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Original Article

# Effects of Anticonvulsant Retigabine on Pain Hypersensitivity Diabetic Rats with Neuropathy

**Marwan Abdulrhman Alshaikh Husian[1], Mohammed Salman Alnajjad[1], Marwan Ahmad Alrayhan[1], Hamzah Mohammed Alarfaj[1], Abdulrhman Mohammed Aljoher[1], Mohammed Abdulrhman Alshaikh Husian[2]**

[1]Medical Intern, King Faisal University, Alahsa, Saudi Arabia, [2]Medical Intern, Department of ICU, King Fahad Hospital Hufuf, Alahsa, Saudi Arabia

## Abstract

**Background:** Diabetes is a chronic condition that affects millions of the world population. One of the most serious complications of diabetes is a neuropathic pain (pain due to nerve injury/dysfunction) that affects about 30–55% of diabetic patients. This nerve dysfunction can lead to numbness, weakness, and spontaneous/ongoing (stimulus independent) pain, and stimulus-evoked (allodynia and hyperalgesia) pain. This pain hypersensitivity is believed to be due to neuronal hyperexcitability. However, the mechanism of this neuronal hyperexcitability is unknown.

**Aims:** Therefore, the aim of this research work was to examine the hypothesis that a subtype of $K^+$ channels known as Kv7 channels (that play an important role in controlling hyperexcitability) is involved in the pathophysiology of diabetic neuropathy.

**Methods and Results:** To examine this hypothesis, we use Darat model of diabetic neuropathic pain (DNP), known as streptozotocin (STZ) model (which involves an injection of 60 mg/kg of STZ; i.p), STZ is a toxin to pancreaticβ-cells that release insulin. We used this model to examine the effects of activating KV7 channels with retigabine (10 mg/kg) on behavioral signs of mechanical allodynia and heat hyperlagisa in the diabetic (STZ treated) rats. Our results show that retigabine (as elective KV7 channel activator that has recently been licensed for treating partial-onset seizures (epilepsy), which, like DNP, is characterized by neuronal hyperexcitability) significantly reduced both allodynia and hyperalgesia.

**Conclusions:** These findings indicate that retigabine may represent a potential therapeutic alternative for DNP. However, further investigations are needed because of the small number of animals tested.

**Key words:** Anticonvulsant, Hypersensitivity, Pain

## INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders resulting in hyperglycemia due to an absolute or relative reduction in insulin production or its action. The chronic state of this disorder will result in multiple serious complications, end organ damage and dysfunction in, i.e., retina, kidneys, cardiovascular system, and nervous system. The International Diabetes Federation in 2011 estimated an overall prevalence of DM to be 366 million, and it is expected to raise to 552 million by 2030. DM has many complications, one of which is neuropathic pain (nerve pain).[1] It is estimated that 28–55% of diabetic patients develop neuropathic pain.[2] Normally, pain is caused directly by noxious stimuli on nerve fiber endings that sense pain or by inflammatory mediators in damaged tissue, but the neuropathic pain is caused by a primary neuronal injury or dysfunction.[3] The nerve dysfunction can cause numbness, weakness, and spontaneous pain as well as stimulus-evoked pain. The pain is spontaneous and sometimes continues, and it has the character of burning, shooting, or shock.[4] The stimulus-evoked pain includes allodynia and hyperalgesia. Allodynia is pain triggered by stimuli that are normally non-painful, and it is induced by very light stimulation, such as a wind or skin contact with clothing while hyperalgesia is increased pain caused by stimuli that are normally painful.[5]

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**Corresponding Author:** Marwan Abdulrhman Alshaikh Husian, Medical Intern, King Faisal University, Alahsa, Saudi Arabia. Phone: +966508111255. E-mail: dr.marwan.sh@live.com

