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ACTIVATION OF KV7 (POTASSIUM CHANNELS) REDUCES PAIN HYPERSENSITIVITY IN A RAT MODEL OF CHEMOTHERAPY-INDUCED NEUROPATHY

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Abstract:

Background: *Most chemotherapeutic agents have serious side effects that can lead to dose reduction or discontinuation of treatment. One of which is damage to the peripheral nervous system. This Neurotoxicity is usually accompanied by chronic peripheral pain mainly in upper and lower extremities. Mechanisms of such chemotherapy-induced neuropathic pain (CINP) are poorly understood, but many researches have suggested that it is directly related to hyperexcitability of peripheral neurons. Many ion channels are believed to be involved in this hyperexcitability, including Kv7, a subtype of voltage gated potassium channels.*

Aim: The aim of this stud was to test whether retigabine, a specific Kv7 opener used to treat epilepsy, which is also characterized by hyperexcitability, is effective in reducing CINP.

Methods: *CINP was induced by injecting Paclitaxel (2 mg/kg) (a common anti-cancer drug) 4 times to male Sprague Dawley rats (n=12). Two evoked pain behaviors were assessed, which are mechanical hypersensitivity/allodynia, and heat hypersensitivity/hyperalgesia. Retigabine (7 mg/kg) was then injected to test its effectiveness in reducing pain hypersensitivity.*

Results: *IP administration of Retigabine had an analgesic effect on paclitaxel-induced mechanical allodynia in the rat model of CINP. Paclitaxel produced a significant reduction in withdrawal response threshold compared to the baseline latency, but after administration of retigabine the withdrawal threshold went back towards normal. However, paclitaxel did not show increase in heat sensitivity in this model.*

Conclusion: *Our findings suggest that Kv7 channels are a good target for developing new analgesics to treat CINP.*

Keywords: *Neuropathy; pain; chemotherapy; Paclitaxel; Retigabine; CINP; Kv7.*

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INTRODUCTION:

Cancer is one of the most common causes of deaths worldwide. It kills millions of people annually. There are a lot of ways to treat cancer, one of which is chemotherapy. Cancer chemotherapy has been used for many years and has been effective in treating different types of cancer. However, most of the commonly used chemotherapeutic agents have serious side effects that can lead to dose reductions or early discontinuation of chemotherapy, reducing the efficacy of cancer treatments (1).

One of These major side effects of chemotherapy is neurotoxicity that may involve many parts of the nervous system, particularly peripheral nervous system (1). As a result of the damage to the sensory nerves, several symptoms appear. Some of these are known as positive symptoms, such as allodynia (pain in response to normally non-painful stimuli), hyperalgesia (hypersensitivity to normally painful stimuli) and spontaneous pain (pain with no stimuli) (1). Other symptoms are called negative symptoms and include abnormal sensations such as numbness and weakness (1). These symptoms are usually symmetrical but can be more severe in one side more than the other (2). These symptoms are more evident in lower and upper extremities, but they usually start in the feet (2). Analgesic and neurotropic drugs are not very effective for treating these symptoms (1). These clinical symptoms of chemotherapy-induced neuropathic pain (CINP) may occur early in treatment, but may continue to be disabling, even several months or years after treatment cessation (3). The prevalence of CINP varies from 10% to 100% depending on a number of factors including the dose and type of the chemotherapeutic agent used, treatment duration and pre-existing neuropathy of other origin, such as diabetes (3).

There are no very effective drugs for treating or preventing CIPN (4) probably because its pathophysiology is poorly understood. Therefore, is important to understand the pathophysiological processes that underlie persistent pain in the debilitating condition of CINP, in order to devise appropriate alleviative or curative strategies.

Many mechanisms have been suggested as possible causes of the peripheral neuropathic damage (2). One of which is hyperexcitability of sensory neurons that leads to increased nerve impulses (action potentials) to the brain and thus, causing pain with little to no stimuli (2).It is believed that abnormalities in ion channels, including sodium, calcium, and potassium channels are involved in the hyperexcitability of sensory neurons, (2). One of the potassium channels that may be involved in this hyper excitability is a voltage-gated K+ channels known as Kv7 channels(5). This is because these ion channels are active (open) near the resting membrane potential and they normally clamp/stabilize the membrane potential near the resting level.

It has been shown that activating the KV7 channels with Retigabine, is effective in treating a type of epilepsy, which is also characterized by neuronal hyperexcitability. (5) Therefore. The aim of this present study was to test whether Retigabine would reduce pain hypersensitivity associated with CINP.

