Available online on 15.01.2016 at http://jddtonline.info
Journal of Drug Delivery and Therapeutics
An International Peer Reviewed Journal
Open access to Pharmaceutical and Medical research

RESEARCH ARTICLE

EXPERIMENTAL STUDY TO EVALUATE THE ANTINOCICEPTIVE ACTIVITY OF FLUOXETINE AND ITS INTERACTION WITH NALOXONE AND ONDANSETRON IN MICE

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Received 18 Dec 2015; Review Completed 01 Jan 2016; Accepted 12 Jan 2016, Available online 15 Jan 2016

ABSTRACT

Objectives: Evaluate antinociceptive activity of fluoxetine and the interaction of fluoxetine with naloxone and ondansetron. **Methods:** 32 albino mice of either sex were divided into 4 groups of 8 mice each: group I received normal saline, group II received fluoxetine; group III received fluoxetine + naloxone; group IV received fluoxetine + ondansetron. Fluoxetine and naloxone were given subcutaneously whereas ondansetron was given intraperitoneally. Eddy's hot plate analgesiometer was used. Latency of licking of paw or jumping from the hot plate was recorded at intervals after giving study drugs in each group. **Results:** Fluoxetine produced a significant increase in the latency of licking of paw or jumping as compared to control at all time intervals except at 120 minutes with onset of antinociceptive effect within 30 min ($p<0.01$) and maximum effect at 60 min time interval ($p<0.001$). There was no significant difference between the control group and the fluoxetine + naloxone or fluoxetine + ondansetron group at any time point. Pre-treatment with naloxone and ondansetron antagonized the antinociceptive effects of fluoxetine. **Conclusion:** Administration of naloxone and ondansetron in fluoxetine treated mice antagonized the antinociceptive activity of fluoxetine. Therefore, antinociceptive activity of fluoxetine may be mediated through μ-opiod and 5HT3 receptors.

Keywords: anti-nociceptive, Eddy's hot plate, fluoxetine, naloxone, ondansetron.

INTRODUCTION

Pain is universally understood as a signal of disease, and is one of the most common symptoms that gets patients', and in turn physicians' attention. Patients of chronic pain are treated with various medications such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids, anticonvulsants, antidepressants like tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs), but none of them have shown outstanding efficacy for relieving chronic pain. The NSAIDs are non-specific analgesics; opioids although the most efficacious have dose-limiting side-effects like sedation, respiratory depression, pruritus, constipation and dependence liability. [1, 2]

Numerous open and controlled studies have shown that antidepressant drugs also have analgesic activity and particularly, SSRIs are effective in mixed and chronic pain. [3] On the other hand there are some studies that have altogether denied the analgesic role of SSRI. [4,5,6] Another challenging aspect is the meager understanding regarding their mechanism of action. The underlying mechanism for the antinociceptive action of these agents probably involves a complex interaction between several neurotransmitter systems and neuroreceptors. [7] There is ample evidence to suggest that pain inhibitory pathway involves monoamines such as noradrenalin (NA) and 5-hydroxy triptamine (5-HT) and SSRI by increasing the

serotonin level may inhibit the release of neurotransmitter carrying the pain sensation from nerve endings. [8,9] A direct activation of the endogenous opioid system or potentiation of a mixed analgesic effect mediated by serotonergic and/or noradrenergic pathways or combinations of these mechanisms are thought to be involved in the antinociceptive action mechanisms of SSRIs. [10]

Despite such an enormity of literature, it is not yet clear whether these can be used as analgesics and if so, what could be the underlying mechanism. Therefore, the present study was planned with the aim of confirming the antinociceptive activity of one of the antidepressants, fluoxetine and delineating the possible receptors involved which mediate its action.

MATERIALS AND METHODS

Ethical considerations

The study began only after obtaining permission from the Institutional Animal Ethics Committee.

