EVALUATION OF ANTI-NOCICEPTIVE EFFECT OF TOLPERISON BY COMPARING ITS EFFECT WITH TRAMADOL IN NEUROPATHIC PAIN

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ABSTRACT
Neuropathic pain is caused by a lesion or disease of somatosensory nervous system and treatment of neuropathic pain remains challenge. The purpose of present study was to evaluate efficacy of Tolperisone, a centrally acting muscle relaxant in reducing neuropathic pain and also attempt to establish the mechanism of action of Tolperisone by comparing its effectiveness with standard drug Tramadol. Neuropathic pain was induced in rats using Sciatic Inflammatory Neuritis Model (SINM). The Successful induction of neuropathic pain was evaluated by observing hyperalgesia and allodynia in rats exposed to various stimuli. Effectiveness of Tolperisone (10mg/kg) in treatment of Neuropathic pain was evaluated by comparing the results obtained with Standard drug Tramadol (10mg/kg). The findings of the present study demonstrate that the Sciatic Inflammatory Neuritis Model (SINM) neuropathic pain result in severe changes in behavioral responses producing mechanical and thermal hyperalgesia and cold-allodynia. Also our results indicate that voltage-gated sodium channel probably contributes to development of hyperalgesia and allodynia in the SIN. This study shows that Tolperisone is a potent anti-hyperalgesic and anti-Allodynic compound in rats and emphasizes the concept that blockade of Na+ channels may be beneficial for pain treatment in humans.

Keywords: Sciatic Inflammatory Neuritis (SIN); Neuropathic pain; allodynia; hyperalgesia; somatosensory system; Tramadol

INTRODUCTION
Neuropathic pain is a pain syndrome caused by primary damage and/or by the dysfunction of the neurotransmission system connecting the peripheral to the central nervous system (CNS). Patients with neuropathic pain frequently complain of sensory abnormality, including increased response to noxious stimulus (hyperalgesia) and pain response to non-noxious stimulus (allodynia) \(^1\). The etiology and underlying mechanisms of such pains are poorly understood and the existing treatments including anti-convulsalant agents, local anesthetics and opioids are often ineffective\(^2\). Hence, it is important to increase our understanding of the mechanisms that underlie neuropathic pain states in order to identify new strategies for the development of effective therapies. A numbers of animal nerve injury models have been developed to study the mechanisms underlying neuropathic pain\(^2\). According to sciatic inflammatory neuritis (SIN) model developed by Bennett JG, by placing a proinflammatory gut suture, dead bacteria or Carrageenan around the sciatic nerve can produce both allodynia and hyperalgesia in the SIN\(^3\). The animals show altered spontaneous behaviour consistent with the presence of lowered threshold and exaggerated responses to thermal and mechanical stimuli\(^2\).

Neuropathic pain develops due to injury or disease of the nervous system...
(including the Peripheral nerve, Dorsal root ganglion (DRG), spinal cord or brain) and is characterized by a combination of spontaneous pain, allodynia (pain due to stimulus which does not normally provoke pain) and hyperalgesia (an increased response to a stimulus which is normally painful). After nerve injury there can be increased expression of sodium and calcium (voltage-gated) channels leading to ectopic discharges and reduction in threshold for activation of nociceptors (peripheral sensitization) leading to altered pain transmission causing hyperalgesia and allodynia.

The allodynia and hyperalgesia induced due to neuropathic pain were assessed by observing the behavioural response of animals to mechanical, thermal and cold stimuli. Alterations in voltage-gated ion channel expression and/or function may have a profound influence on the firing patterns of both primary afferent pathways and central neurones contributing to generation and maintenance of several pain syndromes. Such changes appear to contribute to ongoing, abnormal repetitive discharge from ectopic sites established within primary afferent neurones following injury. Recently, Na\textsuperscript{+} currents and immune system have been identified as important targets for both studying the molecular pathophysiology of pain and discovering new therapeutic agents. Tolperisone, a centrally acting muscle relaxant agent has been widely used as spasmolytics of choice and mainly used for treating muscle spasticity of neurological origin and painful muscle spasms due to rheumatologic conditions. Tolperisone acts at the level of spinal cord by blocking sodium channels and calcium channels.