There are many etiologies of neuropathic pain, such as infection, inflammation, trauma, malignancy, and metabolic disorders such as diabetes, neurotoxins, and neuronal compression.[6] Pain is normally perceived when peripheral sensory nerves and neurons, i.e., in the skin, transmit impulses to the brain. Pain signals are normally generated only when there is actual or threatened tissue damage, but in the case of chronic pain conditions such as during diabetic neuropathy, there is the spontaneous activity of these nerves or neurons so that pain can be felt without any external stimulus. Nerve impulses, also called action potentials, are generated by protein molecules that represent ion channels that regulate the flow of ions mainly sodium (Na+) and potassium (K+) across the neuronal membrane. When Na+ ions move inward by activation of Na+ channels, the neuron becomes more positive inside, and its tendency to transmit nerve impulses is increased. In contrast, when K+ ions move outward by activation of K+ channels, the neuron becomes more negative inside, and its probability of generating nerve impulses is reduced.

There is evidence that during neuropathic pain states, there is an increase in transcription and axonal trafficking of Na+ channels as well as a reduction in expression of K+ channels, including Kv7 channels.[5] The Kv7 channels play an important role in controlling hyperexcitability of neurons (generating more impulse than normal) and normally act as a "brake" on neuronal excitability. However, because of the decrease in the expression of Kv7 channels[7] and increase in Na+ channels during chronic pain states, the neurons become hyperexcitable and generate ectopic activity, i.e., an abnormal activity that is spontaneously generated in an abnormal site.[5]

Although there are some palliative treatments for neuropathic pain that aim to reduce the pain,[8] successful therapy for the debilitating condition of diabetic neuropathic pain (DNP) remains a challenge because the currently available drugs are ineffective and have significant side effects. Therefore, the current study is aimed at assessing the effects of retigabine, a selective activator of Kv7 channels on pain hypersensitivity in diabetic rats with neuropathy. Retigabine has high efficacy and good tolerability in treating adults with partial seizures,[9] a condition, like DNP, characterized by neuronal hyperexcitability. In addition, it has been suggested that retigabine, through specific activation of neuronal KCNQ/Kv7 channels, may have therapeutic potential for neuropathic pain based on findings using a rat model of temporomandibular disorders.[10] Therefore, retigabine may also have therapeutic potential in treating DNP.

## MATERIALS AND METHODS

Twelve male Sprague Dawley rats (250–300 g weight) were used for pain behavioral testing (see below).

### Induction of DM in Rats

Rats were used for induction of diabetic neuropathy. To induce this model, the rats were injected intraperitoneally with 60 mg/kg streptozotocin (STZ), STZ is a toxin to pancreatic β-cells that release insulin. The severity of the induced DM was detected daily by measuring body weights, clinical manifestations, and blood sugar concentration. The observation period was up to 4 weeks. Rats were classed as diabetic if their blood glucose level was >250 mg/dL.

### Drug Administration

Retigabine, the selective activator of kv7 channels was injected subcutaneously into the rat's hind paw to examine the effects activating KV7 channels on pain hypersensitivity in the STZ diabetic model. Retigabine was dissolved in tween 80 and physiological saline (Sigma, St. Louis, MO, USA). The mixture of the vehicle in the present work contained tween 80 and physiological saline in 1:9 ratio (v/v). The drug solutions were given at a volume of 10 μl/kg to the rats intraperitoneally.

### Behavioral Tests

There are many signs represent neuropathic pain behavior in animals of which two types of evoked pain behavior were studied as signs of neuropathic pain. These were mechanical hypersensitivity/allodynia (decreased withdrawal threshold to mechanical force) and heat hypersensitivity/hyperalgesia (decreased withdrawal latency to a noxious stimulus).

An automated von Frey type system known as a dynamic plantar esthesiometer touch stimulator (Ugo Basile, Comerio, Italy) was used to examine the presence of behavioral signs of mechanical allodynia. Mechanical allodynia was indicated by decreased withdrawal thresholds to pressure. This mechanical force (pressure) was applied to the mid-plantar aspect of the hind paw with a blunt metal filament, through an elevated mesh. The rats were placed in plastic chambers on a wire mesh table, and tests were performed after the animals were acclimatized for about 30 min. The mechanical force applied to the rat's hind paw increased gradually to 50 g until the rat showed a withdrawal response. The force (in grams) that elicited a withdrawal response was automatically displayed and recorded. To prevent any tissue damage a cutoff of 50 g was imposed. An interval of 2–3 min between subsequent investigations on the same hind paw was allowed. Allodynia was considered when there was a decrease in withdrawal threshold to the mechanical force. The average of four latency measurements for each hind paw was taken.

