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Centrally Mediated Anti-Hyperalgesic and Anti-Allodynic Effect of Tolperisone in Spared Nerve Injury Model of Neuropathic Pain

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ABSTRACT:

Neuropathic pain is caused by a lesion or disease of somatosensory nervous system and its treatment remains a challenge. The purpose of the present study was to evaluate efficacy of Tolperisone, a centrally acting muscle relaxant in reducing neuropathic pain and also attempt to establish its mechanism of action by comparing its effectiveness with standard drug Phenytoin. Neuropathic pain was induced in rats using Spared Nerve Injury Model (SNIM). The Successful induction of neuropathic pain was evaluated by observing hyperalgesia and allodynia in rats exposed to various stimuli. The Effectiveness of Tolperisone (10mg/kg) in treatment of Neuropathic pain was evaluated by comparing the results obtained with Standard drug Phenytoin (20mg/kg) and results indicate that voltage-gated sodium channel probably contributes to development of hyperalgesia and allodynia in the SNI model. Phenytoin which is sodium channel blocker drug exerts a potent anti-hyperalgesic and anti-allodynic. Tolperisone shows effects similar to Phenytoin and emphasizes the concept that blockade of Na+ channels may be beneficial for treatment of neuropathic pain in humans.

Keywords: Spared Nerve Injury (SNI), Neuropathic pain, allodynia, hyperalgesia, somatosensory system.

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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 painful response to non-noxious stimulus (allodynia) [1]. The etiology and underlying mechanisms of such pains are poorly understood and the existing treatments including Phenytoin, lidocaine and tramadol which 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]. Neuropathic pain develops due to injury or disease of the nervous system (including the Peripheral nerve, Dorsal root ganglion (DRG), spinal cord or brain) results in increasing 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[6]. 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, Na1 currents have been identified as important targets for both studying the molecular pathophysiology of pain and discovering new therapeutic agents.[2]

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[3,4]. Literature survey reveals that tolperisone is voltage-gated sodium and calcium blocker drug acting by the same mechanism as Phenytoin and mexiletine which are most frequently used drugs for treatment of both peripheral and central neuropathic pain.

Hence, the present study was undertaken to evaluate effectiveness of a centrally acting muscle relaxant drug Tolperisone reducing symptoms of neuropathic pain like hyperalgesia and allodynia of neuropathic pain induced using SNI model and to compare its effectiveness with Phenytoin used therapeutically in neuropathic pain.

Materials and Methods

Animals and surgery[5,7]

Experiments were carried out on male or female Wistar strain of albino rats (250–300 g) of either sex. Three to four rats were housed in a cage under a 12 h light/dark cycle with food and water available ad libitum. Ratswere anesthetized with ketamine (80mg/kg;i.p.) and xylazine (10mg/kg;i.p.), the skin on lateral surface of thigh was incised and cranial and caudal parts of the biceps femoris muscle were separated and held apart by a retractor to expose the sciatic nerve and its three terminal braches: the sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were tight-ligated with 5.0 silk and sectioned distal to the ligation, removing 2-4 mm of the distal nerve stump. Great care was taken to avoid any contact with or stretching of the intact sural nerve. Muscle and skin were closed in two layers. The contra-lateral side served as a potential internal sham-operated control. Only animals that recovered completely with no behavioural deficits after post surgerywere used for subsequent behavioural testing. The behavioural response of these rats to various stimuli was assessed 15 days after surgery.

Behavioural Testing (Neuropathic Assay)

Mechanical-hyperalgesia (Pin-Prick test)[8,9]

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.

Mechanical Hyperalgesia (Paw-pressure test)[10]

Response to noxious mechanical stimulation was determined by measuring the 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. Mechanical pressure was increased until withdrawal reflex occurred while rats were lightly restrained. Withdrawal reflex threshold were expressed in grams.