Animals

Thirty two healthy albino mice of either sex (16 males and 16 females) weighing between 25-30 grams, with normal behavior and activity were obtained from Haffkine Institute, Parel, Mumbai. Pregnant animals and those that had delivered once or used previously for any other experimental purpose were excluded from the study. The animals were housed in mice cages in the central animal house of the institution with free access to food and drinking water as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, for a period of 1 month prior to the study, so that they were acclimatized to the environment. A 12-hour light and dark cycle was maintained.

Study drugs

Fluoxetine was obtained from Cadila Pharmaceuticals Limited. Naloxone was obtained from Samarth Pharma Limited. Ondansetron was obtained from Intas Pharmaceuticals Limited. Stock solutions of all the drugs were freshly prepared on the day of the experiment in normal saline (NS; 0.9% NaCl). In previous studies conducted, the effective dose of fluoxetine that did not induce sedation was found to be 5 mg/kg. [11] This dose of fluoxetine was therefore used in our study. Dose of naloxone and ondansetron were calculated based on previous studies.

Experimental study groups and drug administration

The 32 mice were divided into 4 groups of 8 mice (4 male and 4 female mice) each. Group I received normal saline alone, group II received fluoxetine alone; group III received fluoxetine + naloxone; group IV received fluoxetine + ondansetron. Fluoxetine and naloxone were given subcutaneously whereas ondansetron was given intraperitoneally. The treatment groups are summarized in the Table**. (see Table 1)**

Table 1: Treatment groups

Groups	Drug 1	Drug 2
Group 1 (control)	Normal saline	-
Group II	Fluoxetine 5 mg/kg	-
Group III	Fluoxetine 5 mg/kg	Naloxone 2 mg/kg
Group IV	Fluoxetine 5 mg/kg	Ondansetron 0.1 mg/kg

Drugs were administered using 26 G tuberculin syringe using aseptic precautions. Normal saline, fluoxetine, and naloxone were administered subcutaneously (s.c.) in the nape of the neck. Naloxone was administered 10 minutes prior to the administration of fluoxetine in group III. Ondansetron was administered intraperitoneally (i.p.).

Evaluation of anti-nociceptive effect using Eddy's hot plate

The hot plate test was based on that described by Singh et al. and Eddy and Leimbach. [12,13]

A transparent glass cylinder (16-cm-high, 16-cm diameter) was used to keep the mice on the heated surface of the plate. The temperature of the hot plate was set to 55 + 0.5°C by using a thermoregulated pump. The animals were placed on the hot plate and the time until either licking or jumping occurred was recorded by stopwatch. The time of latency was defined as the time period between the zero point when the animal was placed on the hot plate surface and the time when the animal licked its forepaw or jumped off to avoid thermal pain. The animals which showed licking of paw or jumping from the hot plate after 10 seconds were removed from the study.

Post treatment latencies were determined after 30 minutes of administration of study drugs. To minimize tissue damage, a cut-off time (removing from the plate) of 30 seconds was adopted. The latencies of both forepaws' licking and jumping were measured for each animal at 0, 15, 30, 60, and 120 minutes. The accepted "zero time" of this study was 30 min after the administration of study drugs.

Statistical analysis

The data were expressed as the mean + S.D. Unpaired student's "t" test was used to compare groups with each other. Probability (P) value of < 0.05 was taken as level of statistical significance.

RESULTS

The results of the study are described in this section. (See **Table 2 and Figure 1)**

