



Potential of the Antidepressant Effects of Fluoxetine by Administration of Aspirin in a Mouse Model

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Abstract

Background: Previous studies have indicated that major depressive disorder and anxiety disorders are associated with inflammatory progressions. It has also been shown that administrations of anti-inflammatory drugs improve the patient's psychiatric condition. These facts suggest that NSAIDs may be used as an adjunctive therapy in the management of depression. Herein, we investigated whether a combination of suboptimal dose of the selective serotonin reuptake inhibitor, fluoxetine, with aspirin improves the anti depressive effects of fluoxetine.

Methods: Both mouse forced swim and tail-suspended mice, as models of animal depression, displayed respectively, inactive floating position, decreased active swimming and immobility behavior. In these models, mice were treated with single optimal (20mg/kg, ip) or suboptimal (5mg/kg, ip) doses of fluoxetine 30min prior to running experiment.

Results: In contrast to fluoxetine treatment using 20mg/kg, a suboptimal dose induced no significant changes in the immobility duration time, active swimming and climbing trials. Interestingly, co-administration of 400mg/kg of aspirin significantly increased the reduction in immobility duration time in both models. This effect coincided with a significant increase in active swimming. In a subsequent experiment, the anti-immobility effect of this combined treatment was not associated with increase mouse general locomotor activity.

Conclusion: These findings clearly demonstrate that aspirin potentiates the beneficial effects of fluoxetine and that psychostimulant effect does not contribute to the antidepressant value in this mouse model of depression.

Keywords: Fluoxetine; Aspirin; Depression; Forced swim test; Tail suspension test; Mouse

Introduction

Previous studies have demonstrated that major depressive disorder (MDD) is rank fourth position among mood disorders and worldwide is considered as the most prevalent psychiatric condition [1]. However, although the availability of different kinds of antidepressant therapy and launching of selective serotonin reuptake inhibitors (SSRIs), nearly 50% of the patients do not agreeably achieve remission. Indeed, the remarkable association between attempted suicide and delay in the onset of remission of depressive episodes did lead the researchers exploring for a new line of treatments for patients who are suffering from MDD.

The mechanisms behind the etiology of MDD are not yet fully understood. A possible link between depression and inflammation has been found in clinical and preclinical investigations. Numerous studies have demonstrated that immune dis-regulation and an elevation in plasma cytokine production provoke depressive

symptoms in patients with MDD [2]. It has also been shown that activation of the inflammatory pathways play a role in the depressive symptoms [3]. In addition, psychiatric clues were observed during inflammation [4]. Nonetheless, amelioration in the clinical efficacy of patients with major depression [5], and the improvement in the antidepressant efficacy in experimental animals [6] after the concurrent administration of NSAIDs with SSRIs were proved to be mediated by immunological events. A feasible role for pro-inflammatory cytokines and prostaglandins (PG) in regulation of brain norepinephrine and serotonin systems, which are linked to MDD and its treatment, has been reported [7]. Furthermore, higher *in vitro* lymphocyte PGE2 level was observed in patients with MDD than in healthy counterparts [8]. Accordingly, it seems probably that cyclooxygenase (COX) enzyme activity is triggered during depression, leading to pro-inflammatory cascades in the CNS. Clinical studies have showed that celecoxib, a selective

COX-2 inhibitor, enhanced significantly the therapeutic efficacy of reboxetine in patients with MDD [9]. In contrast, other studies have indicated that NSAIDs potentially interfere with the behavioral responses to antidepressants drugs [10].

Therefore, considering these conflict observations in animal and humans' data, the present study was aimed to investigate the mood effect of aspirin, a classical NSAID, in combination with a SSRI, fluoxetine, at a suboptimal dose on the despair behavior in two models predictive of classical behavioral paradigms, the mouse forced swim test and tail suspension test.

