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RESEARCH ARTICLE

Evaluation of analgesic activity of pioglitazone in albino mice

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ABSTRACT

Background: Analgesics selectively relieve pain by acting either on central or peripheral pain pathways. Recently, studies have shown accumulating evidence to implicate N-methyl-d-aspartate receptors (NMDARs) mediation in central and peripheral sensitization and visceral pain leading to the possibility that NMDAR antagonists may be useful in the treatment of pain. **Aims and Objectives:** (1) To evaluate analgesic activity of Pioglitazone (PIO) in mice. (2) To compare the analgesic activity of PIO with the standard drugs tramadol and aspirin, in mice. **Materials and Methods:** Albino mice were divided into four groups, containing six animals ($n = 6$) in each group (control, standard, and test group). Group-I: Control received saline solution 2 ml/kg orally, Group-II: Standard 1 received tramadol at a dose of 10 mg/ kg intraperitoneal, Group-III: Standard 2 received aspirin at a dose of 300 mg/kg orally, and Group-IV: Test received PIO at a dose of 20 mg/kg orally. PIO and normal saline were administered 30 min before, whereas the tramadol and aspirin were administered 15 min before writhing and tail clip methods. The decrease in number of writhes and the delay in reaction time in tail clip method denoted the analgesic activity. **Results:** PIO decreased the number of writhes and delayed the reaction time in tail clip method considerably when compared with control, but less when compared with standard drugs. **Conclusion:** PIO exhibits analgesic activity in both chemical and mechanical pain models in albino mice.

KEY WORDS: Analgesic Activity; Pioglitazone; N-methyl-d-aspartate Receptor; Tail Flick Method; Acetic Acid-Induced Writhing Method

INTRODUCTION

Sensory systems have the role of informing the brain about the state of the external environment and the internal milieu of the organism. Pain is a complex perception, an unpleasant phenomenon composed of sensory experiences that include time, space, intensity, emotion, cognition, and motivation originating from damaged tissue or abnormal physiological condition. International association for the study of pain has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."[1] The term nociception includes all neuronal processes for the recognition of a potentially or actually damaging stimulus. In a neuropharmacology experiment, an afferent neuron is normally labeled nociceptive if it shows a strong response only to stimuli that produce pain in human subject and equivalent reaction in animals.[2]

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Glutamate, the major excitatory neurotransmitter in the brain and spinal cord, exerts its postsynaptic effects through a diverse set of membrane receptors, ionotropic, and metabotropic. N-methyl-d-aspartate receptor (NMDAR) is an ionotropic receptor that directly gate ion channels. There is considerable evidence that pain associated with peripheral tissue or nerve injury involves NMDAR activation.[3] Consistent with this, NMDAR antagonists

have been shown to effectively alleviate pain-related behavior in animal models as well as in clinical situations.[4,5]

Pioglitazone (PIO) an oral antidiabetic agent belonging to the group of thiazolidinediones (TZDs), acts as an insulin sensitizer and is used for the treatment of diabetes mellitus Type 2 in monotherapy and in combination with a sulfonylurea, metformin, or insulin. PIO has also been used to treat non-alcoholic steatohepatitis (fatty liver), but this use is presently considered experimental. It has also been found to reduce the risk of conversion from prediabetes to diabetes mellitus Type 2 by 72%. Beyond its peripheral actions, recent studies indicate that peroxisome proliferator-activated receptors (PPAR) γ agonists regulate central nervous system (CNS) inflammation and that PPARγ is a powerful pharmacological target for counteracting neurodegeneration, ischemic stroke, and spinal cord injury. PIO has also been recently reported to reduce inflammatory pain and neuropathic pain.[6] They have been reported to regulate inflammatory response. In the light of above-described observations, the present study is undertaken to assess the analgesic potential of PIO.

MATERIALS AND METHODS

The study was conducted after getting approval from Institutional Animal Ethical Committee.