Literature survey reveals that tolperisone is centrally acting muscle relaxant drug acting by the same mechanism as Tramadol which is most frequently used drug for treatment of central neuropathic pain. Tramadol is an orally active Opioid analgesic is reported to be effective in neuropathic pain. This compound that block monoamine uptake (5-HT and Nor-epinephrine) potentiate antinociceptive effect of Opioids including tramadol. Hence, anti-nociceptive potency profile of tramadol is due to dual mechanism of Opioids analgesic activity and inhibition of monoamine uptake.

Hence, the present study was undertaken to evaluate anti-nociceptive effect of Tolperisone in treatment of neuropathic pain by comparing its effect with Tramadol used therapeutically in neuropathic pain using (Sciatic Inflammatory Neuritis) SIN model.

**MATERIALS AND METHODS**

**Animals and surgery**

Male Albino rats of Wistar strain (250-300 g) were anesthetized with Ketamine (80mg/kg) and Xylazine (10mg/kg). A 3-cm incision was made posterior to the greater trochanter of the femur and the common sciatic nerves were exposed at the mid-thigh level by blunt dissection through the biceps femoris and gently separated from adjacent tissue. Peri-sciatic immune activation by placing a proinflammatory gut suture, dead bacteria or Carrageenan around the sciatic nerve can produce both allodynia and hyperalgesia in the SIN. For Carrageenan induced hyperalgesia, 1% Carrageenan in volume of 50 µl saline was administered into the instep of right or left hind-paw. The nociceptive duration and threshold was measured at 24 hrs after Carrageenan injection. The results were expressed as percentages of control threshold.

**Behavioural Testing (Neuropathic Assay)**

**Mechanical-hyperalgesia (Pin-Prick test)**

The mechanical-hyperalgesia was assessed by pinprick test: touching (not penetrating) the skin with the point of a safety pin. The surface of the injured hind paw was touched with the point of the bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response. The paw withdrawal duration was recorded in seconds and the normal quick
reflex withdrawal response was given the value of 0.5 s\textsuperscript{11,12}.

**Mechanical Hyperalgesia (Paw-pressure test)**

Response to noxious mechanical stimulation was determined by measuring of withdrawal threshold to paw-pressure using Analgesimeter (Ugo Basile Biological Research Apparatus). Continuously increasing pressure was applied to the dorsal surface of the affected hind-paw using blunt conical probe in Randal Selitto test instrument. On the day before the experiment, nociceptive thresholds of the right or left hind-paw of each rat were measured for 4 times at 1 h intervals. Only animals with stable and reproducible thresholds were selected. Mechanical pressure was increased until withdrawal reflex occurred while rats were lightly restrained. Withdrawal reflex threshold were expressed in grams. Threshold measurements were repeated three times and averaged\textsuperscript{13}.

**Thermal Hyperalgesia (Plantar test)**

The Hargreaves method was used to assess paw-withdrawal duration to thermal nociceptive stimulus. First, the preoperative pain duration of the animals was recorded, and then surgery was performed. In rats, the response to a heat stimulus was tested with the “Plantar Test” device from Ugo Basile. The radiant heat was applied from below to the plantar surface of each hind-paw and the withdrawal duration was measured with a stop-watch. Three measurements were performed on the each hind-paw with at least 1 min intervals to determine mean paw-withdrawal duration (PWD). A preliminary or control threshold was measured for each rat before drug injection. The cut-off value was determined as 30s in order to avoid tissue damage\textsuperscript{131}.

**Drug treatment**

Tolperisone was administered intraperitoneally in dose of (10mg/kg) 30 min prior to behavioral tests on 24 hrs after surgery. Tolperisone was dissolved in normal saline and injected intraperitoneally. In the control group, rats received an equal volume of saline.

**Statistical procedure**

All the Data were expressed as Mean ± SEM and tested with one way ANOVA followed by Tukey’s multiple comparison tests with the help of Graph pad prism-3.

**RESULTS**

Neuropathic pain was induced in all animals using Sciatic Inflammatory Neuritis Model (SINM), and the behaviour of animals to mechanical, thermal was tested at 24 hrs after surgery, respectively.