Materials and Methods

Experimental animals

Experiments were carried out using 72 inbred healthy male mice of the same age, 2 months±one week; and weighting 19 - 30gm of the Albino strain. Mice were procured from the local animal house of Faculty of Pharmacy, University of Tripoli, Tripoli, Libya. Mice were grouped and housed in macrolon cages with no more than six animals per cage, and kept under the same laboratory conditions at temperature of 26 - 28 °C and normal lighting time. They were kindly handled for two days prior to experimental time at the testing room. Mice were allowed a free access to standard laboratory chow, except during testing, while *ad libitum* to water all the time. All experiments were carried out during daylight, starting morning at 10:30 and finished two hours after midday time. Experimental protocols and animals care and use were managed respectively in agreement to the Ethics Committee for Research Programs in University of Tripoli (2015) and to the instructions of animal welfare.

Drugs

The drugs used in this study were fluoxetine HCl (Lilly Co., USA) and aspirin (Panpharma S.A, France). The injected doses were designated according to experimental needs and based on earlier equivalent studies [11,12]. One ml syringe connected to 25gm blunted needle was used for the ip injection.

Experimental design

The present study applied the most widely typical behavioral despair models for assessment of the antidepressant effect; mouse forced swim test and tail suspension test as well as an open field test for general locomotor activity.

Mouse forced swim test

The antidepressant activity was evaluated by the use of forced swim test as described early in rats by Porsolt and his colleagues [13] with a minor modification in mice [14]. This model is characterized by pre-test and test session "six minute each session" within 48 consecutive hours wherein mice had forced to swim. Briefly, a mouse was dropped gently into vertical Plexiglas cylinder contains 15cm depth water maintained at 25±2 °C (height 22cm and diameter 21cm) for a maximum time of six minutes. Thereafter, the mouse was removed, dried and returned back to its macrolon cage. The pre-test session was performed 24 hours before injection

of the test drugs. This pre-test session was executed to facilitate the development of motionlessness behavior "immobility conduct" during the test session. Thereafter, mice were divided into four groups (n=6 mice for each group). Control mice received 10ml/kg normal saline ip. Fluoxetine. was given for the second and third groups at optimal (20mg/kg) and suboptimal (5mg/kg) doses, ip, respectively. The fourth group was treated with a combination of suboptimal dose of fluoxetine and 400mg/kg aspirin, ip. After 30min, forced swim test, the test session, was performed. Mouse retained in the cylinder for the first time was firstly struggle to escape from the water but after certain period of time its movement reduced and established allocated behavior with episodes of immobility and floating of increasing duration. Each mouse was considered as immobile whenever it ceased struggling; adopt a posture upright position, inactively floating in the water keeping its nose above water. The mouse exhibited climbing response when it makes forceful thrashing movements by its forelimbs directed against the walls of the cylinder. Normal movement of the mouse within the center of the cylinder was defined as active swimming. Expression of depressive reactions, immobility time "the absence of escape-oriented behaviors", together with active swimming time, the variables that statistically analyzed, were measured using a digital watch during the last 4min of the 6min observation period. The number of climbing trials was also counted. The antidepressant-like activity was expressed as the decrease in duration of immobility time compared to the control group.

Tail suspension test

The tail suspension test is considered as analogous to the forced-swim test in the mouse and it is a reliable, rapid and simple technique used to evaluate the psychotropic activity and in particular antidepressants activity. The entire time of immobility incited by tail suspension was determined according to the method firstly elucidated by Steru and others [15]. Briefly, the mouse was suspended individually by its tail on a 40cm horizontal metal rod distanced 50cm above the bench and 120cm from the ground using an adhesive tape, fixed one cm from the tip of the tail. During the first minute the mouse was tried to escape from its condition by showing twisting movements. Afterward, mouse developed behavioral despair characterized by disturbing response and immobility, an indication for depression state. Drugs categorized as antidepressants are believed to reduce immobility time displayed by mice following vigorous and unsuccessful trials of escape after suspended by the tail. In this experiment, mice were divided into four groups (n=6 for each group). Thirty minute before experiment, mice were treated ip. with fluoxetine or combination of aspirin and fluoxetine at a suboptimal dose, groups and doses are similar as described early in forced swim test (see above). Control group received 10ml/kg normal saline. Each mouse was then suspended, upside down, for six minutes. The immobility conduct, "mouse hung-down passively and entirely motionless", was observed and duration of immobility (the entire time that the mouse displayed no movement) was recorded during testing period using a digital stop watch. The parameter evaluated was the time, in seconds, spent immobile. The antidepressive activity was expressed as the

