Original Article

Efficacy Of Duloxetine As Analgesic And Anti-Inflammatory Agent In Different Animal Models – An Experimental Study

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DOI: 10.47750/pnr.2022.13.S09.736

Abstract

Background: duloxetine an antidepressant class of drug is a potent and selective inhibitor of 5-HT and NE reuptake in the central nervous system. Several preclinical studies have shown that duloxetine significantly reduces pain behavior across a range of persistent, neuropathic, and inflammatory pain models.

Aims and Objectives: the current study was carried out to evaluate the effect of duloxetine on experimentally induced pain and inflammation in animal models and compare its effect with NSAID Ibuprofen a proven analgesic and anti-inflammatory drug

Materials and Methods: Wistar rats of either sex were selected and divided into four groups control group and ibuprofen, duloxetine (5 mg and 10 mg/kg) each group consist of six animals .analgesic activity was determined by Tail –flick method as described by Haffner. Anti-inflammatory activity was determined by suppression of join oedema in turpentine induced arthritis model. Statistical analysis of the obtained data was done by analysis of variance test and Tukey's honest significance difference post-hoc test

Results: we noticed significant analgesic activity in duloxetine 10 mg /kg group more than ibuprofen treated group and duloxetine 5mg /kg treated group. In turpentine induced arthritis model duloxetine 10mg/kg treated group has shown superior anti-inflammatory activity compared to control and ibuprofen treated groups

Conclusion: present study indicated toward significant analgesic and anti- inflammatory activity of duloxetine. The effect of duloxetine on analgesia and inflammation was found to be dose dependant 10 mg /kg ≥5mg /kg.in summary duloxetine could be an ideal alternative to currently prescribed drugs for analgesia and inflammation.

Keywords— "inflammation", "tail-clip", "analgesia", "turpentine oil", "central modulation".

I.INTRODUCTION

Duloxetine an antidepressant class of drug is a potent and selective inhibitor of 5-HT and NE reuptake *in vitro* and *in vivo* in the central nervous system. [1]

Several preclinical studies have shown that duloxetine significantly reduces pain behavior across a range of persistent, neuropathic, and inflammatory pain models.[2,3,4] Trials involving duloxetine where duloxetine is compared with placebo (placebo- controlled trials) provided evidence of duloxetine's efficacy in reducing osteoarthritis Pain and chronic low back pain.[5.6] A study conducted by Vladimir Skljarevski et al reported that duloxetine is efficacious in treating four distinctively different chronic pain conditions, as demonstrated by clinically significant improvement in pain severity, physical functioning, and patients' ratings of overall improvement suggesting that duloxetine is an effective centrally-acting general analgesic.[7]

Ibuprofen is widely used as analgesic in different medical conditions however injudicious use of ibuprofen is associated with a significantly increased risk of hospital-acquired AKI (acute kidney injury) in hospitalized children, particularly children with CKD chronic kidney disease or in ICU need of intensive care. [8]

Elderly patients and patients with coronary artery disease are at risk for ibuprofen-associated renal impairment requiring renal function monitoring when ibuprofen and other non-steroidal anti-inflammatory drugs are prescribed in those patients [9]

Long- term use of NSAIDS like Ibuprofen is associated with analgesic nephropathy which leads to chronic renal failure.[10]

The efficacy of duloxetine in various clinical studies has shown duloxetine in addition to its antidepressant activity also shows analgesic and anti-inflammatory activity.

Thus considering all the aspects mentioned earlier the current study was carried out to evaluate the effect of duloxetine on experimentally induced pain and inflammation in animal models and compare its effect with NSAID Ibuprofen a proven analgesic and anti-inflammatory drug.

MATERIAL AND METHODS

Albino Wistar rats of either sex (100grams –250 grams) were used for analgesic and inflammation study. The animals were maintained in the animal house for during entire duration of study. Strict acclimatization conditions were maintained for 7 days under standard husbandry conditions. Free access to standard animal diet as prescribed with water supplied *ad libitum* under standard hygienic conditions. The study was carried out in research lab in the department of pharmacology, government medical college, Latur-Maharashtra. Prior approval of the Institutional Animal Ethical Committee was taken before proceeding to the experiments.