Animals

Albino mice (25–35 g) of either sex were randomly selected from central animal facility, JSS Medical College, Mysuru. Animals were housed into groups of 6 per cage at a controlled temperature (23 ± 2°C). Light:dark cycle of 12:12 was followed. The mice had free access to standard pelleted feed and water *ad libitum*.

Drugs and Chemicals

PIO (20 mg/kg) and aspirin (300 mg/kg) dissolved in distilled water immediately before used orally, glacial acetic acid diluted in distilled water to provide 0.06% solution for intraperitoneal injection, tramadol (10 mg/kg), and normal saline.

The mice were divided into four groups containing six animals ($n = 6$) in each group (control, standard 1 and 2, and test group).The test drug PIO 20 mg/kg and normal saline 25 ml/kg were administered orally 30 min prior. Standard drug tramadol 10 mg/kg was administered intraperitoneally 15 min prior, and aspirin 300 mg/kg was administered orally 15 min before the experiment. Significant analgesia with tramadol occurs between 30 and 60 min and with aspirin occurs between 25 and 40 min. The drugs were dissolved in distilled water (vehicle).

- Group 1: Control - normal saline - 25 ml/kg (oral).
- Group 2: Standard 1 - tramadol - 10 mg/kg (intraperitoneal).
- Group 3: Standard 2 - aspirin - 300 mg/kg (oral).
- Group 3: Test - PIO - 20 mg/kg (oral).

Screening of Analgesic Activity

Tail clip method

Mice weighing 25–35 g were used. Haffner's clip was placed at the root of the tail of the mice to apply noxious stimulus. A quick response of the animal was seen as biting the clip or tail, where the clip was placed. The reaction time between application of the clip and the response was noted by a stopwatch. Test drug PIO was administered orally. After 15, 30, and 60 min, same procedure was repeated and reaction time was measured. This model evaluates the central pain.[7]

Acetic acid-induced writhing method

The writhing model represents a chemical nociceptive test based on the induction of peritonitis like condition in animals by injecting irritant substances intraperitoneal. Mice weighing 25–35 g were used. Acetic acid 0.06% was injected intraperitoneal in each animal. The animals reacted with a characteristic stretching behavior that is, a series of constrictions occur that travel along the abdominal wall, sometimes accompanied by turning movements of the body and extension of the hind limbs. This response of writhing was recorded. Test group animals were administered PIO 30 min before administration of acetic acid intraperitoneal. Later, mice were placed individually into glass chambers and a number of writhes were recorded for 15 min. This model evaluates peripheral pain.[8]

$$\%\text{ of inhibition} = \frac{\text{Average number of writhes in control group} - \text{writhes in test group}}{\text{Writhes in the control group}}$$

The time period with the greatest percentage of inhibition was considered the peak time.

Statistical Analysis

The results were analyzed by calculating the mean values, standard deviation, and analysis of variance, *post-hoc* test (Bonferroni). IBM SPSS statistics © IBM Corporation and Other (s) 1989, 2012 software was used for statistical analysis purpose. To test the results of study for the corresponding degrees of freedom, the values were compared at 0.05 level of significance. $P < 0.05$ was considered as significant.

RESULTS

Tail Clip Method

The test drug PIO showed an increase in the reaction time at all-time intervals and was significant when compared to

control at 30 and 60 min ($P < 0.01$). While, the mean reaction time of standard, when compared to control and PIO was more at all-time periods [Table 1].

Acetic Acid-Induced Writhing Method

Mice treated with PIO showed significant reduction in the number of writhes when compared to the control group ($P < 0.01$). However, a number of writhes in the standard group were much less compared to the control and test groups. When compared to control, the percentage inhibition by PIO was 43.9 and that of the standard was 82.64 [Table 2].

DISCUSSION

In the current study, PIO was evaluated for both the central and peripheral analgesic effects and was compared with the standard drugs aspirin and tramadol.