total duration of immobility in treated group relative to the control group.

Open field test for locomotor activity

The ambulatory behavior was measured in an open-field test of general locomotor activity as previously described [16] with a slight modification. Briefly, the open field box (Opto-Varimex monitor, Columbus Instruments, USA) is a rectangular open box made of 44 x 44cm ground floor with 18cm high walls. Before starting, the experiment mice were acclimatized to the open field box for 30min. In order to preclude any unspecific spontaneous motor effect (exploratory behavior) of aspirin, mice were injected with equivalent dose that potentiate the effect of suboptimal dose of fluoxetine in the forced swim and tail suspension tests to have their locomotor activity evaluated. Therefore, mice were treated with normal saline (10ml/kg: group 1), optimal fluoxetine dose (20mg/kg: group 2), suboptimal fluoxetine dose (5mg/kg: group 3) or a combination of suboptimal fluoxetine dose and aspirin (5/400mg/kg: group 4). All tested drugs were given ip. After 30min of drugs administration, the locomotor activity was assessed. In a sound attenuated condition, each mouse was placed in the middle of the box and left to move freely for 4min. The number of squares crossed and traveled by each mouse was counted.

Statistical Analysis

All data are shown as the mean response time \pm SEM. The statistical differences between groups were judged by the use of one-way analysis of variance (one-way ANOVA). When ANOVA

revealed that there is a significant variation among the groups Tukey's *Post hoc* test was applied to output the position of difference between the individual groups. The probability (p) value was considered significant when it is < 0.05. For the analysis of data Graph Pad Prism (Graph Pad Software Inc., version 3.0, San Diego, USA) was functioned.

Result

Forced swim test

There were no remarkable differences between all the groups observed in responsiveness to acute model of forced swimming during the pre-test session (data not shown). After 24h, the control group, during test session, showed observable depressive situation expressed by floating of mice passively in water, which reached to 177.5 (SEM=6.5) sec of immobility time, during 4min observation time (Figure 1). Compared with control group, fluoxetine, at the suboptimal dose 5mg/kg, did not change the development of episodes of immobility and floating of mice to forced swim (Figure 1). Whereas, at 20mg/kg, fluoxetine significantly decreased the immobility duration time in seconds by 44.9% (97.8 \pm 10.9 vs.177.5 \pm 6.5, p <0.001, Figure 1) compared with control group confirming its antidepressant activity. Interestingly, mice in the combination group of aspirin and suboptimal fluoxetine dose showed significant lesser immobility duration time by 52.6% (p <0.001, Figure 1) compared with control mice, indicating that co-administration of aspirin significantly augments the antidepressant activity of fluoxetine in a mouse model of behavioral despair.

