an automated cell counter (ADVIA 60, Bayer).

2.3. Cytokine and NO determinations

TNF, IL-1beta, IFN-gamma and IL-10 concentrations in serum samples were determined by enzyme-linked immunosorbent assay (ELISA) using antibody pairs and recombinant cytokines from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions. The minimum detectable TNF was 5.1 pg/ml, the coefficient of variation 7.3%. For NO measurement, serum concentration of nitrite (NO_2–) after reduction was determined by the Griess reaction. Absorbance of the reaction product at 570 nm was measured using an ELISA reader.

2.4. Statistical analyses

Data were analyzed using Student's *t* test, one-way ANOVA or Newman–Keuls multiple comparison test, using Graph Pad Prism 3.0 software. Differences were considered significant when $P<0.05$.

3. Results

Levels of TNF, IL-1 beta and IFN-gamma of mice pretreated with 100 mg/kg of bupropion were significantly reduced in comparison with those of placebo treated mice after LPS challenge (Fig. 1A–C). Levels of IL-10 were significantly

Table 1
Effects of beta-adrenergic and dopaminergic receptor antagonists on bupropion induced protection against endotoxic shock

Treatment	Percentage of survival (%)	(numbers)
Placebo + Sulpiride	33	(5/15)
Bupropion + Sulpiride	100	(10/10)
Bupropion + Propranolol	20	(1/5)
Placebo + Propranolol	0	(0/5)
Bupropion + SCH23390	40	(2/5)
Placebo + SCH23390	0	(0/5)
Bupropion	100	(10/10)
Placebo	0	(0/15)

Bupropion (100 mg/kg), sulpiride (25 mg/kg), propanolol (1 mg/kg) and SCH23390 (25 μg/kg).