 ISSN: 2250-1177 CODEN (USA): JDDTAO

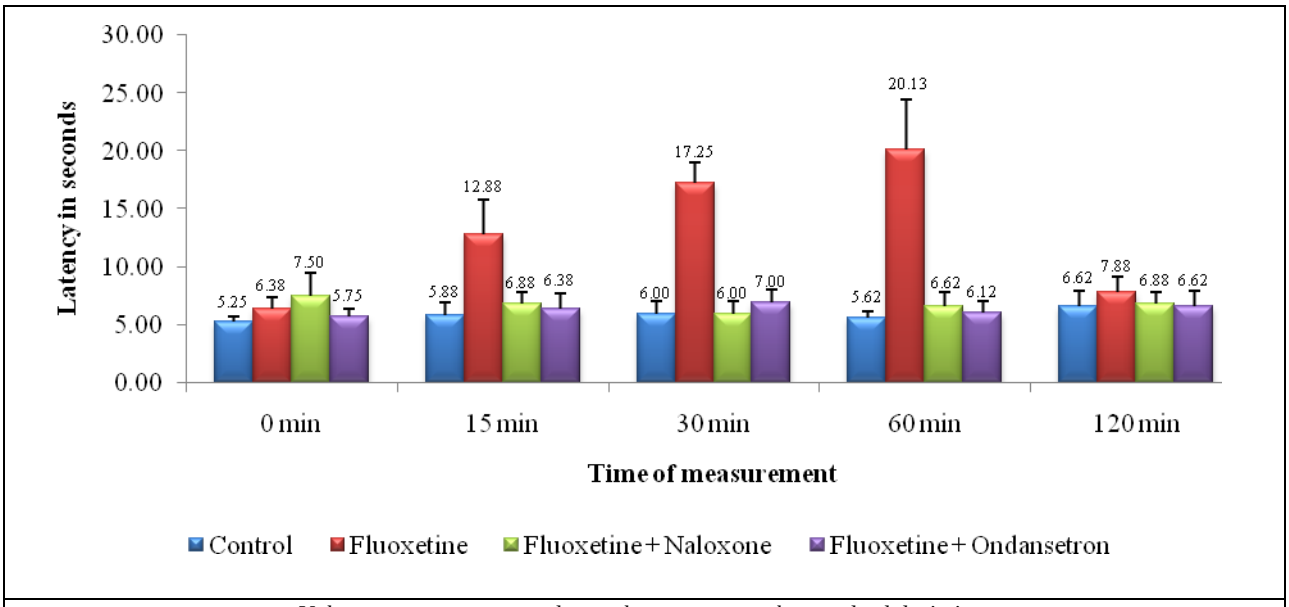
Table 2: Comparison between different groups for latency to animal licking its forepaw or jumping off to avoid thermal pain on Eddy's hot plate

Post drug time interval	0 min	15 min	30 min	60 min	120 min
Control	5.25 ± 0.46	5.88 ± 1.13	6.00 ± 1.07	5.63 ± 0.52	6.63 ± 1.30
Fluoxetine	6.38 ± 1.06*	12.88 ± 2.90$^{**, \alpha}$	17.25 ± 1.75$^{**, \alpha, \beta}$	20.13 ± 4.32$^{**, \alpha, \beta\beta}$	7.88 ± 1.36$^{\beta, \delta, \gamma}$
Fluoxetine + Naloxone	7.50 ± 2.00	6.88 ± 0.99$^{\$}$	6.00 ± 1.07$^{\$}$	6.63 ± 1.19$^{\$}$	6.88 ± 0.99
Fluoxetine + Ondansetron	5.75 ± 0.71$^{\#}$	6.38 ± 1.41$^{\$}$	7.00 ± 1.07$^{\$}$	6.13 ± 0.99$^{\$}$	6.63 ± 1.30

n=8 mice in all groups

****p<0.01, **p<0.001 versus control; $ p<0.001 versus fluoxetine; #p<0.05 versus fluoxetine + naloxone, using one-way ANOVA followed by post-hoc Tukey's test.***

α p<0.001 versus 0 minutes; β p<0.01, ββ p<0.001 versus 15 minutes; δ p<0.001versus 30 minutes, γ p<0.001 versus 60 minutes, using repeated measures ANOVA followed by post-hoc Tukey's test.



Values represent mean and error bars represent the standard deviation.
The accepted "0 min" of this study means 30 min after the administration of study drug.