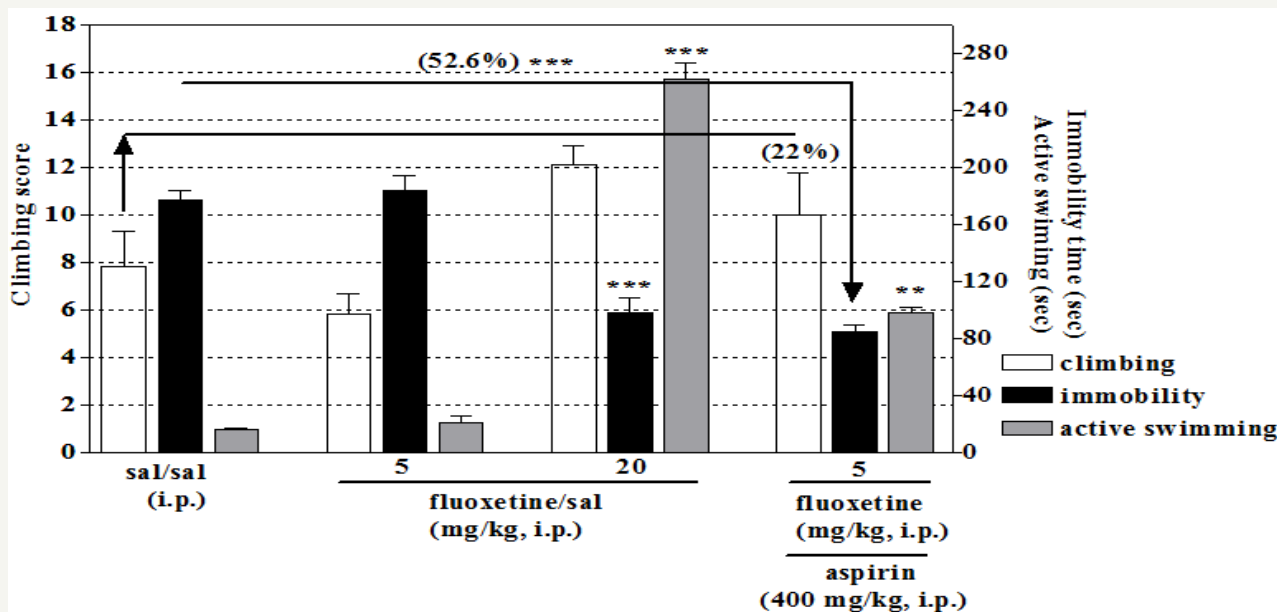


Figure 1: Effects of fluoxetine (20mg/kg) and fluoxetine (5mg/kg) plus aspirin (400mg/kg) on duration of immobility, active swim time and number of climbing in mouse forced swim test. Mice were forced to swim for six min test session. Values are expressed as mean \pm SEM/4 min. (n = 6). ** p <0.01 and *** p <0.001 compared with control group.

Out of 4min run-through period, control mice showed 16.0 \pm 1.2 sec (6.6%) an active swim time. Compared with control group, mice treated by suboptimal fluoxetine dose alone displayed a slightly increase (not significant) in active swimming time by 23.08%

(p =1.2, Figure 1). In the fluoxetine (20mg/kg) treated mice, the mean active swim time was 262.2 \pm 11.2 sec; fluoxetine significantly increases the active swim time by 93.9% (p < 0.001, Figure 1). Similarly, the mean swim time was highly increased by 83.7%

($p < 0.001$, Figure 1) in the combined aspirin with suboptimal fluoxetine dose treated mice. In this group, mice showed 98.2 ± 3.7 sec active swim vs. 16.0 ± 1.2 sec showed by the control group. The number of climbing was unchanged by fluoxetine at suboptimal dose compared with control group. The mean climbing frequency was 5.8 ± 0.9 vs. 7.8 ± 1.5 , respectively (Figure 1). In the fluoxetine (20mg/kg) treated group mice exhibited more struggling to escape than control group; the climbing frequency was increased by 35.5% (not significant, Figure 1). Combination of aspirin with fluoxetine slightly increased the climbing behavior by 22% (from 7.8 ± 1.5 for control group to 10.0 ± 1.8 , not significant, Figure 1). In addition, overall, tested mice diving under water were not observed.

