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EVALUATION OF ANALGESIC ACTIVITY OF TROGLITAZONE AND METFORMIN ON NEUROPATHIC PAIN

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ABSTRACT

Objective: *To evaluate the analgesic activity of the anti-diabetic drugs Troglitazone and Metformin in partial sciatic nerve ligation-induced neuropathic pain.* ***Method:*** *All the animals were subjected to both von Frey's and plantar test (Hargreaves's method) to examine the intensity of the two features namely hyperalgesia and allodynia which resulted from peripheral nerve injury. The Effective Pressure (EP_{50}) and the paw withdrawal latencies were measured and compared among individual animals. Then based on these pain assessment parameters, the animals were divided into five groups of seven animals and dosing was done immediately on day 0 (day on which grouping was done). Troglitazone (10mpk, p.o.) and Metformin (300mpk, p.o.) were tested against Gabapentin (50 mpk, i.p.) as standard drug. The study was done on chronic dosing or multiple dosing. The EP_{50} values and percentage change in paw withdrawal latencies of right and left paws were calculated and statistical analysis was done.* ***Results:*** *Troglitazone produced significant effect on mechanical allodynia on day6 and day9, Metformin produced significant effect on mechanical allodynia on day6, day9 and day12 of treatment and Gabapentin showed significant effect on day 3, day9 and day12 of treatment.* ***Conclusion:*** *The mechanical allodynia parameter of neuropathic pain induced with partial sciatic nerve ligation model (PSNL) which may be attributed to peripheral anti-inflammatory actions, apart from their anti-diabetic activity.*

KEY WORDS

analgesic activity, Neuropathic Pain, Metformin, Troglitazone.

Introduction

Neuropathic pain is defined as 'pain caused by a primary lesion or any dysfunction in the nervous system'.[1] It occurs due to peripheral or central nerves injuries, metabolic disorders, inflammation, neurotoxicity and is characterized by spontaneous pain, hyperalgesia, allodynia, which persist for a long time. The common causes of neuropathic pain are diabetes mellitus, herpes zoster infections, AIDS, traumatic spinal cord injury, multiple sclerosis and neurotoxicity.[3, 4] currently, the clinically available drugs for neuropathic pain have limited efficacy and are associated with a number of intolerable side effects. Thus, neuropathic pain represents a substantial unmet medical need for the development of novel therapies or extending the spectrum of therapeutic potential of the clinically available drugs.[5]

Neuropathic pain reflects both peripheral and central sensitization mechanisms. Peripheral mechanisms include ectopic discharges and ephaptic conduction[12], collateral sprouting[13, 29] and coupling between the sympathetic nervous system and the sensory nervous system[14] and alteration in expression of bradykinin binding sites within dorsal root ganglion neurons.[15] Central mechanisms include spinal cord anatomical re-organisation and spinal cord hyperexcitability.[16, 17, 18,19,36,37]

Stimulus-evoked pain is a common component of peripheral nerve injury or damage and has two key features hyperalgesia is an increased pain response to a suprathreshold noxious stimulus. Allodynia is the

sensation of pain elicited by a non-noxious stimulus and can be produced in two ways: by the action of low threshold myelinated Aβ fibres on an altered central nervous system; and by a reduction in the threshold of nociceptor terminals in the periphery.[2,16,28,29,30,40,41]

Diabetes Mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration called hyperglycemia, caused by insulin deficiency, often combined with insulin resistance[43]*. Troglitazone, a thiazolidinedione class drug, acts as insulin sensitizer. Thiazolidinediones reduce glucose, fatty acid and insulin blood concentrations. They bind to the nuclear receptors called peroxisome proliferator-activated receptors (PPARs) which are present on the nuclear membrane of cells. Thiazolidinediones enter the cell, bind to the nuclear receptors (PPARγ subtype) and affect the expression of DNA. PPARγ agonists have been documented to ameliorate the painful state in the diabetic neuropathy, the carrageenan-induced inflammation and the spared nerve injury-induced neuropathy*[23, 24, 38]*. However, the potential of PPARγ agonists in the partial sciatic nerve ligation induced neuropathic pain is not explored yet.*