MATERIALS AND METHODS:

Twelve male Sprague Dawley rats (250-300g weight) were used in the present experiments. These rats were divided into 2 groups (see below): retigabine group (n=6) and vehicle group (n=6). All the rats were treated with Paclitaxel (Taxol) to induce chemotherapy-induced neuropathic pain (CINP) and they were used for pain behavioral testing (see below).

Animal model of chemotherapy-induced neuropathic pain (CINP):

Several animal models of CINP have been developed to investigate its pathophysiology (3), including the Paclitaxel model, which involves an i.p. injection of Paclitaxel (2 mg/kg) on four alternate days, as described previously (6). CINP was induced in 12 male Sprague Dawley rats by intraperitoneal (i.p) injection of Paclitaxel (2 mg/kg).

Pain behavioral testing

Two forms of evoked pain behaviors were used as described previously (7-11). These were mechanical hypersensitivity (mechanical allodynia) and heat hypersensitivity (heat hyperalgesia). Before conducting this testing, rats were acclimatized for 7 days and their postures for standing, walking, and resting were monitored daily up to 7 days post operation.

1. **Mechanical hypersensitivity (Allodynia):**

mechanical allodynia was assessed as described previously (7-11). To assess for this behavior, rats were placed in plastic boxes on an elevated mush floor and a blunt metal filament was applied to the mid-plantar surface of the rat`s hind paw, using an automated von Frey type system known as a dynamic plantar esthesiometer touch stimulator (Ugo Basile, Italy). The mechanical force that was applied increased from 0 to 50g over 15-seconds period. The mechanical force applied to the rat's hindpaw increased gradually until the rat showed a withdrawal response. The force increased from 0 to 50g over a 15

seconds period and a cutoff of 50 g was imposed to prevent tissue damage. This force (in grams) was recorded and displayed automatically. Each paw was tested 4 times and the average force was calculated and considered as a paw withdrawal threshold. A time interval of at least 1 minute between each trial was allowed. Mechanical allodynia in Taxol treated rats was indicated by decreased withdrawal thresholds to pressure (mechanical force).

2. Heat hypersensitivity (hyperalgesia):

heat hyperalgesia was indicated as described previously (7-11) using a planter (Hargreaves) analgesymeter (Ugo Basil, Comerio, Italy) (7). The test was conducted by placing each rat in a chamber on a glass floor (2-mm-of thickness) under which the heat source was applied. Testing started by pressing a timer which automatically stopped upon withdrawal response. Each paw was tested 3 times and the average force was calculated and considered as a paw withdrawal latency. At least 5 min intervals between sequential trials were allowed to avoid the possibility of sensitization. Hyperalgesia was indicted in Paclitaxel rats by decreased withdrawal latency to heat stimuli.

DRUGS:

Two drugs were used in the present experiments. These were Paclitaxel to induce CINP (see above) and Retigabine to activate Kv7 channels and to determine its effect on pain hypersensitivity in Paclitaxel rats.

Paclitaxel solution was prepared as described previously (12). A stock solution (6 mg/ml in Cremophor/EL) of Paclitaxel was diluted with saline to a concentration of 2 mg/ml and injected IP at 2 mg/kg on four alternate days (D0, D2, D4 and D6) for a cumulative dose of 8 mg/kg as described previously (12). Control rats received matched injections of the vehicle.

Retigabine, was injected subcutaneously (7 mg/kg) into the rat`s hind paw to examine the effects of pharmacologically activating KCNQ/KV7 channels on pain hypersensitivity in the Paclitaxel injected rats. Retigabine was dissolved in tween 80 and physiological saline (Sigma, St. Louis, MO, USA). The mixture for the vehicle used in this study contained tween 80 and saline (0.9% NaCl, autoclaved before use) in 1:9 ratio (v/v). The drug solutions were administered at volume of 10 ml per kilogram to the rats intraperitoneally.

DATA ANALYSIS:

Rats were divided into 2 groups: Retigabine group (n=6) and vehicle group (n=6). Statistical analysis was performed using IBM SPSS statistics software (the 17th edition, USA) for windows. The data groups were normally distributed; they are therefore presented as mean ± SEM. Comparison between pre-drug and post-drug mean values was made using paired t-test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS:

12 rats were used to test whether Retigabine is effective for treating CINP or not. After 4 weeks of Paclitaxel injection, 6 rats were injected with Retigabine solution and the other 6 were injected with only the vehicle for control. Then, several tests were done to them.