Heat hyperalgesia was indicated as reported previously[11] by reduced paw withdrawal latency to a noxious heat stimulus applied to the plantar aspect of the hind paw using a planter (Hargreaves) analgesy-meter (Ugo Basile, Comerio, Italy). A laser radiant heat source was placed under 2-mm thick glass floor on which each rat was placed. Once the stimulus began a timer was activated, and the timer stopped automatically once a photocell detected a withdrawal response. During each session, an average of three latency measurements for each hind paw was taken. An interval of 5 min between successive stimuli on the same hind paw was allowed to reduce the possibility of sensitization.

### Data Analysis

Statistical analysis was performed using IBM SPSS statistics software (the 19th edition, USA) for windows. Most of the data showed normal distribution; they are hence presented as a mean ± standard error of the mean. Comparison between pre-drug and post-drug mean values was made using a paired *t*-test. $P < 0.05$ was used as the criterion of statistical significance.

## RESULTS

To confirm that rats treated with streptozotocin (STZ) developed pain behaviors of mechanical and heat hypersensitivity (allodynia and hyperalgesia), we compared pain behavior values 4 weeks after induction of diabetes with pretreatment (baseline) values. A significant reduction in the mean paw withdrawal threshold (MWT) from a mechanical stimulus [Figure 1], or in the mean paw withdrawal latency (HWL) from a noxious heat stimulus [Figure 2] was taken as indicators of mechanical allodynia and heat hyperalgesia, respectively. These results indicate that STZ rats developed DNP.

Having established that the STZ rats developed DNP, we then examined the effects of activating KV7 channels with retigabine, a selective blocker of Kv7 channels, on behavioral signs of mechanical allodynia and heat hyperalgesia in these diabetic rats (diabetic neuropathy model). We compared pain behavior values 2–3 h after retigabine injection with those of vehicle (0.9% physiological saline). 12 rats were used for testing heat hyperalgesia and mechanical allodynia. Six rats were injected with retigabine and the other six injected with vehicle for each test. The results show that retigabine caused a significant increase in both the MWT ($P < 0.01$) and in the mean paw withdrawal latency ($P = 0.045$) [Figure 1], indicating that retigabine reduced mechanical allodynia ($P < 0.01$) and heat hyperalgesia ($P = 0.045$). The average mean of withdrawal threshold (mechanical allodynia) in rats treated with the vehicle was 22.15 g

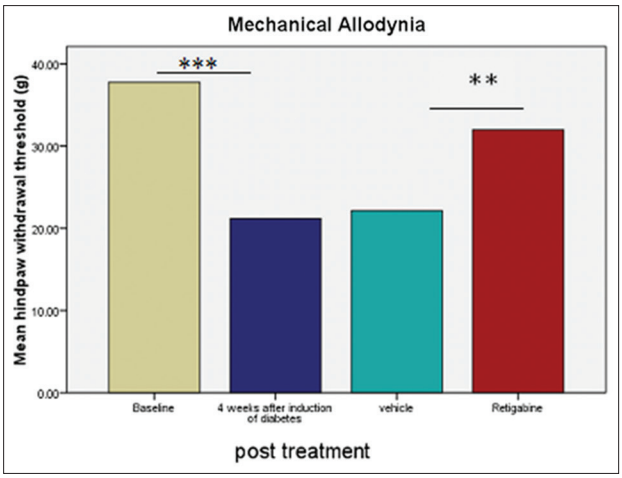


**Figure 1: Effects of retigabine on mean hind paw withdrawal threshold in rats 4 weeks after streptozotocin treatment. Baseline: Normal rats without diabetic neuropathy, vehicle: 2–3 h after vehicle injection as compared 2–3 h after retigabine injection, ***$P < 0.001$, **$P \leq 0.01$**