Metformin, an oral anti-diabetic drug in the biguanide class, is the first-line medication for the treatment of type 2 diabetes. In vivo and in vitro studies in the past have demonstrated that metformin stimulates the insulin-induced component of glucose uptake into skeletal muscle and adipocytes in both diabetic individuals and animal models.[25] *Inflammation, including nuclear factor-κB (NF-κB) signalling, is increasingly recognized as a significant contributing factor to diabetes mellitus and several previous studies have found that Metformin inhibits NF-κB signalling, including in vascular tissue and recently in hepatocytes.*[8] *Gabapentin, an anticonvulsant, is used as standard, since it has proven to be a useful adjunct for treating neuropathic pain with minimum side effects. Gabapentin was effective in the treatment of painful diabetic neuropathy, postherpetic neuralgia, and other neuropathic pain syndromes.*[6,7,30] *It relieved symptoms of allodynia, burning pain, shooting pain, and hyperesthesia.*[20,21,22] *Adverse effects were typically mild to moderate and usually subsided within approximately 10 days from the initiation of treatment.*

Animal models: *The majority of currently used neuropathic pain models share alterations in hind-limb cutaneous sensory thresholds following partial injury of a peripheral (usually sciatic) nerve as a common feature.*[1,35]*Neuropathic pain can be induced by the three most commonly used models namely, chronic constriction injury (CCI) of sciatic nerve, the partial sciatic nerve ligation model (PSNL) and the spinal nerve ligation model (SNL).*[1] *Partial injury to somatosensory nerves sometimes causes causalgia in humans which is a neurological syndrome, with a rapid onset of spontaneous constant burning pain exacerbated by several factors such as light touch (allodynia), temperature change, movement of the involved limb, visual and auditory stimuli and emotional disturbances.*[10,11,31,32,33] *The pain in causalgia sometimes spreads beyond the cutaneous distribution of the damaged nerve. The combination of hyperalgesia and allodynia i.e., pain response to low threshold stimuli, characterizes the hyperesthetic state of causalgia.*[9]

Materials and methods

Experimental animals: *Male Wistar rats weighing 250-300g were used for the study. They were housed in the animal cages with free access to water and standard laboratory pellet chow diet.*

Drugs and reagents: *Troglitazone (10 mpk) and Metformin (300 mpk) were administered orally as a suspension in 10% Tween and 90% carboxy methyl cellulose (CMC). Gabapentin (50 mpk) was administered intraperitoneally in 0.9% saline*

Induction of neuropathic pain by PSNL technique*: Peripheral neuropathic pain was induced by PSNL as described by Seltzer et al. 1990. In brief, the rat was deeply anesthetized with ketamine (50mg/kg i.p.) and xylazine (5mg/kg i.p.). The skin of its lateral surface of the left thigh was incised and a cut made directly through the biceps femoris muscle to expose the sciatic nerve and its three terminal branches (the sural, common peroneal and tibial nerves). Thereafter, a tight ligation was created around 33-50% of the sciatic nerve, leaving the rest of the nerve uninjured. The muscle and the skin were closed in two layers. A decrease in blood flow through the vasa nervorum has been implicated in the development of diabetic neuropathy. Vasa nervorum*

are small arteries that provide blood supply to peripheral nerves.[1] Sham controls were performed by exposing the sciatic nerve without inducing any lesion. All the surgical procedures were carried out under normal sterile conditions.

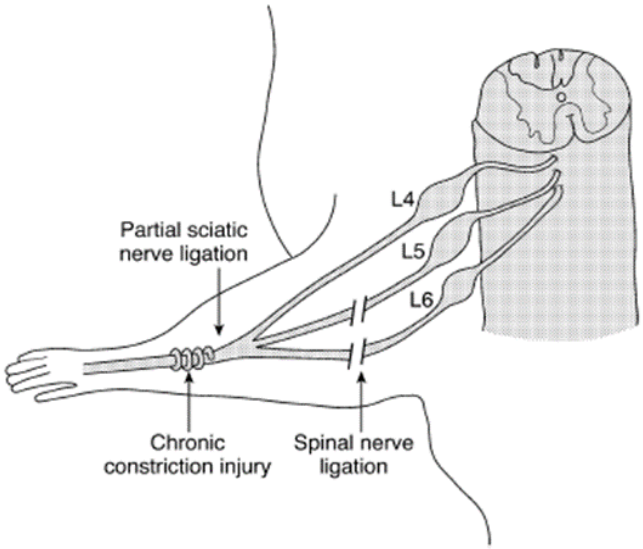

Fig.1 Schematic drawing of the PSNL (tight ligation of 33±50% of the sciatic nerve trunk), CCI (loose ligations of the sciatic nerve trunk), and SNL (tight ligation and transection of the L5 and L6 spinal nerves) animal models of neuropathic pain.[1]