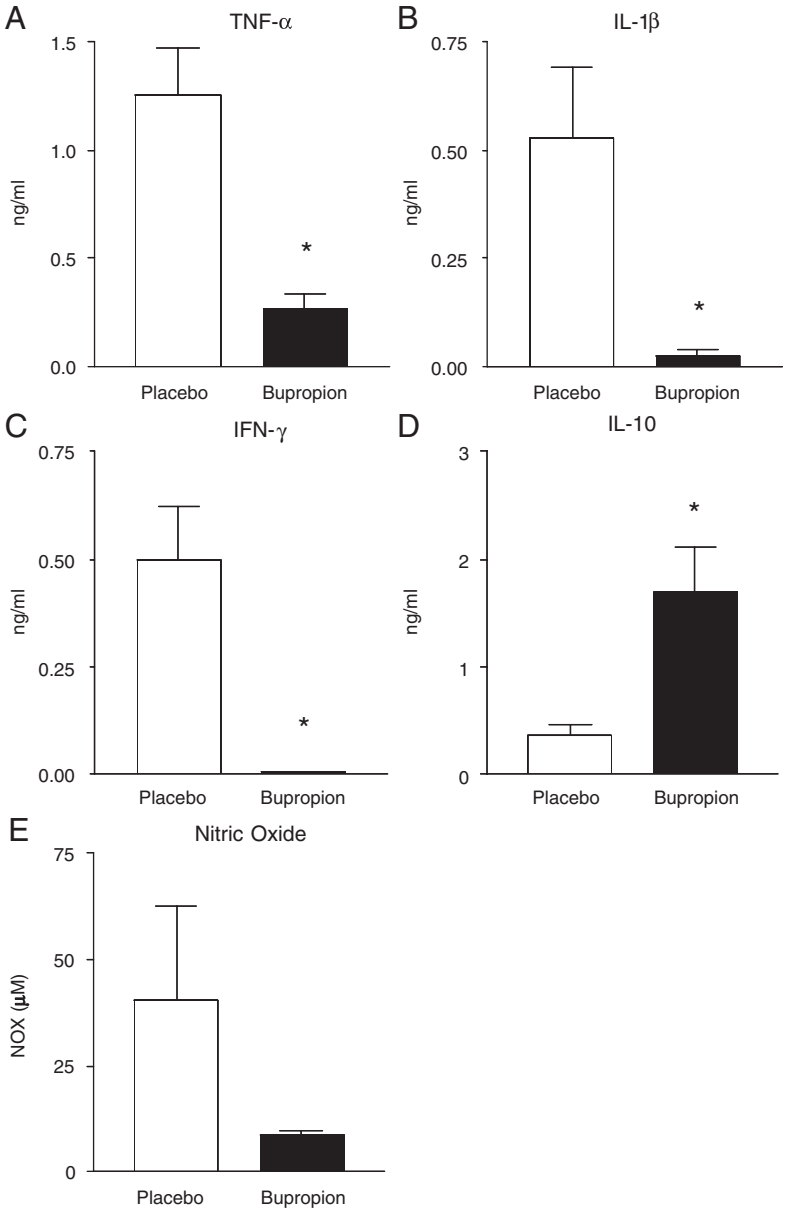

Fig. 1. Effects of bupropion treatment in cytokine and NO production in mice challenged with LPS. Male BALB/c mice were injected with 100 mg/kg of bupropion or with placebo i.p. in saline 1 h before challenge with 600 μg of *E. coli* LPS by i.p. route. Mice were killed 90 min (A, B and D) or 2 h (C) later for detection of serum cytokines by sandwich ELISA. Values represent means ± SEM of 5 mice per group. (E) Pooled sera of 5 mice/group were assayed for nitric oxide production by the Griess method. *$P < 0.05$.

raised compared with placebo treated mice (Fig. 1D). NO production was four-fold reduced in bupropion treated mice compared to placebo treated mice (Fig. 1E).

Bupropion mediated survival in LPS-induced shock in a dose dependent manner (Fig. 2A). Signs of endotoxemia, such as diarrhea, immobility, lethargy, piloerection and shivering were milder or absent in mice treated with 100 mg/kg of bupropion, than in placebo treated mice. Platelet counts 4 h after LPS challenge in bupropion-treated mice were significantly higher than placebo mice and similar to those of normal, untreated mice (Fig. 2B). Bupropion treated mice had higher leukocyte levels (Fig. 2C) compared with placebo treated mice.

Non-selective beta-adrenergic receptor antagonist propranolol and D1 selective dopamine receptor antagonist SCH23390 were able to partially block bupropion mediated survival (Table 1). Neither propranolol nor SCH23390 alone protected mice from a lethal challenge with LPS. The D2

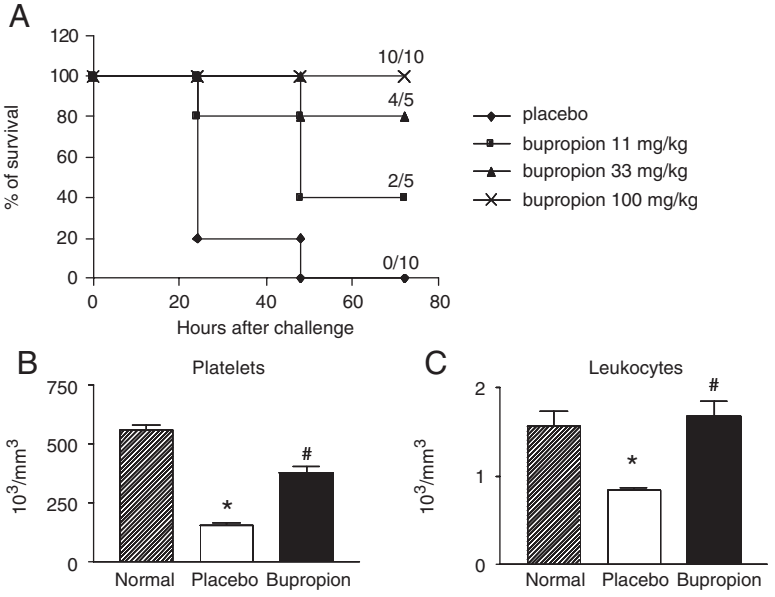

Fig. 2. Bupropion protects mice against a lethal challenge of LPS. Groups of BALB/c mice were injected with bupropion or with placebo i.p. in saline 1 h before challenge with 600 μg of LPS (DL-100) by i.p. route. (A) shows mortality of mice (5–10 per group) challenged with a lethal dose of LPS. (B and C) Show platelet and leukocyte numbers evaluated in peripheral blood samples collected 4 h after LPS challenge in an automated cell counter. *$P<0.05$ compared to normal group of untreated mice and #$P<0.05$ when compared to LPS+placebo group.

antagonist sulpiride alone gave partial protection but did not change effects of bupropion (Table 1).

