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Bosentan, a mixed endothelin receptor antagonist, induces antidepressant-like activity in mice

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HIGHLIGHTS

- Bosentan reduces immobility time in the forced swimming test and tail suspension test.
- Bosentan increases circulating IL-6 levels in mice exposed to forced swimming test.
- This is the first evidence that endothelin mediates depression-like behavior.

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ABSTRACT

Endothelins are peptides described initially as potent vasoactive mediators. Recently, studies reported that endothelins can modulate the production and release of cytokines by immune cells. In turn, cytokines are involved in depression disorders and also in the effectiveness of some antidepressants. Therefore, we investigated the effects of treating mice with bosentan, a mixed endothelin receptor antagonist, in widely used models for assessing antidepressant activity of compounds, the forced swimming (FST) and the tail suspension tests (TST). Moreover, the influence of bosentan treatment on circulating IL-6 levels was also addressed after FST. The results show that bosentan treatment induced a bell shaped dose-dependent antidepressant-like effect with increase in circulating IL-6 levels in animals exposed to FST. Bosentan also presented antidepressant-like effect in TST. Similar results were obtained with nortriptyline treatment in the FST and TST. Possible anxiogenic effect of bosentan was excluded using the elevated plus maze test. Therefore, this is the first study to demonstrate the antidepressant-like activity of bosentan in mice, unveiling a previous unrecognized role of endothelin in depression and its possible relation with increased circulating IL-6 levels.

1. Introduction

Endothelins were first described as 21-amino acids peptides derived from endothelial cells with potent vasoactive properties [1]. In mammals, the endothelin family comprises 3 ligands (ET-1, ET-2 and ET-3) and 2 endothelin-receptors (endothelin ET_A and ET_B) [2–4]. Immune cells such as macrophages [5], monocytes and lymphocytes [6] produce endothelin and also express endothelin receptors, indicating the autocrine effect of endothelin in those cells, which is related to modulation of cytokine production [7–9].

Cytokines are cellular mediators that drive innate and adaptive immune responses to various stimuli. The ability of nervous tissue to respond to cytokines allows immediate and complex adaptive peripheral [10,11] and central [12] responses of the organism. Interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α are pro-inflammatory cytokines that have been related to the pathophysiology of depression. The IL-1β and TNF-α can induce depressive-like symptoms in animal models of helplessness (or despair) like Porsolt's forced swimming test (FST) [13,14]. In contrast, the systemic administration of IL-6 induces antidepressant activity in FST, stimulates the hypothalamo–pituitary–adrenocortical (HPA) axis, and increases tryptophan levels and serotonin metabolism in several brain regions [15]. Moreover, the modulation of circulating levels of these cytokines has proved to be necessary to reverse depressive-like symptoms by antidepressants such as imipramine, venlafaxine and *Hypericum perforatum* [16–18].

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Endothelin receptor antagonists inhibit the production of cytokines during inflammation [7–9]. Therefore, we reason that targeting endothelin receptors could affect depressive-like behavior by modulating the production of cytokines. In this sense, the effects of bosentan, a mixed endothelin ET_A/ET_B receptor antagonist, was investigated in FST, tail suspension test (TST) and elevated plus maze test (EPM). Moreover, we also investigated the effects of bosentan on circulating levels of IL-6 after exposure to FST. Bosentan was selected since it was the endothelin receptor antagonist prototype and the first to enter clinical use [19].

2. Materials and methods

2.1. Animals

Male Swiss mice of 20–25 g from Universidade Estadual de Londrina were used in this study. Mice were maintained at the Department of General Psychology and Behavior Analysis of the same University, at least two days before the experiments with free access to water and food, and temperature of 23 °C ± 2. A 12/12 h light/dark cycle was used with lights on at 6 and off at 18 h. All behavioral tests were made between 12–17 h. Animal care and handling procedures were approved by Ethics Committee of the Universidade Estadual de Londrina (OF. CIRC. CEUA N° 07/2011). Every effort was made to minimize the number of animals used and their suffering.

2.2. Protocol of treatment

Bosentan (a mixed endothelin ET_A/ET_B receptor antagonist, diluted in 1% solution of xanthan gum in saline) was administered per orally (p.o.) with the aid of a gavage needle at the doses of 0.3–300 mg/kg, 1 h before FST or at the dose of 3 mg/kg 1 h before EPM and TST. The vehicle group received solution of xanthan gum 1% in saline (p.o.), 1 h before the tests. Nortriptyline (15 mg/kg, i.p., 1 h) was used as control drug. All groups were of 10 mice.