Behavioural examination

Test for mechanical allodynia (von Frey's method): *Mechanical allodynia was assessed by the von Frey's test using von Frey's filaments of different sizes (5, 7, 10, 11, 12 and 15 with forces 9.8, 14.7, 39.2, 78.4, 98 and 637.5 mN respectively). The animal was placed in a chamber with a mesh metal floor, covered by an opaque plastic dome. The dome enabled the animal to walk freely but not to rear up on its hind limbs. This enables the experimenter to reach the plantar surface of the paws from beneath, unobserved by the animal. The filaments were placed perpendicularly onto the hind paw of the animals and pressure was applied by bending the filament to U-shape. The measurements of von Frey's hair pain threshold was done by applying 10 stimuli, each for 6 seconds with 4 seconds interval between each. Effective pressure 50 (EP_{50}) was calculated using Graph pad prism (linear regression method) and compared with control.*

Test for thermal hyperalgesia-Plantar test (Hargreaves's method): *Thermal nociceptive threshold, as a measure of thermal hyperalgesia was assessed by the Hargreaves's apparatus, which is an instrument designed for the measurement of hyperalgesia to thermal stimulation in unrestrained animals. Here, heat stimulation can be applied to the animals without any physical contact between the animal and the heat source.[26, 27] The instrument consists of three parts, an electronic gear, a paw stimulation platform and a light source or test head. The light source consists of three Teflon balls at its bottom, for sliding. The animals were placed in the individual compartments. After an acclimatization period, the animals were tested directly by focusing the hind paw with a light of 1% idle intensity and applying stimulus of 15% active intensity. The nociceptive threshold with respect to licking of the hind paw and jumping response was recorded in seconds. A cut-off time of 20sec was maintained to prevent tissue injury. The paw withdrawal latencies were measured and compared with control.*

Experimental protocol

Grouping of animals was done based on pain assessment. Generally, pain is induced three days after the sciatic nerve ligation. On the third day after ligation, all the animals were subjected to both von Frey's and plantar test (Hargreaves's method). The EP_{50} and the paw withdrawal latencies were measured and compared among individual animals. Then the animals were grouped based on these parameters. Five groups with seven animals each were formed.

Table 1: Grouping of animals

Group	Group size	Dose
Disease Control	7	0.25% T/CMC
Sham Control	7	-
Troglitazone	7	10mpk, p.o.
Metformin	7	300mpk, p.o.
Gabapentin	7	50mpk, i.p.

The day on which grouping of animals was done was considered as day 0. Immediately after grouping, all the animals were dosed. The sham control group of animals were not administered any dose, since they were not subjected to any surgical procedures. The current study was based on chronic dosing or multiple dosing i.e., the animals were administered daily throughout the study period. On day 3, all the animals were subjected to von Frey's test to assess mechanical allodynia and plantar test (Hargreaves's method) to assess thermal hyperalgesia in presence of the test drugs. The EP_{50} values and percentage change in paw withdrawal latencies of right and left paws were calculated and were compared to that of disease control and sham control groups. In a similar fashion, the animals were tested on day 6, day 9 and day 12 of treatment. The EP_{50} values and percentage change in paw withdrawal latencies of right and left paws were calculated.

Statistical analysis

All the results were expressed as mean ± standard error of means (S.E.M.). The data from the behavioural results were statistically analysed by Student's t-test. The P-value <0.05 was considered to be statistically significant.