4. Discussion

Our data provides evidence that the commonly used antidepressant bupropion interferes with in vivo LPS-stimulated cytokine production in mice. Production of NO is known to be stimulated via LPS-induced TNF and IFN-gamma, and blocked by IL-10. NO decrease we saw therefore may have been direct, or have resulted from bupropion mediated inhibition of TNF and/or IFN-gamma, and/or by the increase in IL-10 production. Partial abrogation of protection by norepinephrine and/or D1 dopaminergic antagonists indicate that bupropion may lower inflammatory cytokines by increasing signaling at beta-adrenergic and/or D1 receptors.

Lymphocytes synthesize and bear receptors for dopamine [8,9]. Mice with targeted deletions of the same dopamine transporter that bupropion inhibits, show similar cytokine changes to those that we here demonstrate [10]. These mice also show immunological consequences that one would expect from these changes—increased antibody and decreased cell mediated responses to antigen [10].

There is a well-established inverse relationship between intracellular cAMP levels and TNF synthesis [11,12]. D1 dopamine receptor subtype positive coupling to andenylate cyclase [13], the rate limiting enzyme in cAMP synthesis, and D2 receptor subtype negative coupling to adenylate cyclase [13] leads to the obvious hypothesis that immunomodulation by bupropion is mediated by increased agonist effects at D1 dopaminergic or beta-adrenoceptors on macrophages and/or lymphocytes, resulting in increased intracellular cAMP with consequent decreased TNF synthesis.

LPS induced “cytokine storm” is universally fatal in these mice unless given protection. Some protection and a decrease in TNF was seen using sulpiride, concordant with the findings of others who found decreased TNF from LPS-treated monocytes in vitro after the D2 antagonist haloperidol [14,15]. Negative coupling of D2 receptors to adenylate cyclase and hence to cAMP levels results in D2 agonists decreasing cAMP therefore D2 antagonists will tend to prevent D2 mediated cAMP lowering. Note also that D2 receptors have intrinsic activity in absence of ligand, so sulpiride and haloperidol and most other clinically used anti-psychotic medications are really inverse agonists and will therefore increase intracellular cAMP even when no dopamine signaling is present at D2 receptors. The partial reversal of bupropion protection by the selective D1 antagonist SCH 23390 (see Table 1) again indicates that bupropion is affecting TNF levels via D1 stimulation through intracellular cAMP increase.

Considerable expense and iatrogenic morbidity are seen with long term use of current anti-inflammatory treatments. Uncontrolled case reports have indicated bupropion reduces disease activity in several inflammatory diseases where TNF is thought to be pathophysiologically active: atopic dermatitis and psoriasis [16], Crohn's disease [3,4], recurrent aphthous oral ulcers not associated with Crohn's disease [17], and hepatitis B [5]. Strong evidence exists that TNF mediates important pathogenic steps in several malignancies, for example chronic lymphocytic leukemia [18] and multiple myeloma [19]. Bupropion may be of use in these as well.

Our results are concordant with previous work, for example that showing tricyclic antidepressants' suppression of LPS induced TNF in vitro [20], where increased intracellular cAMP was documented and correlated with TNF suppression [20], and work showing the non-tricyclic antidepressant fluoxetine suppresses non-stimulated in vitro production of TNF by human whole blood cultures with indications this occurred by an increased cAMP mechanism [21].

Does bupropion lower TNF in any human disease? Urgent research should be directed to definitively answering this question. Bupropion suppression of TNF synthesis may prove to be beneficial in a wide variety of inflammatory and malignant human diseases.

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