Thermal Hyperalgesia (Plantar test)[11]

The Hargreaves method was used to assess paw-withdrawal duration to thermal nociceptive stimulus. The preoperative pain duration of the animals was recorded before surgery. 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 each hind-paw using plantar test device from Ugo Basile with at least 1 min intervals to determine mean paw-withdrawal duration (PWD). A preliminary or control paw withdrawal threshold was measured for each rat before drug injection. The cut-off value was determined as 30s in order to avoid tissue damage.

Cold-allodynia (Chemical test)[12]

The rats were housed in transparent plastic boxes with a floor made of 6 mm wire mesh. After a 5–10-min accommodation period, 0.5 ml of acetone was sprayed onto the plantar surface of the rat's hind leg from below the grid with a syringe with a blunt needle. Rats that developed cold allodynia withdraw the injured limb from the floor, when acetone was applied, and the amount of time spent with the leg raised was used as a measure of pain. Both hind legs were tested in each animal, starting with the control leg, with an interval of 3 min

between them. The cut-off duration was determined as 30s.

Drug treatment

Tolperisone was dissolved in normal saline and injected intraperitoneally in dose of (10mg/kg) 30 min prior to behavioural tests on 15 days after surgery.

Statistical procedure

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

Results

Spared nerve injury model

a) Effect of Tolperisone on mechanical hyperalgesia in Spare Nerve Injury induced Neuropathic pain in rats.

Spare nerve injury 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. The paw-withdrawal duration to pin-prick significantly increased (*p 0.001) and paw-withdrawal threshold to application of pressure significantly decreased (*p 0.001) in ipsilateral paw, 15 days after surgery as compared to pre-surgery value of control group. Administration of std. Drug Phenytoin (20mg/kg;i.p.) and test drug Tolperisone (10mg/kg;i.p.) to spare nerve injured rats significantly reduced (*p 0.05) paw-withdrawal duration and significantly increased (*p 0.05) paw-withdrawal threshold of ipsilateral hind-paw in response to noxious pin-prick stimulus and application of paw-pressure test, as compared to post-surgery values of control group. (Table 1 & 2)

b) Effect of Tolperisone on thermal hyperalgesia in Spare nerve injury induced Neuropathic pain in rats.

Spare Nerve Injury induced Neuropathic pain in rats resulting in development thermal hyperalgesia as evidenced by application of thermal stimuli to ipsilateral paw showed significant reduction (*p 0.001) in paw-withdrawal duration at 15 days after surgery, as compared to pre-surgery values. Administration of std. Drug Phenytoin (20mg/kg;i.p.) and test drug Tolperisone (10mg/kg;i.p.) to spare nerve injured rats significantly increased (*p 0.05) paw-withdrawal duration 15 days after surgery , as compared to post-surgery level of control group. (Table 3)

c) Effect of Tolperisone on cold-allodynia in Spare nerve injury induced Neuropathic pain in rats.

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

Group (treatment)	Paw-withdrawal Duration (sec)	
	Before surgery	15 daysAfter surgery
Control (Normal saline)	0.9553±0.08734	7.400±0.1216*
Std (Phenytoin; 20mg/kg)		2.464±0.09723#
Test (Tolperisone; 10mg/kg)		2.792±0.08656#

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

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

Group (treatment)	Paw-withdrawal Threshold (g)	
	Before surgery	15 daysAfter surgery
Control (Normal saline)	15.43 ±0.3244	5.343±0.1840*
Std (Phenytoin; 20mg/ kg)		12.570±0.2315#
Test (Tolperisone;10 mg/kg)		11.610±0.2359#

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

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

Group (treatment)	Paw-withdrawal Duration (sec)	
	Before surgery	15 days After surgery
Control (Normal saline)	0.9233± 0.07954	9.763±0.1561*
Std (Phenytoin; 20mg/kg)		3.175±0.08366#
Test (Tolperisone;10mg/kg)		3.773±0.07953#

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

Table 4: Effect of Tolperisone on SNI cold-allodynia (chemical test)

Group (treatment)	Paw -withdrawal Duration (sec)	
	Before surgery	15 daysAfter surgery
Control (Normal saline)	No response observed	12.92±0.2273
Std (Phenytoin; 20mg/kg)		4.707±0.1470#
Test (Tolperisone;10mg/kg)		5.210±0.096480#