Table 2: Dose-response effect of Troglitazone and Metformin on mechanical allodynia in PSNL animals (von Frey's test)

Percentage change in 50 % paw withdrawal threshold between right and left paw (Mean± SEM)

Group	Day 0	Day 3	Day 6	Day 9	Day 12
Disease control	84.4±29.1#	110.5±28.3	119±26.9#	84.7±23.8#	90.6±27.7#
Sham control	211.9±14.1	215.3±40.7	252.2±11.7	244.4±19.1	214±25.9
Troglitazone	104.6±30.5	173.7±36.2	239.7±8.6*	190.9±19.1*	178±31.2
Metformin	51.6±26.6	168.5±32.2	202.9±26.5*	187.8±26.1*	181±29.2*
Gabapentin	99±36.1	222.1±18*	216.9±38	203.3±32.5*	210±36.6*

 # p<0.05 Vs sham control (t-test); *p<0.05 Vs Disease control (t-test)

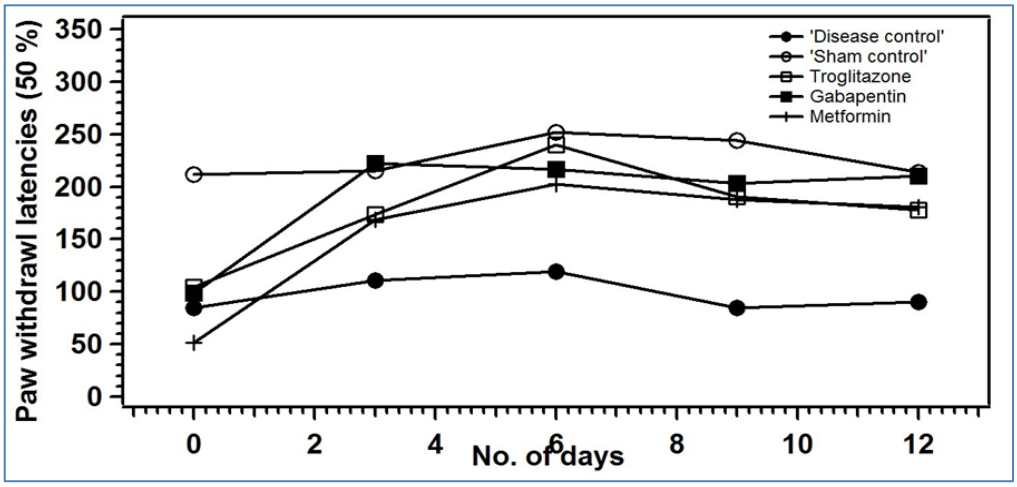

Fig.2 Effect of Troglitazone and Metformin on PSNL induced neuropathic pain in rats (von Frey's method) Troglitazone produced significant effect on mechanical allodynia on day 6 and day 9, Metformin produced significant effect on day 6, day 9 and day 12 and Gabapentin (standard) showed significant effect on day 3, day 9 and day 12 of treatment.

Table 3: Dose-response effect of Troglitazone and Metformin on thermal hyperalgesia in PSNL animals (Hargreaves's test)

Percentage change in paw withdrawal latency of right and left paws (Mean ±SEM)

Group	Day 0	Day 3	Day 6	Day 9	Day 12
Disease control	15.2±6.1	5.8±6	26±6	11.9±9.9	16.9±8.9
Sham control	8.3±8.8	19±8.4	10.7±17.9	-5.6±11.4	16±9.5
Troglitazone	24±4.9	3.6±12	18.3±8.1	8.5±7.7	0.8±0.6
Metformin	18.6±7.4	3.3±12	14.1±3.2	-0.7±6.4	-40.1±30.5
Gabapentin	15.5±13.5	6.1±6.5	16.2±8.5	2.5±7.4	-4.9±9