The rats were divided into separate groups and kept in polypropylene cages. The cages were maintained in a room at ambient temperature of 23±1°C with the help of air conditioners with enough humidity on a 12 h light-dark cycle. Similar conditions were maintained in research laboratory while performing experiments. The study was conducted during daytime (between 09.00 Morning and 06.00 evening).
Following groups were made each having six animals.

- Group (1): Control group (normal saline 10 ml/kg)
- Group (2): Standard drug group Ibuprofen (100 mg/kg)
- Group (3): Duloxetine (5 mg/kg)
- Group (4): Duloxetine (10 mg/kg).

All the drugs were given through intraperitoneal route.

Analgesic Method

Tail clip method

The analgesic activity of the test drug was determined by the tail-clip method as described by Haffner (1929) [11].
Haffner's method is simple and has the advantage that the reflex mechanism on which it is based involves the higher centers.The animal has to identify exactly the place where the noxious stimulus is applied, and it carries out coordinated movements to remove it.

30 minutes after administering group- specific drugs, an artery clip is applied at the root of the tail (approximately 1 cm from the body). The animal quickly responds to the noxious stimulus by biting the clip or tail near the location of a clip. The time interval between the application of clip and the response is considered as reaction time (latency period). The reaction time was recorded by a stopwatch.
The readings (latency period) were noted at basal and after 30 minutes after the drug dosing, according to their respective groups.

Anti-inflammatory method

Turpentine induced arthritis

In this method prior baseline recording of the joint diameter was made by using a micrometer screw gauge. Acute non-immunological inflammatory joint oedema was produced by injecting 0.1 ml of turpentine oil into the synovial cavity of the right knee joint using tuberculin syringe[12]

30 min after the drug administration, the lateral knee joint was measured at 1hrs, 2hrs, 3hrs, 4hrs using a micrometer screw gauge. Changes in lateral diameter were noted for each rat.

Calculation:

Percentage anti-inflammatory effect of particular drug was calculated as follows:

$$\text{Percentage anti-inflammatory effect} = \frac{\text{Mean difference in lat. diameter in control} - \text{Mean difference in lat.diameter in test}}{\text{Mean difference in lat.diameter in control}} \times 100$$

Statistical Analysis

All data collected during the study presented as Mean ± SE. Data were evaluated by means of one-way analysis of variance ANOVA. Comparisons between the groups were made using Tukey HSD *post hoc* test, to establish the statistical difference if any between groups. The criterion for statistical significance was fixed at $P<0.05$.and <0.01

RESULTS

Table 1 Tail-Clip Method

Drug group	Reaction time Before treatment(in seconds) Mean ±SE	Reaction time After treatment(in seconds)	% Inhibition
Control	1.45 ±0.25	1.53±0.26	05.22
Ibuprofen	3.39 ±1.32	7.17±1.30[b]	52.71
Duloxetine(5mg/kg)	1.72±0.14	4.01±0.75	57.10
Duloxetine(10mg/kg)	2.50±0.56	7.11±0.59[b,c]	64.83
a$P\leq0.05$ versus control, b$P\leq0.01$ versus control, c$P\leq0.05$ versus ibuprofen d$P<0.01$ versus ibuprofen			

Tail clip analgesic method

The table 1 shows reaction time in seconds and percentage of inhibition in tail clip analgesic method, after comparing the difference between the means in different groups it shows that

- At 30 min in the tail-clip analgesic method the Ibuprofen treated group is showing significant analgesic activity.
- Duloxetine5mg/kg treated group is not displaying significant increase in reaction analgesic as compared with the control group
- the duloxetine at 10 mg/kg is showing significant analgesic effect
- On comparing the difference between means the Duloxetine 10mg/kg group is showing the highest difference between the means as compared to control means the analgesic activity of Duloxetine is more than the Ibuprofen group, this may be due to fact that the Duloxetine is having centrally mediating action in pain pathway.