2.3. Forced swimming test

FST was performed as previously described [20]. Briefly, mice were placed into a cylinder of 10 cm diameter and 30 cm height with water at 25 °C ± 1 reaching 19 cm of water column to avoid the tail touch in bottom [20]. Sessions were recorded and the immobility time (depression-like behavior) was registered in seconds by an experimenter unaware of the groups. Immobility was considered when the animal moved just to keep his head out of the water in the last 4 min of the total swimming session of 6 min.

2.4. Tail suspension test

The TST was used as described before [21] to corroborate the antidepressant activity of treatment with bosentan. Mice received bosentan (3 mg/kg, p.o.), vehicle or nortriptyline (15 mg/kg, i.p.), 1 h before the TST. A short piece of adhesive tape was used to attach mice by the tail about 20 cm above the floor. Mice were recorded during 6 min and the immobility time (depression-like behavior) was registered in seconds by an experimenter unaware of the groups.

2.5. Elevated plus maze

In order to discard possible anxiogenic or anxiolytic activity of bosentan, which in turn would also modulate the immobility time in forced swimming test [22], the EPM test was used as described before [23]. Mice received bosentan (3 mg/kg, p.o.), vehicle or nortriptyline (15 mg/kg, i.p.), 1 h before the EPM test. The number of entries and the time spent in the open and the enclosed arms were recorded during the session of 5 min.

2.6. Cytokine measurement

Mice were treated with bosentan (0.3–300 mg/kg, p.o.), vehicle or nortriptyline (15 mg/kg, i.p.), 1 h before FST. In the sham group, the cytokine levels on blood samples were determined 6 min after swimming for just 3 s. At the end of the test, mice were terminally anaesthetized with 1.5% isoflurane (Abbott Park, IL, USA) and the blood samples were collected in microcentrifuge tubes. The samples were centrifuged at 1300 rcf for 20 min and the supernatant collected. IL-6 levels were determined by ELISA using eBioscience kits as indicated by manufacturer. The results are expressed as picograms (pg) of IL-6 per ml of serum ($n = 10$).

2.7. Statistical analysis

Since the variables were only the treatments, we used analysis of variance with one criterion (One-way ANOVA) followed by the multiple comparison Tukey's test. Differences were considered significant for $P < 0.05$.

3. Results

3.1. Treatment with bosentan reduces depression-like behavior in FST and TST

Treatment with bosentan showed significant reduction of the immobility time in the FST in a dose-dependent manner (Fig. 1A). A bell shaped curve was observed with no antidepressant-like activity at the dose of 0.3 mg/kg of bosentan, maximal effect with the dose of 3 mg/kg and reduction of effect with the dose of 30 mg/kg up to no effect with the dose of 300 mg/kg of bosentan. Therefore, the dose of 3 mg/kg was selected for the next experiments at Fig. 1B–D. Treatment with bosentan significantly reduced the immobility time in TST (Fig. 1B), but showed no significant difference in the percentage of entries into the open arms (Fig. 1C) or in the time spent in the open arms (Fig. 1D), neither in entries into the closed arms in EPM (data not shown) test. Taken together, these data suggest that bosentan reduced immobility time in FST due to an antidepressant-like activity, exclusively. The control drug nortriptyline at a dose selected from the literature [24] presented antidepressant-like activity as expected (Fig. 1A and B). Curiously, the treatment with nortriptyline showed an acute anxiolytic activity in EPM test by increasing both the percentage of entries into and time spent in the open arms (Fig. 1C and D).

3.2. Treatment with bosentan increases IL-6 serum levels in mice exposed to FST

Exposure to FST did not alter the serum levels of IL-6 (Fig. 2). However, the treatment with bosentan increased IL-6 serum levels (Fig. 2). Again, a bell shaped curve was observed with significant increase in IL-6 serum levels in the groups treated with bosentan at the doses of 3 and 30 mg/kg, as well as nortriptyline group. The doses of bosentan of 0.3 and 300 mg/kg, or vehicle did not alter significantly the serum levels of IL-6 (Fig. 2).

4. Discussion

The Porsolt's forced swimming test (FST) is the most widely used test for assessing pharmacological antidepressant activity of compounds [20]. In the present study, we used FST to investigate a