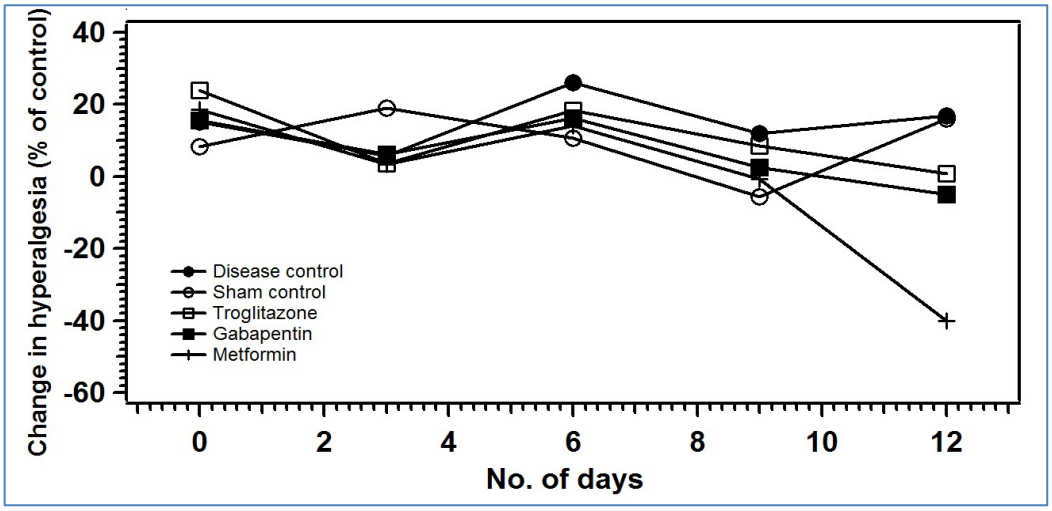

Fig.3 Effect of Troglitazone and Metformin on PSNL induced neuropathic pain in rats ((Hargreaves's method).

Results and Discussion

In the present study, peripheral neuropathic pain was induced using PSNL technique which resulted in a significant development of thermal hyperalgesia and mechanical allodynia. These features of pain appeared from day 3 of the surgery. Then animals were subjected to plantar or Hargreaves's test and von Frey's test to check the intensities of hyperalgesia and allodynia respectively. Then they were grouped based on the pain assessment parameters, and then dosed with the test drugs Troglitazone and Metformin and standard drug Gabapentin in their respective groups. All the animals were given multiple dosing (means, dosing throughout the study period). Then again, the animals were tested in the presence of drugs on day 3, day 6, day 9 and day 12.

Under the von Frey's method of testing mechanical allodynia, the difference in 50% paw withdrawal threshold was recorded in milliNewtons. Troglitazone showed significant effect on day 6 ($p<0.01$) and on day 9 ($p<0.01$); Metformin showed significant effect on day 6 ($p<0.05$), day 9 ($p<0.05$) and day 12 ($p<0.05$) and Gabapentin showed significant effect on day 3 ($p<0.01$), day 9 ($p<0.05$) and day 12 ($p<0.05$).

Under the Hargreaves's method of testing hyperalgesia, the percentage change in paw withdrawal latencies of right and left paws were recorded in seconds. Both the test drugs did not show any significant effect on all days tested ($p>0.05$)

PSNL is the main cause of causalgiform pain disorders in humans. The sciatic nerve provides about 25% of the hind paw's skin sensory innervation. This includes 100% of plantar skin, where most of the sensory tests are performed.

In the current study, we employed von Frey's and Hargreaves's test to assess the two main symptoms of neuropathic pain i.e., allodynia and hyperalgesia respectively, which were developed as a result of PSNL. These responses started from day 3 onwards after the surgery. Animals were grouped based on pain assessment and were subjected to multiple dosing i.e., they were administered daily throughout the study period. All the animals were tested on day 3, day 6, day 9 and day 12 of treatment. The EP_{50} values and percentage change in paw withdrawal latencies of right and left paws were calculated and the results were expressed as mean ± S.E.M. then comparison of results was done among different groups. The animals in the sham control group showed higher EP_{50} values, which indicates that the pain produced was either very less or there was no pain produced at all. This suggests that, the pain produced in the other groups was because of a sciatic nerve injury rather than tissue damage. In the von Frey's test conducted to assess mechanical allodynia, Troglitazone produced higher EP_{50} values on day6 and day9, Metformin showed higher EP_{50} values on day 6, day 9 and day 12 of the study. Gabapentin which was taken as the standard compound showed higher EP_{50} values on day 3, day9 and day12 of treatment.

Conclusion

Based on above study, the plantar test (Hargreaves's test), both the drugs did not show any significant effect ($p>0.05$) but in the von Frey's test, the drugs showed higher EP_{50} values on day 3, day 6, day 9 and day 12 of the study. In conclusion, the anti-diabetic drugs Troglitazone and Metformin showed the ability to attenuate neuropathic pain associated with PSNL, which may be attributed to peripheral anti-inflammatory actions, apart from their antidiabetic activity.

Competing interests

None declared.

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