Table 2 Turpentine Induced Arthritis

Drug group	Basal(mm)Mean±SE	Difference with basal in (mm) Mean ±SE			
		1 hr	2hr	3 hr	4 hr
Control	7.52±0.11	2.41±0.21	3.91±0.41	6.79±0.01	5.76±0.02
Ibuprofen	6.83±0.31	0.507±0.15[b]	0.80±0.22[b]	1.03±0.33[b]	1.14±0.01[b]
Duloxetine 5mg/g	7.65±0.20	0.675±0.05[a]	0.89±0.09[b]	1.76±0.01[b]	1.71±0.26[b]
Duloxetine 10 mg/kg	7.55±0.27	0.44±0.06[b]	0.87±0.15[b]	0.84±0.21[b]	1.08±0.35[b]
a$P\leq0.05$ versus control, b$P\leq0.01$ versus control, c$P\leq0.05$ versus ibuprofen d$P<0.01$ versus ibuprofen					

Turpentine induced arthritis

Table 2 shows comparison of basal right knee diameter as measured by micrometer screw gauge and the difference with length of basal knee joint diameter after administering drugs at 1 hr, 2h, 3h and 4 hrs

- The table shows that all the drug treated groups are showing significant decrease in increment of joint length diameter after giving turpentine at right knee joint of animal
- The comparison between Ibuprofen treated and Duloxetine 5mg/kg treated group indicate that ibuprofen is having higher inhibitory activity as compared to the Duloxetine 5mg/kg treated group.
- The comparison between standard drug Ibuprofen verses duloxetine 10 mg/kg group gives indication that the duloxetine at 10 mg/kg is having higher inhibitory activity compared to ibuprofen.

DISCUSSION

The current study has assessed analgesic activity of duloxetine and ibuprofen in haffner tail clip analgesia model. The haffer's tail clip analgesic model is based on higher central action since animal has to locate exact site of noxious stimulus [11].our study finding shows that Duloxetine at 5 mg /kg is showing non inferior/ less analgesic activity as compared with Ibuprofen however on comparison with 10 mg / kg Duloxetine treated groups its evident that superior analgesic effect is noted. This might be because of duloxetine's modulatory action on pain pathways. Significant anti inflammatory effect compared with control group is shown by duloxetine tested groups. Although ibuprofen was found to have significant anti-inflammatory activity compared to duloxetine in 5 mg /kg treated groups. Duloxetine at 10 mg / kg is showing higher antinnflammatory action in turpentine induced arthritis model.

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter and that contributes to the regulation of various physiological functions by its actions in the central nervous system (CNS) and in the various organ systems. Peripheral 5-HT is also a potent immune modulator and affects various immune cells through its receptors and via the process of serotonylation. Alterations in 5-HT signaling have been described in inflammatory conditions of the gut, such as inflammatory bowel disease. Changes in 5-HT levels have also been reported in patients with allergic airway inflammation and rheumatoid arthritis. [13]

Norepinephrine modulates the inflammatory and proliferative phases of wound healing after inflammation in a temporally defined, cell-specific manner, by increasing recruitment of innate immune cells. [14]

Evidence suggests that noradrenalin has an anti-inflammatory action in the central nervous system (CNS) via its ability to suppress microglia and astrocytic activation, and inhibit production of inflammatory mediators. [15]

Joan B. O'Sullivan, Karen M. Ryan et al demonstrated that acute treatment of rats with the noradrenalin reuptake inhibitors (NRIs) desipramine and atomoxetine elicited anti-inflammatory actions in rat cortex following a systemic challenge with bacterial lipopolysaccharide (LPS). Characterized by a reduction in cortical gene expression of the pro-inflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), the enzyme inducible nitric oxide synthase (iNOS), and the microglial activation markers CD11b and CD40,also in the study they found there was reduced activation of nuclear factor-kappa B (NF-κB); transcriptions factor a major regulator of inflammation in the CNS.[15]