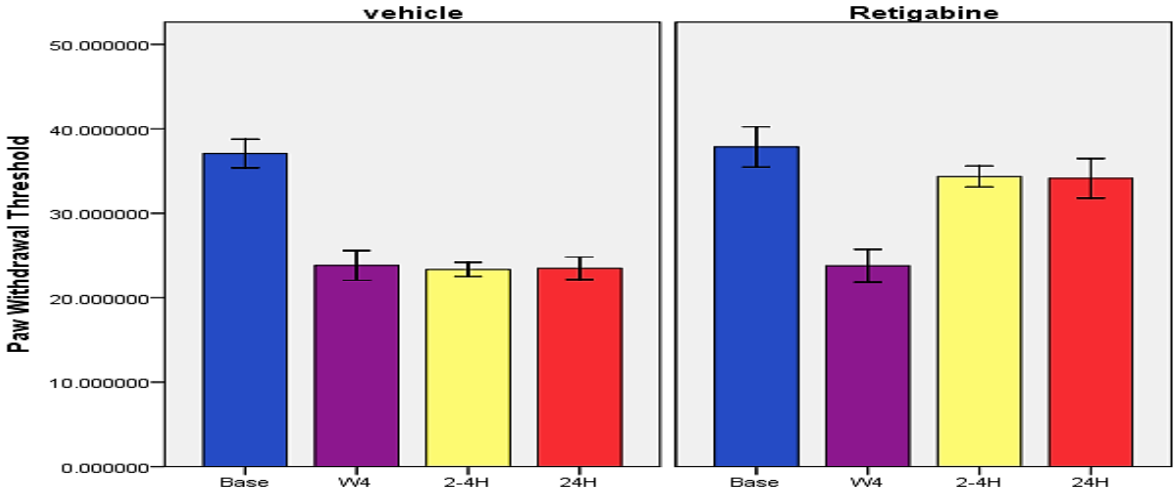

*Figure 1. Effect of Retigabine on Paclitaxel induced mechanical allodynia. The bars represent the mean ± S.E.M of values obtained from 6 animals. (a) The bars represent the control group (6 rats) that were injected with only the vehicle. (b) The bars represent the Retigabine group (6 rats) that injected with Retigabine solution. *** = P value < 0.001. ** = P value < 0.01. Base stands for the baseline threshold. W4 stands for 4 weeks after Paclitaxel injection. 2-4H stands for 2-4 hours after vehicle/Retigabine injection. 24H stands for 24 hours after vehicle/Retigabine injection.*

Mechanical Allodynia effect of Paclitaxel. Mechanical Allodynia was indicted in Paclitaxel rats by decreased withdrawal threshold to mechanical stimuli. After 4 weeks of Paclitaxel administration, the mechanical sensitivity was measured by timing the paw withdrawal response and was compared to the baseline latency using a dynamic plantar esthesiometer. Paclitaxel produced a significant reduction in withdrawal response threshold compared to the baseline latency (P value < 0.001 figure 2). The withdrawal threshold became 23.801 ± 1.251 grams, whereas the withdrawal threshold was 37.464 ± 1.399 grams before paclitaxel injection as shown in figure2. That means the rats s howed significant signs of mechanical allodynia.

Treatment of Paclitaxel induced mechanical allodynia with Reaigabine solution.

Injection of Retigabine was dissolved in saline and tween 80 and was administered subcutaneously in the rats' hind paw. The mixture was injected only once at a dose of 10ml/kg into 6 rats while the other 6 were injected with only the vehicle. After 2-4 hours and after 24 hours of Reaigabine injection, the mechanical responses were tested. The group that is injected with only the vehicle showed no changes in the paw withdrawal threshold (P value > 0.05 figure 1). On the other hand, the group that is injected with showed a significant increase in the paw withdrawal threshold (P value < 0.01 figure 1). The withdrawal threshold became 34.362 ± 1.243 grams after 2-4 hours and 34.137 ± 2.345 grams, whereas the withdrawal threshold was 23.801 ± 1.251 grams after 4 weeks of paclitaxel injection as shown in figure 1b.

Heat hyperalgysia assessment of Paclitaxel.

