THE ROLE OF TRPV4/P38 SIGNALING PATHWAY IN DIABETIC NEUROPATHIC PAIN

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ABSTRACT

Introduction*: Diabetic neuropathy is one of the most common and the most disabling of diabetes mellitus chronic complications. The purpose of this study is to investigate the role of transient receptor potential vanilloid (TRPV4) in diabetic neuralgia and its potential mechanism.*

Materials and methods*: Male Wistar rats (180-200g) were used to construct diabetic models using streptozotocin (STZ). Western blot analysis was used to detect the expression of protein TRPV4, P38 and p-P38. The assessment of mechanical allodynia was evaluated using paw withdrawal threshold (PWT). The assessment of thermal cold allodynia was evaluated using paw withdrawal latency (PWL).*

Results*: Our results showed that the mechanical and thermal cold pain thresholds of STZ-induced diabetic rats (DM group) were both significantly lower than that of the control group from the week-2. Inhibitory of TRPV4 was accompanied with the decreased expression of p38 and P-p38 protein. Inhibition of TRPV4 had no effect on serum glucose, lipid parameters and body weight in diabetic rats, while had no effect on serum glucose, lipid parameters and body weight in diabetic rats.*

Conclusion*: Our data revealed that TRPV4 plays an important role in regulating neuropathic pain in diabetic rats, providing more evidence for the treatment of peripheral neuropathy in clinical diabetic patients, and providing new insights for its treatment.*

Keywords: *Diabetic neuropathic pain; TRPV4; P38; PWT; PWL.*

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Introduction

Diabetic neuropathy, as one of the most common and disabling chronic complications of diabetes, has a prevalence ranging from 10% to 95%[1,2]. The most common type of diabetic neuropathy is peripheral neuropathy, often manifested as symmetrical numbness of distal limbs[3]. Some patients will experience abnormal pain, including spontaneous pain, hyperalgesia (severe pain caused by mild stimulation) and hyperalgesia (harmless stimulation such as light, touch can also cause obvious pain), which is called diabetic neuropathic pain[4].

At present, the mechanism of neuropathic pain in diabetes mellitus is not completely clear, so there is no radical treatment, and symptomatic pain relief is still the main treatment.

The rat model of sustained compression of dorsal root ganglion (DRG) is a typical neuropathic pain model in experimental study[5]. There are many ion channels on the membrane of DRGs neurons[6]. The transient receptor potential vanilloid (TRPV4) in the transient receptor potential ion channel family, one of the transmembrane ion channels, has attracted considerable attention in recent years[7,8]. Studies have shown that TRPV4 is

involved in the process of neuropathic pain[9,10]. But whether it plays a role in diabetic neuropathic pain and its mechanism is unclear

In this study, we found that TRPV4 expression was significantly increased in L4-6 DRGs of diabetic rats. We also demonstrated that inhibition of TRPV4 may alleviate the mechanical allodynia in diabetic rats via p38 pathway. These results provide a new perspective for the treatment of diabetic neuropathic pain.

Materials and methods

Animals

Male Wistar rats (180-200g) were purchased from the Experimental Animal Center of Shanghai No. 6 People's Hospital (Shanghai, China). In vivo studies were approved by the Ethics Committee of the Shanghai No. 6 People's Hospital. The animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Streptozotocin (STZ) was used to induce diabetes mellitus as previously described11. In brief, STZ was dissolved in 1% citrate solution and injected intraperitoneally at a dose of 70 mg/kg. The same volume of citrate buffer was injected intraperitoneally in the control group. Diabetes induction was confirmed by assessment of fast blood glucose 3 days after STZ injection (>15.0mmol/L meant successful).

In order to assess the role of the TRPV4 channel on the development of mechanical and cold allodynia, HC-067047, a selective TRPV4 channel antagonist was subcutaneously injected once a day, at the dose of 1 mg/kg after diabetes induction. At the same time, vehicle (1% DMSO and 0.25% Tween 80 in saline solution) was injected instead in the control rats. The serum glucose, lipid parameters and body weight were monitored once a week in rats using commercial kits until tissue samples were taken.

