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Ameliorative potential of pioglitazone and ceftriaxone alone and in combination in rat model of neuropathic pain: Targeting PPARγ and GLT-1 pathways

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ABSTRACT

Background: The relation between glutamate homeostasis and PPAR gamma has got tremendous importance in nerve trauma and pain. Present study has been designed to elucidate the interaction between the GLT-1 activator (ceftriaxone) and PPAR gamma agonist (pioglitazone) in the spinal nerve ligation induced neuropathic pain.
Methods: Male SD rats were subjected to spinal nerve ligation to induce neuropathic pain. Pioglitazone, ceftriaxone and their combination treatments were given for 28 days. Various behavioral, biochemical, neuroinflammatory and apoptotic mediators were assessed subsequently.
Results: In the present study, ligation of L5 and L6 spinal nerves resulted in marked hyperalgesia and allodynia to different mechanical and thermal stimuli. In addition there is marked increase in oxidative–nitrosative stress parameters, inflammatory and apoptotic markers in spinal cord of spinal nerve ligated rats. Treatment with pioglitazone and ceftriaxone significantly prevented these behavioral, biochemical, mitochondrial and cellular alterations in rats. Further, combination of pioglitazone (10 mg/kg, *ip*) with ceftriaxone (100 mg/kg, *ip*) significantly potentiated the protective effects as compared to their effects *per se*.
Conclusion: Based on these results we propose that possible interplay between the neuroprotective effects of pioglitazone and ceftriaxone exists in suppressing the behavioral, biochemical, mitochondrial, neuroinflammatory and apoptotic cascades in spinal nerve ligation induced neuropathic pain in rats.

Introduction

Till date only fewer therapeutic strategies are available with limited success in neuropathic pain (NP) relief [1]. Intricate interplay between wide variety of the pathophysiological mechanisms involved in the development and progression of NP makes it difficult to design effective therapeutic strategies [2]. So, there is an imperative need for the development of better therapeutic regimens with improved efficacy and minimal side effect profiles.

Peroxisome proliferator-activated receptor gamma (PPARγ) receptors and their downstream mechanisms are involved in nociception [3]. Inflammatory mediators released from activated macrophages, glial cells will play critical role in the pathology of both inflammatory and NP. PPARγ ligands are known to transrepress the expression of these inflammatory mediators via different mechanisms [4–6]. Further, their role in neuroinflammatory, oxidative stress process has been considered as protective strategies in different neuronal disorders [7–9]. Pioglitazone, a PPARγ agonist with better blood brain barrier permeability, is known to affect the neuro-immune mechanisms in the spinal cord [10]. Oxidative stress and free radicals generated because of damage to neuronal tissues further alter the mitochondrial enzyme complex activities, caspase activation and neuronal death [11]. Mitochondrial impairment resulting in severe deficiency of energy production and neuropathy has been suggested in different chemotherapeutic agents as well as in diabetes induced neuropathy [12,13]. Further, mitochondrial poisons worsen the NP by targeting the sensory neuronal mitochondria [14]. However, injury induced NP has remained unexplored and the data related to mitochondrial enzyme complexes is sparse. Traditionally, PPARγ is

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known for its antidiabetic, antiinflammatory activities, but recent studies suggest that activation of PPARγ is involved in the alteration of glutamate transport [15] and further pioglitazone is shown to be associated with non-genomic PPARγ mechanism [10].

Glutamate concentration at synapse is maintained and controlled totally by glutamate/excitatory amino acid transporters (EAAT). Of the five types (EAAT 1–5) of these transporters cloned till date, EAAT2 (GLT-1) is responsible for more than 90% of glutamate clearance in central nervous system [16]. Growing body of evidence has reported a decrease in the spinal cord expression of GLT-1 in different neurodegenerative disorders including NP [17,18]. Selectively increasing the expression of this transporter is suggested as one of the protective mechanism involved in preventing the glutamate mediated neuronal toxicity [19]. NP is known to be associated with increased release of glutamate in synaptic cleft thereby causing hyperexcitability of second order neurons [16]. Indeed, glutamate antagonists have been tried for NP relief with limited success because of their narrow therapeutic window and associated side effects profile [20]. So, reducing the dose as in combination therapy will further provide a way for effective pain relief with increased safety profile. We therefore hypothesized that simultaneously targeting the PPARγ and GLT-1 could be useful for effective pain relief.