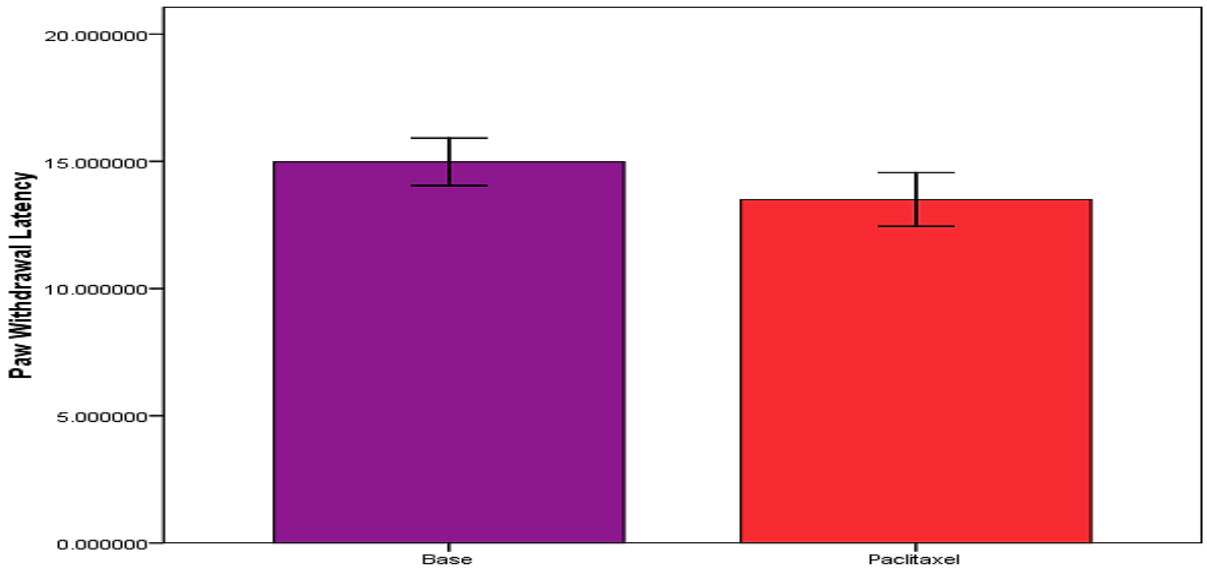

Figure 2. Effect ofF Paclitaxel on heat sensitivty of rats. Each bar represents the mean ± S.E.M of values obtained from 6 animals. This is a comparison between the withdrawal baseline latency and the wthdrawal latency after Paclitaxel. Base stands for the baseline latency (in seconds). Paclitaxel stands for the withdrawal latency after 4 weeks of Paclitaxel injection (in seconds).

Hyperalgesia was indicated in Paclitaxel injected rats by decreased withdrawal latency to heat stimuli. Paclitaxel was injected intraperitoneally at a concentration of 2 mg/ml and a dose of 2 mg/kg in four alternate days. After four weeks of Paclitaxel injection, the heat sensitivity was measured by timing the paw withdrawal response and was compared to the baseline withdrawal latency using a planter analgesymeter. The rats didn't show significant changes in paw withdrawal threshold compared to the baseline latency before Paclitaxel injection (P value > 0.05 figure 2). The withdrawal latency became 13.5 ± 1.056 seconds, whereas the withdrawal latency was 14.983 ± 0.932 seconds before paclitaxel injection as shown in figure2. That means they didn't show significant heat hyperalgesia.

DISCUSSION:

This present study was aimed at testing whether an anticonvulsant drug, Retigabine, reduces pain hypersensitivity in a rat model of chemotherapy induced neuropathic pain (CINP). The findings of the current study demonstrate that Retigabine reduced mechanical hypersensitivity induced by the chemotherapeutic agent, paclitaxel.

Retigabine has been shown in other studies to have anticonvulsant effects for treating epilepsy (5) which, like CINP is characterized by neuronal hyperexcitability. Our findings that Retigabine reduced mechanical hypersensitivity in the paclitaxel model of CINP are in agreement with a recent study showing that other anticonvulsant drugs, such as E139 have ant nociceptive effects in rodent models of chemotherapy induced neuropathic pain (15). Our results are also in agreement with previous studies showing that Retigabine has antinociceptive effect on other models of chronic pain (12, 14).

In the present, study we have shown that paclitaxel significantly reduced the withdrawal response threshold of the rats caused by mechanical stimuli indicating that this anticancer drug reduces mechanical hypersensitivity. These results are in agreement with previous studies which suggest that paaclitaxel significantly reduce the paw withdrawal threshold on the same and on different animal models of chronic pain (12, 15). On the other hand, our study shows that paclitaxel did not significantly increase the paw heat sensitivity (paw withdrawal latency caused by heat stimuli), a finding that is in agreement with a previous study showing that paclitaxel does not significantly increase heat sensitivity in the same animal model (12). However, there is another research that shows that paclitaxel does increase heat

sensitivity, but it was done on a mouse model of CINP (15).

CONCLUSION:

This present study shows that Retigabine has therapeutic potential for treating chemotherapy induced neuropathic pain. It also suggests that Kv7 channels are a good target for developing new analgesics to treat chemotherapy induced neuropathy, and thus stop the discontinuation of chemotherapy treatment for patients who can't tolerate it. By doing that, patients can continue their lives as usual.

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