In tail clip model, which is highly sensitive for centrally acting drugs, tramadol was chosen as the standard drug as it is a centrally acting opioid analgesic. When compared to control, the mean reaction time of PIO started increasing gradually at 15 min and peaking at 60 min. However, the mean reaction time of standard, when compared to PIO was more at all-time periods.

Acetic acid-induced writhing method is used to detect peripheral analgesic activity of a test compound. Accordingly, aspirin was chosen as the standard drug. Although aspirin has a central component of action, it predominantly produces analgesia through a peripheral action by inhibiting prostaglandin synthesis. Here, PIO showed significant decrease in the number of writhes ($P < 0.01$) and a percentage inhibition of 43.9 was observed. However, a study by Bhamare *et al*.[9] observed that PPARγ agonists did not significantly affect the peripheral pain mechanisms. Studies have shown that PPARγ agonists alleviate neuropathic pain[10] and have anti-inflammatory activity.[11]

Table 1: The analgesic activity in mechanical pain model - tail clip method

Groups	15 min	30 min	60 min
Control	5±1.41	6±1.41	5.6±1.21
Standard 1 (tramadol)	7.33±1.63*	13.16±1.17*	22.33±1.75*
Test	6.83±1.47*	12±2.37*	17.83±2.64*

Data are expressed as mean±SD of n=6. *P<0.05 compared with control, SD: Standard deviation

Table 2: The analgesic activity in chemical pain model - writhing method

Groups	Number of writhes	Percentage inhibition
Control	32.67±2.94	-
Standard 2 (aspirin)	5.67±1.21*	82.64
Test	18.33±2.33*	43.9

Data are expressed as mean±SD of n=6. *P<0.05 compared with control, SD: Standard deviation

NMDARs are ionotropic glutamate receptors with glutamate-gated cation channels having high calcium permeability. NMDAR hypofunction can result in cognitive defects, whereas overstimulation causes excitotoxicity and subsequent neurodegeneration. Therefore, NMDARs are important therapeutic targets for many CNS disorders including stroke, hypoxia, ischemia, head trauma, Huntington's, Parkinson's, and Alzheimer's diseases, epilepsy, neuropathic pain, alcoholism, schizophrenia, and mood disorders.[12] Excitatory synaptic transmission releases L-glutamate, an excitatory neurotransmitter from presynaptic terminals that diffuses across the synaptic cleft and binds to postsynaptic NMDARs. However, individual excitatory synaptic inputs received during baseline activity do not result in calcium (Ca^{2+}) influx due to its pronounced voltage dependence.

PPARs are a transcription factor belonging to the nuclear receptor superfamily. The regulation of gene transcription by nuclear receptor ligands is commonly referred to as the "classical" or "genomic" pathway. Responses mediated by the genomic pathway typically have latencies of at least 30 to 60 min (and up to days) and are associated with changes in protein synthesis. The pleiotropic actions produced by PPARs are not only mediated through slow-response genomic (transcription-dependent)[13,14] but also by rapid non-genomic (transcription-independent) mechanisms.[15] One of its isoform PPARγ mediates numerous physiological functions of which its role as a lipid sensor is of greater clinical significance. Recent studies have suggested that PPARγ is a powerful pharmacological target for counteracting neurodegeneration, ischemic stroke, and spinal cord injury.[16]

TZDs are selective agonists for nuclear PPARγ. PIO, a PPARγ agonist on binding to the receptor, activates insulin-responsive genes that regulate carbohydrate and lipid metabolism and acts as insulin sensitizers. They represent an important pharmacotherapy in the treatment of glucose intolerance. PIO is also known to exert antidepressant and anticonvulsant effect through nitric oxide pathway.[17,18] PPARγ agonists by effectively attenuating oxidative stress, inflammation, and apoptosis in the CNS[19] have been proven to have neuroprotective potential in the treatment of cerebral ischemia, Parkinson's disease, Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis. Studies have demonstrated that PPARγ agonists have anti-inflammatory effects and have a therapeutic potential in neuropathic pain syndromes.[20,21] PIO has been known to improve memory function by acting on the glutamatergic pathway in the brain where it ameliorates the Ca^{2+}-mediated excitotoxicity by its antagonistic action on NMDAR.

