Antidepressant behavioral effects of duloxetine and fluoxetine in the rat forced swimming test[1]

Efeitos antidepressivos da duloxetina e da fluoxetina no teste do nado forçado em ratos

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ABSTRACT

Purpose: To compare the effects of the antidepressant drugs duloxetine and fluoxetine on depressive behaviors in rodents. **Methods:** Eighteen male Wistar rats were given systemic injections of duloxetine, fluoxetine, or saline prior to a Forced Swimming Test (FST). Immobility and number of stops were measured. **Results:** Rats given injections of fluoxetine displayed significantly less immobility ($p = 0.02$) and fewer stops than the control group ($p = 0.003$). Duloxetine significanlty reduced the number of stops ($p = 0.003$), but did not effect immobility ($p = 0.48$). **Conclusion:** Duloxetine and fluoxetine reduced depressive behaviors in the Forced FST. However, our findings suggest that fluoxetine is more effective than duloxetine.
Key words: Physiological Effects of Drugs. Fluoxetine. Depression. Animal Experimentation.

RESUMO

Objetivo: Comparar o efeito antidepressivo da droga cloridrato de duloxetina com a fluoxetina. **Métodos:** O teste do nado forçado, teste comportamental que avalia a atividade antidepressiva em ratos, foi utilizado em 18 ratos Wistar, machos adultos, divididos em três grupos iguais: duloxetina, fluoxetina e controle. **Resultados:** Os dados do teste do nado forçado foram analisados pelo teste One-way ANOVA, Mann Whitney e Kruskall-Wallis.Houve diferença significativa ($p = 0,003$) entre o número de paradas dos grupos duloxetina e fluoxetina e o grupo controle. **Conclusão:** A duloxetina e a fluoxetina tiveram frequência de paradas similares. A fluoxetina mostrou ser mais efetiva que a duloxetina no teste do nado forçado em ratos.
Descritores: Efeitos Fisiológicos de Drogas. Fluoxetina. Depressão. Experimentação Animal.

Introduction

Depression has been described as normal alterations in humor as well as a group of specific disturbances. Sadness and unhappy feelings are common in situations where one faces loss, separation, failure, or interpersonal conflicts. The biological etiology of depression is hypothesized to be due to a deficiency in monoaminergic neurotransmitters, and more specifically, deficiencies in norepinephrine (NE) and serotonine (5-hidroxitriptamina; 5-HT). The Diagnostic and Statistical Manual of Mental Disorders [1] describes the emotional symptoms of depression as sadness, lack of interest, guiltiness, and suicidal thoughts. Although this definition emphasises the presence of emotional symptoms, physical symptoms are also recognized as a key component of the depressive syndrome. These physical symptoms include a lack of energy, sleep disorders, pain, headaches, changes in appetite, gastrointestinal disorders, and changes in psychomotor function. Depressed patients visit doctors and hospitals more frequently than the non-depressed population [3] and have more limited activity levels 4. Because the emotional symptoms of depression are more easily recognized, the physical symptoms are often underestimated, which makes a proper diagnosis and prognosis more difficult [2]. Antidepressant drugs that potently inhibit 5-HT/NE reuptake, such as duloxetine (Cloridrate of (+)-(S)-N-metil-y-(1-naftanelinoxi)-2-tiofenopropanamine, formula: C18H19NOSHCl) [2], have been developed to treat major depression, especially in patients with pain and urinary incontinence. Duloxetine weakly inhibits the reuptake of dopamine and does not have a significant affinity for muscarinic, histamine, beta-adrenergic, dopaminergic, serotonergic, or opioid receptors. Duloxetine Cloridrate is not chemically related to other selective inhibitors of serotonin and NA reuptake, tryciclics, tetracyclics, or other available drugs prescribed for the treatment of major depression disorder [2]. In animals, duloxetine lowers brain 5-HT levels via chloroanphetamine and hypothalamic NE and epinephrine depletion [5]. Using in vitro and in vivo analyses, Kasamo et al.[6] showed that duloxetine is a double inhibitor of 5-HT and NE reuptake. Moreover, the results of various experiments indicate that duloxetine has preferential effects on the 5-HT transporter Antidepressants that inhibit the reuptake of 5-HT and NE, such as duloxetine, have fewer reported side effects than tryciclic antidepressants, and thus are ideal for the treatment of chronic pain associated with depression [7]. Iyengar et al. [8] recently found duloxetine to be more effective than gabapentine, an antiepileptic drug, in the treatment of neuropathic pain. In addition, Reneric [9] demonstrated that fluoxetine, or compounds with mixed activity for inhibiting NE and 5-HT reuptake, has effects similar to those of desipramine alone and may have serotonergic antidepressant behavioral effects due to the selective reuptake of NE inhibitors. The forced swimming test (FST) is a behavioral task used in rodents that predicts the efficacy of antidepressant treatments in humans. In this task, the rodents are placed in a tank of deep water for an extended period of time, and the length of time the rodent remains immobile, making only those movements necessary to keep its head above water, is measured [10]. This immobilization represents a state of desperation in the rodents, which is a symptom seen in depression. Antidepressant drug treatment reduces this immobility time, and thus results in increased activity, which is reflective of the efficacy of the antidepressant drug[10]. This test is relatively selective for antidepressant drugs, as few other psychoactive drugs elicit effects in the FST. One study, in which fluoxetine was administered with either desipramine or bupropion (to enhance either both 5-HT and NE or dopamine transmission) indicated that these drug combinations could simultaneously increase two distinctive active behaviors in the FST. However, the overall behavioral pattern of the fluoxetine-desipramine combination was similar to that of desipramine alone, and duloxetine versus fluoxetine alone was not tested. The purpose of the present study was to compare the behavioral effects of the antidepressant drugs duloxetine and fluoxetine on the antidepressive behaviors of Wistar rats submitted to the FST.