Figure 1. Comparison between different groups for latency to animal licking its forepaw or jumping off to avoid thermal pain on Eddy's hot plate

Fluoxetine produced a significant increase in the latency of licking of paw or jumping as compared to control at all time intervals except at 120 minutes with onset of antinociceptive effect within 30 min i.e. 0 min time interval ($p<0.01$) and maximum effect at 60 min time interval i.e. after 90 min of administration of fluoxetine ($p<0.001$).

There was no significant difference between the control group and the fluoxetine + naloxone or fluoxetine + ondansetron combination group at any time point. Thus, it appears that pre-treatment with naloxone and ondansetron antagonized the antinociceptive effects of fluoxetine as the values are comparable to the control group.

Pretreatment with naloxone and ondansetron in fluoxetine treated mice group significantly reduced the analgesic effect of fluoxetine at all time intervals ($p<0.001$ for fluoxetine + naloxone and fluoxetine + ondasetron versus fluoxetine alone at 15 min, 30 min, and 60 min). Thus it reflects that the antinociceptive effect of fluoxetine is antagonized by naloxone as well as ondansetron.

There was no significant difference found when the combination group of fluoxetine + naloxone was compared with the combination group of fluoxetine + ondansetron at any time point.

DISCUSSION

In daily life the pain sensation is specifically evoked by potential or actual noxious (i.e. tissue damaging) stimuli applied to the body such as heat, squeezing a skin fold or over-rotating a joint. The predictable correlation between the noxious stimulus and the pain sensation forces us towards avoiding behavior and situations that evoke pain. Pain during disease is different from "normal" pain. It occurs in the absence of external noxious stimuli, during mild stimulation or in an unpredictable way. [14] Pain is a symptom of many diseases requiring treatment with analgesics. Severe pain due to chronic conditions needs the use of strong analgesics such as opioid drugs. The addiction liability

of opioids has led to intensive search for compounds without this hazard. [15] Tricyclic antidepressants, even though their mechanism of action is not clear, have been used for decades for the treatment of chronic pain in patients without depression. Because of numerous undesirable side effects of traditional tricyclics, SSRIs with favorable side effects profile are preferred. [10] Antidepressants are reported to be more effective than opioid analgesics in treating neuropathic or differentiation pain. Inhibition of the reuptake of monoamines is considered to be a major effect of antidepressants. [11] The analgesic effect of SSRIs has been shown both in animal models [16,17,18] and human cases suffering from chronic [19,20] but not in acute pain. However there appears to be some disparities in the results of these studies. Some studies indicated the presence of analgesic effects to SSRIs whereas others negated this observation. Hence the present study was undertaken to evaluate the antinociceptive activity of fluoxetine, a long acting SSRI, and to find out its site of action.

Central analgesic activity of fluoxetine was tested through an acute pain model of hot plate analgesiometer. In previous studies, the analgesic effects of fluoxetine was evaluated at a dose of 5 mg/kg [11,20] and the same dose was used for the current study. The doses of naloxone and ondansetron were also selected based on the previous studies. [10,11, 15]

The antinociceptive effects of fluoxetine and its interaction with naloxone and ondansetron were observed 30 minutes after the administration of fluoxetine i.e. at 0,15,30,60 and 120 min intervals, based on the previously published reports. [10,11, 20]

The number of mice to be used for this experiment was decided after reviewing the CPCSEA guidelines and previous study references to have minimum number of animals for particular animal experiment to get statistically significant data. [21]