During the course of pain transmission primary afferent nociceptors are responsible for conveying the noxious information received to the projection neurons in the DH dorsal horn of the spinal cord, subset of these projection neurons in turn transmit these sensory information up to the thalamus reaching the somatosensory cortex through the spinothalamic tract providing information on the intensity and the location of the noxious stimulus. During the course of pain transmission several modulation studies have indicated role of neurotransmission in pain modulation.[16]

Monoamines NA and 5-HT are the neurotransmitters involved in the descending control pain modulatory pathway. NA plays a prominent role in inhibition of spinal cord activity that originates in supraspinal areas. Interaction with the noradrenergic system via release of NA and activation of α1, α2C and β-adrenoceptors is essential mechanism underlying the peripheral anti-nociception induced by the non-steroidal anti-inflammatory drugs.[17]

Duloxetine a balanced SNRI decrease the uptake of these neurotransmitters indirectly and increase the level of serotonin and norepinephrine via central action. Duloxetine thus can play a vital role in the pain modulation.

Various studies have indicated towards antinociceptive as well as inhibitory action of duloxetine on inflammation .

In the study conducted by Yuan, M., Tang, T., Ding, Z. *et al.*[18] involving patients undergoing total knee arthroplasty found duloxetine reduce acute postoperative pain in the immediate postoperative period and decrease the opioid consumption as well as accelerated postoperative recovery, without increasing the risk of adverse medications .

Duloxetine showed an analgesic effect on the spontaneous pain behaviors and hyperalgesia induced by the formalin injection [19], a model of chronic inflammatory pain.

YuyaKawarai,SumihisaOrita et al[20] investigated the efficacy of duloxetine on hyperalgesia, histopathological and radiographic findings, pain-related sensory innervations of dorsal-root ganglia (DRG), and spinal changes in a rat model of induced hip osteoarthritis (OA).

MIA administration into the hip joint led to mechanical hyperalgesia of the ipsilateral hind paw. A single injection of duloxetine significantly attenuated it in induced hip OA and suppressed the number of Iba(ionized-calcium-binding adaptor molecules)in microglia of the ipsilateral dorsal horn . Results suggest that a single injection of duloxetine suppressed mechanical hyperalgesia and influenced the expression of Iba1 ionized-calcium-binding adaptor molecule 1 (Iba1) in the microglia of the ipsilateral dorsal horn in the MIA-induced hip OA.

A study indicate that the use of lower doses (20-40 mg) of oral duloxetine had significant beneficial effect of improving pain symptoms which improves function and quality of life in patients with knee osteoarthritis .[21]

A study by Choi *et al.* [22] has shown neuroprotective effect of duloxetine due to its anti-inflammatory action. Researchers examine the effect of duloxetine on seizure behavior excitotoxic neuronal damage in mouse hippocampal region following kainic acid given via intraperitoneal route.

Duloxetine at 10 mg/kg reduced neuronal death in hippocampus region after giving kainic acid. Duloxetine also suppressed kaininic acid induced activation of microglia and astrocyte and increase of inflammatory factors TNF –alpha and Interleukin -beta.

In the present study we evaluated analgesic and anti-inflammatory activity of Duloxetine a balanced serotonin and norepinephrine reuptake inhibitor in different animal models. Duloxetine increase level of this neurotransmitters serotonin and epinephrine in central pathways which induce modulatory effect in pain pathway transmission. Duloxetine alter neuroimmune interaction Duloxetine administration leads to suppression / inhibition of inflammatory cytokines and mediators like TNF and interleukin beta this could be considered as possible mechanism related to anti-inflammatory effect of duloxetine.

Further studies are needed to confirm these finding in clinical settings

CONCLUSIONS

In the present study duloxetine provided evidence of analgesic and anti-inflammatory effect. In the clinical viewpoint duloxetine could be used as alternative to currently prescribed mainline drugs like opioids and NSAID for inflammation and painful conditions, especially in the chronic conditions requiring long term medications. There is reciprocal relation between pain and depression; often it is found that chronic depression is associated with pain as co-symptom. In various psychosomatic disorders like fibromyalgia patients often complain of chronic pain and there is associated depression. An antidepressive drug like duloxetine providing analgesic and anti-inflammatory effects with having additional safety profile could be boon in such patients.

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