There is widespread expression of NMDAR on the sensory neurons in the cell bodies as well as the peripheral and central

processes of the primary sensory neurons, in the lumbar dorsal root ganglion.[22] Nociceptive C and Aδ fibers also express NMDARs. NMDA agonists have shown to produce pain behaviors while NMDA antagonists effectively alleviate pain-related behavior in animal models as well as in clinical situations.

Peripheral release of glutamate and subsequent activation of NMDAR is critical in the development of neuropathic pain. NMDARs located in peripheral somatic tissues and visceral pain pathways play an important role in nociception. The number of NMDARs on peripheral nerve fibers increases during inflammation, and this may contribute to peripheral sensitization in inflammation.[23] The nociceptive responses induced by injection of glutamate into the mouse paw appear to involve not only peripheral but also spinal and supraspinal NMDARs and are largely mediated by release of nitric oxide.[24] Changes in the periphery after trauma lead to the phenomenon of peripheral sensitization and primary hyperalgesia. Central sensitization is the state where dorsal horn excitability is increased and, as a consequence, its response to sensory input is facilitated.[3] Convincing evidence has demonstrated that the development of spinal hyperexcitability and persistent pain involves activation of NMDARs. The increased NMDAR function is expressed as an increase in channel openings.

Normally, NMDARs do not participate in synaptic transmission due to their voltage-dependent block by extracellular magnesium. However, following tissue damage, persistent noxious stimuli can depolarize membrane strong enough to permit participation of NMDARs in synaptic transmission. Nociceptive input to the dorsal horn is further increased through positive feedback through presynaptic NMDARs. Ca^{2+} entry causes activation of protein kinases and results in phosphorylation of NMDARs. As a consequence, the magnesium block at resting membrane potentials is decreased and channel opening time is prolonged. This explains the mechanism of pain mediated by NMDARs.[25] NMDAR antagonists alleviate pain by reducing NMDAR mediated Ca^{2+} currents and transients, and thereby, blocks prolonged depolarization evoked by dorsal horn neurons.

The analgesic actions of PIO might be mediated through its antagonistic effect on NMDAR which depletes Ca^{2+} flux by reducing NMDAR-mediated Ca^{2+} currents and transients, and thereby, blocks prolonged depolarization evoked by dorsal horn neurons. The study highlights the action of PIO as an analgesic acting on both central and peripheral pain pathways by antagonizing the NMDARs. The study is limited by the fact that other models such as thermal model and *in vitro* models for the evaluation of analgesic activity could not be included in the study. However, considering the safety issues involved with PIO, and availability of many drugs having superior analgesic activities, the routine use of PPARγ agonists as analgesics cannot be justified. However, as PIO provides the combined benefit of reducing hyperglycemia, hyperalgesia, and central and peripheral sensitization, it can be suggested that TZDs represent a novel pharmacotherapy in patients with Type 2 diabetes-associated pains.

The key to the successful utilization of NMDA antagonists as an analgesic lies in activating enough peripheral NMDAR to block pain transmission, but keeping the systemic concentration low enough to avoid unwanted CNS side effects such as memory impairment, psychotomimetic effects, ataxia, and motor incoordination, as NMDARs are important for normal CNS function.

CONCLUSION

The test drug PIO has shown significant analgesic activity when compared to control in both central (tail clip method) and peripheral (writhing method) experimental models of pain. Thereby, it can be concluded that PIO possibly has analgesic activity by influencing both the central and peripheral pain pathways through antagonistic action on NMDARs.

NMDARs are critically involved in the induction and maintenance of neuronal hyperexcitability after noxious events. The central and the peripheral somatic and visceral NMDARs play a greater role in the mechanism of pain. Thus, NMDAR antagonists are quite likely to emerge as a viable strategy for pharmacological treatment of pain.

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