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

Neuropathic Pain induced due to SNI resulted in development of cold-allodynia evidenced by increasing in paw-withdrawal duration 15 days after surgery when acetone was sprayed on the plantar surface of paw. The std. Drug Phenytoin (20mg/kg;i.p) and test drug Tolperisone (10mg/kg;i.p.) exhibited anti-allodynic effect by significantly decreasing (*p0.05) duration of paw-withdrawal, as compared to post-surgery values of control group. (Table 4)

DISCUSSION

The Spared Nerve Injury model is the most commonly employed animal modelfor inducing Neuropathic pain[7]. The SNI procedure induced neuropathic pain which was evidenced by a chronic behavioural mechanical, thermal and cold hypersensitivity of immediate onset and prolonged action. The territories of the spared sural nerve, and to a significantly less extent the saphenous nerve, were both affected, but there was no change in the contra-lateral paw. A feature of SNI model is the substantial co-mingling of intact axons with denervated cell in the nerve distal to the lesion, which for C-fibres might include intact axons, contacted by the same cell that envelope a degenerating C-fibre[13,14].

The injured nerves exhibit spontaneous or ectopic firing particularly in large A-fibres which is dependent on voltage-gated sodium channel activity and contributes to development of hyperalgesic and allodynic characteristic of neuropathic pain. Increased electrical excitability of dorsal root ganglion neurons, together with spontaneous activity arising from primary afferent A-fibre, arises as a consequence of axonal ijury[14]. Much of this injury induced remodelling of sensory neuron function has been attributed to dynamic regulation of voltage-gated Na^+ channel expression within dorsal root ganglion neurone and at the site of injury[15].

The current study was designed to explore the SNI induce neuropathic pain and to evaluate the effect of drug treatment on the associated behavioural changes.

The drug under study, Tolperisone displayed a lower withdrawal duration and higher withdrawal threshold in operated animals as compared to control animals in SNI model of neuropathic pain. The sensitivity to cold-stimulation was also attenuated by Tolperisone. The results of Tolperisone correlate with anti-hyperalgesic and anti-allodynic response of standard drug Phenytoin. Phenytoin is an anti-epileptic drug which blocks voltage-gated sodium channel in axonal neurone. Since Tolperisone produced activity similar to Phenytoin, it can be proposed that Tolperisone may be acting by inhibiting voltage-gated sodium channels thus reducingimpulse production by afferent fibres and suppressing hyperalgesia and allodynia.

Neuropathic pain is a chronic condition with early and late phases. The early/acute reaction phase is generally attributed to a direct effect on nociceptors with concomitant activation of Aβ and Aδ fibres as well as high threshold C nociceptive afferent fibres and the late phase of neuropathic pain which depends on combination of inflammatory reaction in peripheral tissue and functional changes in dorsal horn of spinal cord. During early phase of neuropathic pain, Tolperisone probably blocks axonal conduction through its action on voltage gated sodium channels and during late phase it may be due to higher number of C-fibres firing during this period causing higher level of depolarization and making them more sensitive to blockade of voltage gated sodium channel by Tolperisone[16,17]. Thus, Tolperisone is effective in both early/acute and late phase of neuropathic pain.

CONCLUSION

The findings of the present study demonstrate that the Spared Nerve Injury Model of neuropathic pain results in severe changes in behavioural responses producing mechanical and thermal hyperalgesia and cold-allodynia due to voltage-gated sodium channel. The drug under study, Tolperisone is found to possess therapeutic potential for treatment of neuropathic pain. It is possible that Tolperisone may be blocking voltage gated sodium channels like Phenytoin for relieving neuropathic pain. This study shows that Tolperisone is a potent anti-hyperalgesic and anti-allodynic compound in rodent animals and emphasizes the concept that blockade of Na+ channels may be beneficial for pain treatment in humans as supported by clinical evidences. Additional pre-clinical studies are required to establish the exact mechanism of Tolperisone in the treatment of neuropathic pain.

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