**Sciatic Inflammatory Neuritis Model**

a) Effect of Tolperisone on mechanical hyperalgesia in Sciatic Inflammatory Neuritis induced Neuropathic pain in rats

Sciatic inflammatory neuritis (SIN) in rats induced neuropathic pain resulting in significant development of mechanical hyperalgesia in response to pin-prick stimulation and application of pressure on lateral surface of ipsilateral hind-paw. In SIN rats, 24 hrs after surgery, the paw-withdrawal duration of ipsilateral paw was significantly increased (*P < 0.05) in response to noxious pin-prick stimulation and paw-withdrawal threshold of ipsilateral paw was significant decreased (*P < 0.05) in response to application of pressure on ipsilateral paw as compared to control group.

Administration of Standard drug Tramadol (10mg/kg;i.p.) produced significant reduction (*P<0.05) in Paw-withdrawal duration and significantly increased (*P<0.05) Paw-withdrawal threshold of ipsilateral paw as compared to post-surgery values. Tolperisone was administrated at doses of 2.5, 5, and 10 mg/kg. Administration of Tolperisone, at 10 mg/kg significantly reduced (*P<0.05) paw-withdrawal duration and significantly increased (*P<0.05) paw-withdrawal threshold as compared to post-surgery values of control. The low doses of Tolperisone 2.5 and 5 mg/kg
did not significantly reduce the paw-withdrawal duration and paw-withdrawal threshold of ipsilateral paw in Neuropathic rat.

**Table 1: Effect of Tolperisone on SIN induced mechanical hyperalgesia (pin-prick test)**

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>Paw-withdrawal Duration (sec)</th>
<th>Before surgery</th>
<th>24 hr After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline)</td>
<td>0.9608 ± 0.02337</td>
<td>4.854±0.01587</td>
<td></td>
</tr>
<tr>
<td>Std (Tramadol;10mg/kg)</td>
<td>1.8050±0.01498</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;2.5mg/kg)</td>
<td>3.792±0.01584</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;5mg/kg)</td>
<td>3.093±0.01870</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;10mg/kg)</td>
<td>1.558±0.01854</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed Mean ± SEM, (n=6), *p<0.001 compared to control group (pre-surgery group), #p<0.05 compared to test group. One way ANOVA followed by Tukey's Multiple Comparison Test.

**Table 2: Effect of Tolperisone on SIN induced mechanical hyperalgesia (paw-pressure test)**

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>Paw-withdrawal Threshold (g)</th>
<th>Before surgery</th>
<th>24 hr After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline)</td>
<td>14.790 ± 0.01163</td>
<td>7.0201±0.01079</td>
<td></td>
</tr>
<tr>
<td>Std (Tramadol;10mg/kg)</td>
<td>12.9234±0.0151</td>
<td>0¹</td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;2.5mg/kg)</td>
<td>8.3076±0.01627</td>
<td>0¹</td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;5mg/kg)</td>
<td>9.27±0.01423</td>
<td>0¹</td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;10mg/kg)</td>
<td>13.2389±0.0123</td>
<td>0²</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed Mean ± SEM, (n=6), *p<0.001 compared to control group (pre-surgery group), #p<0.05 compared to test group. One way ANOVA followed by Tukey's Multiple Comparison Test.

**Figure 1: Effect of Tolperisone on SIN induced mechanical hyperalgesia (pin-prick test)**

Each bar Expressed Mean±SEM. (n=6), *p<0.001 compared to control group (pre-surgery group), #p<0.05 compared to test group. One way ANOVA followed by Tukey's Multiple Comparison Test.

**Figure 2: Effect of Tolperisone on SIN induced mechanical hyperalgesia (paw-pressure test)**

b) **Effect of Tolperisone on thermal-hyperalgesia in Sciatic Inflammatory Neuritis induced Neuropathic pain in rats**

In SIN rats, 24hrs after surgery, the paw-withdrawal duration of ipsilateral paw in response to noxious thermal stimuli was significantly increased (*p<0.05) as compared to pre-surgery values of control group.
Administration of Standard drug Tramadol (10mg/kg,i.p.) produced significant reduction (*P < 0.05) in paw-withdrawal duration of ipsilateral paw as compared to post-surgery values of control group.