Methods

Eighteen male Wistar rats, weighing between 200 and 300 g, were housed in groups of six in polycarbonate cages. They were maintained on a 12-h light-dark cycle in a temperature-controlled (22°C) colony room and had free access to food and water. The experiments were performed according to the Guide for the care and use of laboratory animals, and the Ethics Committee for Experiments on Animals approved all procedures.

Drug treatment

The rats were divided into three groups of six: Fluoxetine group, Duloxetine group, and Control group. All injections were given intraperitoneally at 24, 5, and 1 h before the SFT. The Duloxetine HCl (Lilly Co.) group received 40 mg/kg duloxetine, dissolved in sterile water 1 mL/kg. The Fluoxetine HCl (Lilly Co.) group received 10 mg/kg fluoxetine. The Control group received 0.25 ml of saline solution.

Forced swimming test

The procedure used has been previously described by Porsolt et al. [10]; Porsolt et al. [11]; Porsolt [12], Detke [13], and Reneric [9]. The animals were forced to swim inside a cylinder filled with water, without the possibility of escaping. The resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. When the animals ceased all movements except those necessary for survival (keeping the head above the

water), the behavior was considered to be immobile. This was classified as induced depression. On the first day (pre-session test) the rats were forced to swim for 15 min in an acrylic cylinder (29 cm diameter x 50 cm tall), containing 25°C water 40 cm deep. The water was changed after every swimming test to eliminate urine, excrement, and fur. After the swimming session, the rats were removed from the cylinder, dried with towels, and placed under a light bulb (32°C) for 15-30 min. They then received the first dose of their respective drug and were returned to their home cage. Twenty-four h later, the rats were tested under the same conditions for 5 min (test session). They received their second and third doses of their respective drugs at 5 and 1 h prior to the test session. Immobility was measured (sec) as well as the number of times they stopped swimming (number of stops). Immobility referred to the absence of movement, with the body inclined forward, passively floating, and the paws immobile. The pretest and test sessions were videotaped (Panasonic video color camera and recorder) for scoring the movements and behavior of the animals.

Statistical analysis

Behavioral scores of the duloxetine, fluoxetine and control groups were compared using a one-way ANOVA and multiple comparisons were analyzed by the Tukey test. Nonparametric analyses were compared with the Kruskal-Wallis and Mann Whitney tests. $p < 0.05$ was considered statistically significant.

Results

As shown in Table 1, there was no significant difference in weight between the Duloxetine and Fluoxetine groups ($p = 0.69$); however these two groups were significantly different from the control group ($p = 0.002$). The Duloxetine and Fluoxetine groups did not differ in the number of stops ($p = 0.240$). The control group, however, made more stops than the two experimental groups ($p = 0.003$). Again, there was no difference between the Duloxetine and Fluoxetine groups in immobility.

TABLE 1 - Drug effects on weight and FST behavior

Variable	Duloxetine	Fluoxetine	Control (Saline)	p
Weight (g)	284.5 ± 19.96	274.17 ± 18.09	232.67 ± 26.31	0.002A
Number of stops	5.33 ± 3.39	3.00 ± 1.79	18.5 ± 8.02	0.003B
Immobility (min)	14.2 ± 6.28	4.7 ± 3.0	25.6 ± 7.98	0.108C

Mean ± standard error; A One-way ANOVA; B Kruskal-Wallis Test; C Mann Whitney Test

Of the two experimental groups, only the Fluoxetine group displayed significantly lower immobility than the Control group ($p = 0.02$). The Duloxetine and Control groups had similar immobility times ($p = 0.48$). Analysis of the two antidepressant groups together (Duloxetine + Fluoxetine) resulted in a significant reduction in immobility time ($p = 0.005$) and number of stops ($p = 0.005$) when compared to the control group.

Discussion

In the present study, we used the FST to compare the effects of duloxetine and fluoxetine on antidepressive behaviors, including immobility. Antidepressant drugs, as well as electroconvulsive shocks and REM sleep deprivation, have been shown to reduce immobility. Porsolt et al [11] found that such immobility is reflective of a low-mood state in the rats, which is sensitive to antidepressant treatment. The FST technique, described in detail by Porsolt et al.[12], has been widely used to evaluate depressive behavior in rats, dogs [14], and in children separated from their parents [15]. According to Harloe et al. [16], young monkeys separated from their mothers demonstrate two very distinctive behavioral stages. The first stage consists of high levels of calmness, then screams and protests; in the second stage the monkeys cease all activity, clearly showing need, sadness, and depression. Similar behaviors were observed in the control group in the present study. However, rats given an antidepressant drug treatment displayed significantly less immobility and stops than the control rats. Thus, the drugs made them more active and less depressed. Consistent with the findings of Mendes-da-Silva et al.[17] and Dableh [18], in the present study fluoxetine reduced both immobility and the number of stops in the FST. Although the fluoxetine group displayed a slightly lower number of stops than the duloxetine group, the difference was not significant. However, contrary to Reneric [9], we did not find a difference between the duloxetine and control groups in the time spent immobile. This finding may indicate that fluoxetine is a stronger antidepressant drug than duloxetine. It is also likely that experimental variations or the smaller sample size contributed to the lack of an effect of duloxetine on immobility, as there was a non-significant trend. When duloxetine and fluoxetine were analyzed together as a group, these antidepressant drugs were significantly effective at reducing depressive behaviors, as compared to the control group.

Conclusion

The results of the present study suggest that fluoxetine (10 mg/kg) is more effective than duloxetine (40 mg/kg) in reducing FST-induced depressive behaviors.

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