Tail suspension test

The results of the tail suspension test are displayed in Figure 2. In

all the groups, mice showed normal behavior before tail suspension among all controls and treated animals (data not shown). After 30min, mice that had received normal saline displayed, after tail suspension, clear aberrant behavior expressed by complete motionless for 160.2 ± 11.1 sec as appraised by the time of mobility during the period of experiment (Figure 2). Injection of suboptimal fluoxetine dose did not change the development of events of immobility to tail suspension compared with control mice (Figure 2). Whereas, fluoxetine, at 20mg/kg, produced significant increase in the mobility time and greatly suppress the development of tail suspension induced episodes of motionless behavior by 35.2% ($p < 0.01$). Alike, and compared with control group, co-injection of aspirin with suboptimal fluoxetine dose induced a highly significant increase in mobility time by 91.9% ($p < 0.001$, Figure 2).

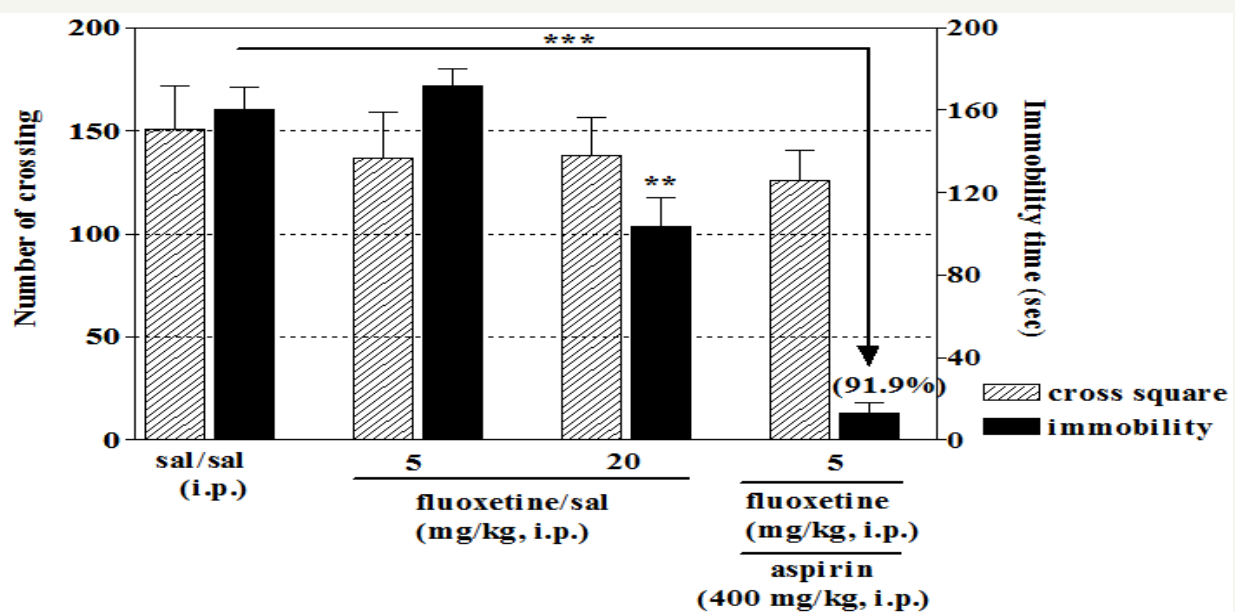


Figure 2: Effects of fluoxetine (20mg/kg) and fluoxetine (5mg/kg) plus aspirin (400mg/kg) on total locomotor activity in open field test. Mice were tail suspended for six min. Values are expressed as mean \pm SEM/4 min (n=6). ** $p < 0.01$ and *** $p < 0.001$ compared with control group.

Open field of locomotor activity

In order to exclude the possibility that the decrease in the immobility time produced by co-administration of aspirin is due to a psychostimulant effect, such as amphetamine [13], the general locomotor activity of mice was measured. Figure 2 show that there are no significant changes were observed in the ambulatory movement expressed by number of squares crossed by each mouse among all the treated groups. These findings give evidence that no psychostimulant effect was induced in these mice.