It was observed that fluoxetine possesses significant antinociceptive effect with onset of action within 30 minutes and maximal effect at 90 minutes post-administration of the drug. These findings are in conjunction with the findings of Kurlekar et al. and Sikka et al. which showed dose dependent antinociceptive activity of fluoxetine.[11,20] The combination groups of fluoxetine + naloxone and fluoxetine + ondansetron were comparable to the control group. This indicated that the antinociceptive effect of fluoxetine was blocked by naloxone and ondansetron. This suggests the contribution of opiodergic and serotonergic mechanisms in mediating antinociceptive action of fluoxetine. Even though there are no earlier studies on fluoxetine and ondansetron combination, a study conducted by Duman et al. on paroxetine, another SSRI found that ondansetron significantly inhibited the antinociceptive effect of paroxetine which is similar to the findings of the current study. [10] Opioid or Opioid-like activity of fluoxetine and some SSRIs has been reported in few binding assays. Other studies have disputed the affinity of fluoxetine for opioid receptors. [11] However in the present study the involvement of opioid receptor in the analgesic activity of fluoxetine was observed.

Rafiran-Kopaei and Swell and Singh et al. also reported that Fluoxetine-induced

antinociception was blocked by naloxone. [22,17] Gray AM also showed that antidepressants produce antinociception that can be blocked by opioid antagonist thus suggesting direct agonist-like actions of the antidepressants at opioid receptors. [23] Thus it is likely that fluoxetine acts through opioid pathways involving the μ opioid receptor.

Also in the present study ondansetron, a 5HT3 receptor antagonist significantly inhibited the antinociceptive effect of fluoxetine. This suggests the contribution of 5-HT3 receptors in the antinociceptive action of fluoxetine. Certain published reports have also stated that both α-adrenoceptor and 5-HT receptor antagonists inhibit the antinociception mediated by antidepressants in various studies. Similarly, depletion of central NA systems with α-methyl-p-tyrosine and 5-HT systems with p-chlorophenylalanine inhibits antinociception by antidepressants. Within the spinal cord, activation of both NA and 5-HT receptors produces analgesia and NA and 5-HT mechanisms interact significantly. [24]

In another study by Abdalla S et al. it has been observed that fluoxetine blocks selectively the uptake of 5-HT, while mirtazapine is believed to increase both noradrenergic and serotonergic neurotransmission via blockade of central alpha-2 adrenergic auto and heteroreceptors increasing the availability of both amines at the synapse. Noradrenergic and serotonergic descending inhibitory and excitatory pathways are believed to play an important role in endogenous analgesic systems. Both noradrenaline and serotonin produce membrane hyperpolarization while decreasing the excitatory transmitter release from primary Aδ and C afferent fibers presynaptically and increasing the inhibitory transmitter release, including both GABA and Glycine, from the interneurons. [2] This further emphasizes the role of 5HT3 in analgesic activity of fluoxetine. Studies by Duman EN et al. and Patil R et al. also support these findings. [10, 15]

In the present study when we compared the combination of fluoxetine + naloxone with the combination of fluoxetine + ondansetron, difference was not significant. Thus it shows that analgesic activity of fluoxetine was blocked by both naloxone and ondansetron, hence indicating the involvement of opiodergic and serotonergic mechanisms. According to explanation provided in the previously published studies it was thought that 5HT descending system plays an important role in morphine analgesia which mediates through μ opioid receptor. The analgesic action of systemic opioids can be blocked by depletion of 5HT by inhibiting its synthesis. In the present study we have also found that analgesic activity of fluoxetine is mediated through μ opioid receptor and 5HT3 receptors. [17]

Thus there can be an interplay between both the receptors i.e. μ opioid receptor and 5HT3 receptors in mediating the antinociceptive activity of fluoxetine so

even blockade one receptor can significantly inhibit the antinociceptive activity of fluoxetine.

So from the present study it is confirmed that fluoxetine has antinociception potential that is antagonized by naloxone and ondansetron. This combined finding suggests an involvement of opiodergic system by increasing the release of endogenous opioid peptides or opioid receptors activation and serotonergic mechanisms through serotonergic receptors activation induced by fluoxetine.

CONFLICT OF INTEREST: None

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 ISSN: 2250-1177 CODEN (USA): JDDTAO