Ceftriaxone, a β-lactam antibiotic, because of its capability to specifically up-regulate the GLT-1 expression is shown to be efficacious in different neuronal disorders including NP [17,21]. Despite the fact that ceftriaxone is effective in different NP models data showing its efficacy in L5/L6 spinal nerve ligation (SNL) model is lacking and needs further investigation.

Materials and methods

Animals

Male SD rats (180–220 g) bred in Central Animal House, Panjab University, Chandigarh were used in this study. Animals were acclimatized to the laboratory conditions prior to experimentation. They were maintained on 12-h light/dark cycle and have free access to food and water. All the experimental procedures were performed between 9:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University (Protocol no. 504/2/4/14/UIPS-42) and carried out in accordance with the guidelines of Indian National Science Academy Guidelines for the use and care of experimental animals. All the behavioral studies were carried out by a person who is blinded to the treatment groups.

Spinal nerve ligation

The procedure of ligation of the spinal nerves was performed as per the modified method of Chung [22]. Briefly, under chloral hydrate (350 mg/kg, *ip*) anesthesia, L5–L6 spinal nerves were carefully exposed and tightly ligated distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve with (6–0) silk thread. Following complete hemostasis, the wound was sutured. The procedure of the sham group was identical to that of the experimental group except spinal nerve ligation. All surgical procedures were carried out under normal sterile conditions.

Drug and treatment schedule

Study protocol includes 10 treatment groups, consisting of 10 animals in each group. Pioglitazone, ceftriaxone (Panacea Biotech, Mohali) were used in the present study. Pioglitazone was suspended in 0.25% sodium carboxy methyl cellulose (CMC) and ceftriaxone was dissolved in normal saline. Drugs were administered by the intra-peritoneal (*ip*) route as per body weight (5 ml/kg). Different animal groups (Table 1) received respective drug treatments daily in the morning 10:00 h, for 28 days starting from the day after SNL. Drug doses selection was made on the basis of earlier literature reports [17,23] and all the behavioral experiments were performed 30 min after the drug administration.

Assessment of nociception

Mechanical allodynia

Rats were placed individually in a clear plastic cage containing mesh (1 cm^2 perforations). Animals were adapted to the testing environment for at least 15 min before assessment of mechanical allodynia by vonfrey apparatus (IITC Life Science, Woodland Hills, CA). A polypropylene rigid tip of 0.5 mm diameter was used to apply the force to the plantar region of the hind paw. The force that leads to paw withdrawal was recorded by the anesthesiometer. The test was repeated for three times at the intervals of 5 min and the mean value was recorded [24].

Chemical allodynia

The cold allodynia was assessed by spraying a 200 μl of acetone onto the plantar surface of rat paw (placed over a wire mesh), without touching the skin. The total time duration that animal spent on lifting, shaking or licking against acetone treatment that was recorded for 2 min immediately after acetone application [25].

Mechanical hyperalgesia

Paw pressure threshold was assessed with Randall–Selitto paw pressure analgesia meter (IITC Life Science, Woodland Hills, CA). Increasing pressure at a linear rate of 10 g/s was applied to the center of the hind paw. Pressure at which animal withdraws its paw was recorded by an analgesia meter and expressed in mass units (g), with a cut-off of 250 g to avoid potential tissue injury.

Table 1
Grouping of experimental animals.

S. no.	Group name	Treatment (mg/kg)
1.	Naive	Without treatment
2.	Sham	Sham (surgery performed, vehicle administered)
3.	SNL (Control)	L5/L6 spinal nerves were ligated + vehicle administered for 28 days
4.	SNL + Pio (5)	L5/L6 spinal nerves were ligated + Pioglitazone (5 mg/kg, *ip*)
5.	SNL + Pio (10)	L5/L6 spinal nerves were ligated + Pioglitazone (10 mg/kg, *ip*)
6.	SNL + Pio (20)	L5/L6 spinal nerves were ligated + Pioglitazone (20 mg/kg, *ip*)
7.	SNL + Cef (100)	L5/L6 spinal nerves were ligated + Ceftriaxone (100 mg/kg, *ip*)
8.	SNL + Cef (200)	L5/L6 spinal nerves were ligated + Ceftriaxone (200 mg/kg, *ip*)
9.	SNL + Pio (5) + Cef (100)	L5/L6 spinal nerves were ligated + Pioglitazone (5 mg/kg, *ip*) + Ceftriaxone (100 mg/kg, *ip*)
10.	SNL + Pio (10) + Cef (100)	L5/L6 spinal nerves were ligated + Pioglitazone (10 mg/kg, *ip*) + Ceftriaxone (100 mg/kg, *ip*)