Discussion

The present study was carried out to evaluate the mood effect of aspirin in behavioral despair models of depression in mice. The effects obtained after combination of aspirin and suboptimal fluoxetine dose was determined. Our data show that aspirin do augment the antidepressant efficacy of fluoxetine in mice. In the two behavioral models, mouse forced-swim test and tail-

suspension test, it was clearly observed that combination of aspirin with fluoxetine produced an antidepressant-like effect to a greater extent than fluoxetine alone, as demonstrated by a mean decrease in duration of immobility time from baseline values. In addition, the effects of co-administration of aspirin on the swimming time in mouse forced-swim suggest that the serotonergic pathway rather than the noradrenergic pathway is involved in the obtained antidepressant-like effect.

In the present study it has been observed that no difference between the performances of treated groups in forced-swim test during pretest session, indicating that mice behavioral are comparable at baseline. But, during test session, mice exhibited significantly different behavioral in both models. Our data are in line with the hypothesis that depression is, in part, associated with inflammation [17]. On comparing the effect of the drugs treated groups during test session mice after co-injection of aspirin with fluoxetine shows a great increase in mobility time indicating

exhibition of antidepressant activity. Thus, it appears probably that administration of aspirin raised the un-detectable antidepressant activity of fluoxetine when given at suboptimal dose. Hence, aspirin, in combination with fluoxetine, is able to increase the antidepressant effects. Contrary with the latter, neither Lavakumar [18] nor Păunescu [19] observed a promising effect after co-administration of celecoxib, ibuprofen or parecoxib with fluoxetine in mice exposed to mouse forced-swim test. This indicates that since the decrease in the immobility duration time induced by NSAIDs cannot be attributable to psychostimulant effect NSAIDs, either selective or nonselective COX inhibitors, differently interact with the antidepressants efficacy of fluoxetine.

Although SSRIs are usually effective and reliable as first-line drug in treating MDD, these medications need several weeks to reach complete efficacy. During this holdup period patients are exposed to a raised risk for suicide and continue to be functionally ruined. The present study, on the other hand, does not address the query that the onset of antidepressant efficacy would be reduced. Indeed, no animal model that reliably the delay in efficacy in humans that typify all existing antidepressant medications. The pharmacological effects of aspirin in humans are immediate and the highest effect takes 2-4 hours to achieve. Consistent with this evidence, our data suggest that the ability of aspirin to pharmacodynamically potentiate the fluoxetine effects require short time. Furthermore, the toxicity of either drug can be attenuated, while augmenting their efficacy as antidepressants. Therefore, further studies are required to clarify if interactions between aspirin and antidepressants can permit the use of different dosages that far from those with the possibility to produce unwanted effects.

The anti-immobility effect induced by the injection of aspirin appears not to be related to motor effects since mice treated with aspirin alone do not show increased locomotion activity when examined in the open-field of general locomotor activity (data not shown). This may indicate that the psych-stimulant effect is not at play and may also suggest that the obtained antidepressant effect is specific.

Alteration in level of inflammatory cytokines in MDD has been reported. Recent immune-mediated concepts on the etiology of depression in preclinical studies using forced-swim test include increased pro-inflammatory cytokine interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) [20]. Furthermore, aspirin has significantly shown decreases forced-swim test induced increase IL-6 and TNF- α level [20]. In the meantime, down-regulation of COX-2 expression and reducing PGE2 levels have been shown to be involved in the antidepressive effects in rodents [21]. Thus, our results suggest that the obtained antidepressant activity most probably attributed to the anti-inflammatory effects of aspirin since fluoxetine, at a suboptimal dose, in combination with aspirin at a low dose (100mg/kg), a non-anti-inflammatory dose, did not change the immobility time of mice in forced-swim test (unpublished observation).

Conclusion

This study concludes that aspirin at the anti-inflammatory

dose is capable to enhance the antidepressant activity of fluoxetine without affecting psychostimulant deeds. Furthermore, it suggests that the serotonergic pathway is triggered by aspirin since the effect of fluoxetine was enhanced.

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