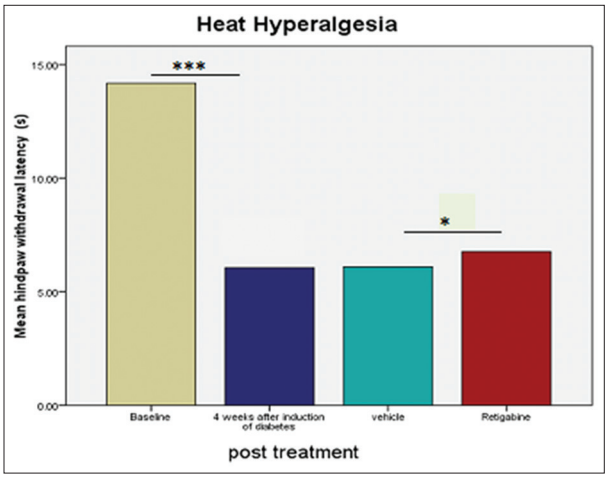


**Figure 2: Effects of retigabine on mean hind paw withdrawal latency in rats 4 weeks after streptozotocin treatment. Baseline: Normal rats without diabetic neuropathy, vehicle: 2–3 h after vehicle injection as compared to 2–3 h after retigabine injection, ***$P < 0.001$, *$P < 0.05$**

compared to 31.98 g caused by retigabine injection. The mean withdrawal latency in rats treated with retigabine was longer than that of vehicle rats (6.76 vs. 6.09 s) [Figure 1].

## DISCUSSION

The main aim of the current work was to examine if activating Kv7 channels with the anticonvulsant retigabine, blocks or reduces pain hypersensitivity in diabetic rats with neuropathic pain (DNP). Interestingly our results show that activation of these channels by subcutaneous injection of retigabine into the rat's hind paw reduced the behavioral signs of both mechanical allodynia and heat hyperalgesia.

As mentioned in the introduction, neuropathic pain is a serious complication of DM that has a negative impact on the nervous system causing hyperalgesia, allodynia, and spontaneous/ongoing pain in human patients. A few animal models of DNP have been developed to investigate its pathophysiology[12] including the widely used STZ rat model, which involves the injection of STZ (60 mg/kg, i.p.). The interesting thing about this model is that the pain hypersensitivity that is manifested in human patients with DNP is also manifested in this rat model of DNP. Therefore, we used this model to assess the effects, on pain hypersensitivity in this model, of the anticonvulsant retigabine, a selective agonist of the KV7 channels (which play a key role in controlling neuronal hyperexcitability)[7] and which are believed to be involved in chronic pain states (see introduction).

To the best of our knowledge, there have not been any previous studies evaluating the effects of retigabine on DNP. However, there was a study that evaluated the analgesic effect of retigabine on temporomandibular joint pain.[10] This study suggested that retigabine may be therapeutically beneficial for temporomandibular joint pain[10] through suppression of central hyperexcitability by specific activation of neuronal KCNQ/Kv7, thus supporting our findings. Moreover, retigabine may prove to be useful in the treatment of a diverse range of disease states in which neuronal hyperexcitability is a common causative factor according to a stud.[13] In addition, another research showed that retigabine had high efficacy and good tolerability in treating human adults with refractory partial seizures.[9] Taken these studies together, retigabine has good potential in alleviating DNP.

## CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study together with the previous findings of animal studies using other models of chronic pain suggest that retigabine may alleviate hyperalgesia and allodynia associated with chronic pain conditions including diabetic neuropathy. However, further investigations are needed because of the small number of rats that were used in the present experiments. It will also be interesting to see whether lower doses of retigabine would also alleviate pain hypersensitivity associated with DNP. These findings are not unexpected given that retigabine has recently been released to market for treatment of epilepsy which, like DNP, is characterized by neuronal hyperexcitability.

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