Assessment of mechanical allodynia

The assessment of mechanical allodynia was evaluated using paw withdrawal threshold (PWT). Rats were put in a glass box (22 cm *12 cm *22 cm) which placed on a metal screen. After 30 minutes of silence, von Frey filament was used to stimulate the middle part of the plantar of the hind limb operation side of rats vertically for a duration of less than 4 seconds. The behavior of raising or licking the foot of rats was regarded as a positive reaction, whereas the negative reaction. The test begins at 6 g. When the stimulus could not cause positive reaction, the stimulus of adjacent large first-order intensity was given. Oppositely, if the positive reaction occurred, the stimulus of adjacent small first-order intensity was given. This continued until the junction of positive and negative reactions appeared. Then Four consecutive measurements were made, with an interval of about 2 minutes. Ultimately, the lowest intensity of the positive reaction of more than three times in five consecutive needling sessions was shown as the PWT of the mouse. In order to avoid the injury of rat sole, 15g was the maximum strength.

Assessment of thermal cold allodynia

The assessment of thermal cold allodynia was evaluated using paw withdrawal latency (PWL). Rats were put in a glass box which placed on a 3 mm thick glass plate and the soles of rats were irradiated with a heat stimulator according to Hargreaves method. The PWL was observed from the beginning of irradiation to the occurrence of leg-raising avoidance in rats. The automatic cut-off time was 20 seconds to prevent tissue damage. Each rat was measured five times at intervals of 3 minutes, and the average value of three times was the rat's PWL value.

Western blot analysis

Protein was extracted from Dorsal Root Ganglion (DRG) and lysed in Ripa buffer. The same amount of protein was exposed to 12% of SDS-PAGE gel and transferred to PVDF membrane. TRPV4, p38 and p-p38 were as the primary antibodies and GAPDH was as the control.

Statistical analysis

All data were presented as mean ± SD by using SPSS 17.0 (SPSS ver. 17, SPSS Inc., Chicago IL, USA). Comparisons were assessed using one-way ANOVA and $p<0.05$ was considered statistically significant.

Results

Comparison of serum glucose, lipid parameters and body weight between the two groups

The serum glucose level of diabetic rats was significantly higher than that of the control group after successful modeling. The TC and TG levels of diabetic rats increased significantly compared to

the control group. The weight gain of control group was remarkably higher than that of the DM group from the initial (0 weeks) to the final (4 weeks), as shown in Table 1.

Group	Glucose (mM)					TG (mM)	TC (mM)	Weight gain (g)
	PRE	Week 1	Week 2	Week 3	Week 4			
Control group	6.51±0.84	6.52±0.83	6.49±0.61	6.47±0.74	6.49±0.72	1.63±0.22	0.70±0.12	30.2±2.2
DM group	6.47±0.79	15.92±0.34*	17.95±0.80*	18.63±0.87*	20.43±0.80*	2.25±0.46*	1.98±0.16*	15.8±2.6*

Table 1: Comparison of blood glucose, HbA1c, serum lipid parameters and body weight gain between the two groups.

*Data are means ± SD, * means P<0.05 compared with control rats. TG, Triglyceride; TC, Total cholesterol.*

Characterization of mechanical and thermal cold allodynia in diabetic rats

To explore the characterization of mechanical and thermal cold allodynia in diabetic rats, the PWT and PWL were measured before and 1, 2, 3 and 4 weeks after the establishment of the model. The results showed that the mechanical and thermal cold pain thresholds of STZ-induced diabetic rats (DM group) were both significantly lower than that of the control group from the week 2 (Fig. 1A-B).