The low doses of Tolperisone 2.5 and 5 mg/kg did not significantly reduce the duration of ipsilateral paw-withdrawal in Neuropathic rats. However, administration of Tolperisone in dose of 10 mg/kg significantly reduced (*P < 0.05) paw-withdrawal duration as compared to post-surgery values of control group.

Table 3: Effect of Tolperisone on SIN induced thermal hyperalgesia (plantar test)

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>Paw-withdrawal Duration (sec)</th>
<th>Before surgery</th>
<th>24 hr After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline)</td>
<td>0.9177 ± 0.01497⁷</td>
<td>7.944±0.01262²#</td>
<td></td>
</tr>
<tr>
<td>Std (Tramadol;10mg/kg)</td>
<td>2.438±0.01522⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;2.5mg/kg)</td>
<td>5.324±0.01263⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;5mg/kg)</td>
<td>4.985±0.01048⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (Tolperisone;10mg/kg)</td>
<td>2.056±0.01367⁹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed Mean ± SEM, n=6. *p<0.001 compared to control group (pre-surgery group), #p<0.05 compared to test group. One way ANOVA followed by Tukey's Multiple Comparison Test.

DISCUSSION

In Sciatic Inflammatory Neuritis model (SINM), inflammation of sciatic nerve at the level of mid-thigh produced neuritis due to minor structural damage to axon or glia resulting in neuropathic pain sensation. This was evidenced by increasing paw withdrawal duration (pin-prick and thermal stimulation) and decreasing paw-withdrawal threshold (paw-pressure test) as compared to pre-surgery values. It has been reported that the normal response to pin-prick is due to input from Aδ nociceptors that the pain evoked by Aδ input is resistant to Opioids analgesic like Morphine. The hyperalgesic response to pin-prick on neuritis side is due to input from C-nociceptors and this pain is susceptible to Opioids analgesics like morphine.

Recent studies have reported that endoneuronal infiltration of immune cell expose Aδ and C-nociceptors axons to pro-inflammatory cytokines like TNF-α which produces ectopic discharge in Aδ and C-nociceptors and sensitize them due to sensitization of nociceptors result in mechanical and thermal hyperalgesia, were produced. Hence, one hypothesis suggests that immunosuppressive therapy should have therapeutic benefit in neuropathic pain. This is supported by report of a study involving use of corticosteroids which reported that systemic injection of corticosteroids reduces neuropathic pain in animals with experimental neuritis due to its immunosuppressive effect.

Tramadol is an orally active Opioid analgesic is reported to be effective in neuropathic pain. This compound that block monoamine uptake (5-HT and Nor-epinephrine) potentiate anti-nociceptive effect of Opioids including tramadol. Hence, anti-nociceptive potency profile of tramadol is due to dual mechanism of Opioids analgesic activity and inhibition of monoamine uptake.

In our study we observed that the drug under study Tolperisone reversed abnormal reactivity neuropathic rats to mechanical and thermal stimuli with statistically significant
difference compared to post-surgery values of control group. These results are in correlation with the results obtained with standard drug tramadol. As discussed earlier we can thus conclude that Tolperisone may be therapeutically benefited in neuropathic pain due to mechanism similar to tramadol. It may be exerting immunosuppressive effect or may be acting by inhibition of monoamine uptake. Further studies are needed to establish exact mechanism of action.

CONCLUSION
The findings of the present study demonstrate that the Sciatic Inflammatory Neuritis Model of neuropathic pain results in severe changes in behavioural responses producing mechanical and thermal hyperalgesia and allodynia. However the results obtained with the SIN model indicate that hyperalgesia associated neuritis is probably due to sensitization of Aδ-fibers and C-nociceptors.

The drug under study, Tolperisone is found to possess therapeutic potential for treatment of neuropathic pain. It may be acting by exerting immunosuppressive effect or inhibition of uptake of monoamine like 5HT and Nor-epinephrine similar to Tramadol. Additional pre-clinical studies are required to establish the exact mechanism of Tolperisone in the treatment of neuropathic pain.

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