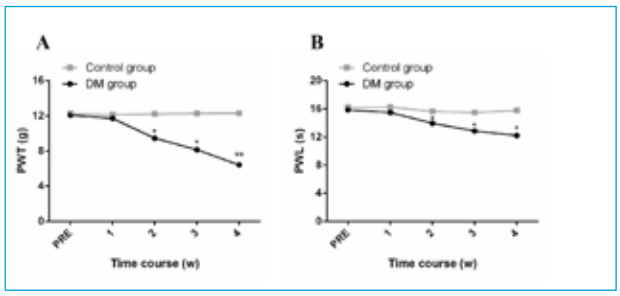

Fig. 1: Characterization of mechanical and thermal cold allodynia in diabetic rats. **A**. The mechanical allodynia was evaluated by PWT; **B**. The thermal cold allodynia was evaluated by PWL. *P < 0.05; **P < 0.01.

Expression of TRPV4 and p38 were up-regulated in L4-6 DRGs of diabetic rats

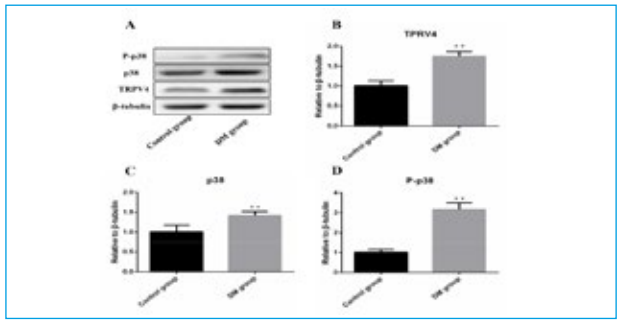

Fig. 2: Expression of TRPV4 and p38 were up-regulated in L4-6 DRGs. **A**. Proteins were examined via western blot analysis; **B-D**. Expression of TRPV4, p38 and P-p38 protein increased in L4-6 DRGs in DM group. **P < 0.01.

To study the role of TRPV4 and p38 in mechanical and thermal cold allodynia in diabetes, we explored the protein expression of TRPV4 and p38 in L4-6 DRGs by western blot firstly. The results indicated that the expression of TRPV4, p38 and P-p38 protein in L4-6 DRGs in DM group increased significantly compared with the control group (Fig. 2A-B).

Inhibition of TRPV4 may alleviate the mechanical allodynia in diabetic rats via p38 pathway

Thus, HC-067047, a selective TRPV4 channel antagonist, was used to investigate the role of TRPV4 on the development of mechanical and cold allodynia. Data demonstrated the inhibition of TRPV4 could significantly reverse the mechanical pain threshold in diabetic rats from the week 2 (Fig. 3A).

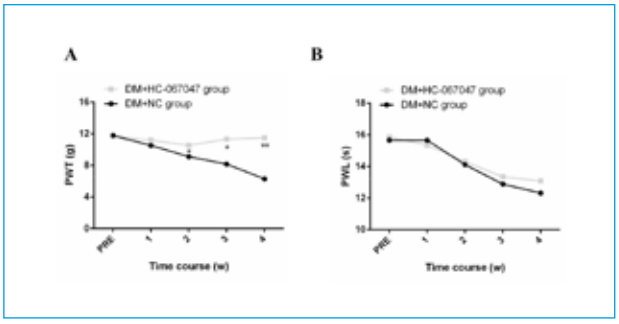

Fig. 3: Inhibition of TRPV4 alleviate the mechanical allodynia in diabetic rats. **A**. The mechanical allodynia was evaluated by PWT; **B**. The thermal cold allodynia was evaluated by PWL. *P < 0.05; **P < 0.01.

Unfortunately, HC-067047 exerted no role in the thermal cold allodynia (Fig. 3B). Besides, western blot showed that inhibitory of TRPV4 was accompanied with the decreased expression of p38 and P-p38 protein (Fig. 4A-C).

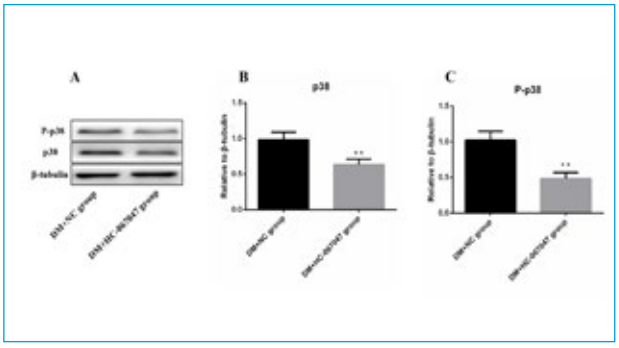

Fig. 4:HC-067047 may attenuate the mechanical allodynia via p38 pathway. **A**. Proteins were examined via western blot analysis; **B-C**. HC-067047 decreased the expression of p38 and P-p38 protein. **P < 0.01.

Inhibition of TRPV4 had no effect on serum glucose, lipid parameters and body weight in diabetic rats

In order to access the effect of TRPV4 antagonism on physiological parameters, we compared the serum glucose, lipid parameters and body weight between the DM+NC group and the DM+HC-067047 group. The results indicated that Inhibition of TRPV4 had no effect on serum glucose, lipid parameters and body weight in diabetic rats, as shown in Table 2.

Group	Glucose (mM)					TG (mM)	TC (mM)	Weight gain (g)
	PRE	Week 1	Week 2	Week 3	Week 4			
DM+NC group	6.59±0.73	15.99±0.73	17.58±0.92	19.01±0.93	20.68±0.76	2.36±0.59	1.94±0.36	14.9±3.1
DM+HC-067047 group	6.38±0.83	16.28±0.45	17.95±0.79	18.49±0.77	20.01±0.99	2.22±0.65	1.88±0.47	15.6±2.7

Table 2: Effect of HC-067047 on blood glucose, HbA1c, serum lipid parameters and body weight gain in diabetic rats.

Data are means ± SD. TG, Triglyceride; TC, Total cholesterol.

Discussion

Currently, the specific mechanism of diabetic neuropathy is not clear, and the main purpose of treatment is to relieve symptoms[12]. STZ-induced diabetic rat model has been widely used in basic research of diabetes mellitus[13], and our research is to select adult Wistar male rats as diabetic neuropathic pain animal model.

As a core component of the nervous system, neurons are involved in processing and transmitting signals, including inflammatory response signals[14]. In DRGs, small neurons may be the most important neurons to produce and transmit pain signals[15]. Normally, DRG neurons do not produce electrical signals directly or spontaneously[16]. TRPV4, a gene that has been proved to have regulatory function in neuropathic pain, has also been shown to have regulatory relationship with p38 in the MAPK family[17,18]. Previous studies have also shown that p38 can be involved in the pathophysiological process of neuralgia[19]. PWT and PWL are classical important indicators for assessing pain thresholds[20].

In this study, found that the mechanical and thermal cold pain thresholds of STZ-induced diabetic rats (DM group) were both significantly lower than that of the control group from the week 2. In addition, the expression of TRPV4 and p38 and p-P38 in L4-6 DRGs increased significantly. Inhibition of TRPV4 could alleviate the mechanical allodynia in diabetic rats via p38 pathway.

Interestingly, inhibition of TRPV4 had no effect on serum glucose, lipid parameters and body weight in diabetic rats.

In conclusion, we found an increase in TRPV4 and P38 expression in DRGs of diabetic neuralgia. Further use of TRV4 antagonists demonstrated that TRV4 regulates diabetic neuralgia and may play a role through P38. These results reveal that TRPV4 plays an important role in regulating neuropathic pain in diabetic rats, providing more evidence for the treatment of peripheral neuropathy in clinical diabetic patients, and providing new ideas for its treatment.

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