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Metformin attenuates hyperalgesia and allodynia in rats with painful diabetic neuropathy induced by streptozotocin

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ABSTRACT

Painful diabetic neuropathy is a common complication of diabetes mellitus, which often makes the patients suffer from severe hyperalgesia and allodynia. Thus far, the treatment of painful diabetic neuropathy remains unsatisfactory. Metformin, which is the first-line drug for type-2 diabetes, has been proved to attenuate hyperexcitability in sensory neurons linked to chemotherapy-induced neuropathic pain, highlighting its potential in alleviating pain related with painful diabetic neuropathy. The present study was designed to investigate the potential beneficial effect of metformin on hyperalgesia and allodynia in diabetic rats. The mechanical sensitivity, heat nociception, and cold allodynia were examined. The levels of malondialdehyde, superoxide dismutase, and advanced glycation end-products in the blood were measured. The expression of adenosine monophosphate-activated protein kinase (AMPK) phosphorylation and AMPK target genes were examined in the sciatic nerves of the animals. It was found that metformin was capable of attenuating diabetes-induced mechanical hyperalgesia, heat hyperalgesia and cold allodynia. In addition, metformin was capable of decreasing malondialdehyde and glycation end-products levels in blood, as well as increasing superoxide dismutas activity, indicating the inhibitory effect of metformin against diabetes-induced oxidative stress. Further studies showed that metformin could activate AMPK and increase the AMPK target genes in sciatic nerves in diabetic rats. In conclusion, metformin is able to attenuate diabetes-induced hyperalgesia and allodynia, which might be associated its anti-oxidative effect through AMPK pathway. Metformin might be used as an effective drug, especially with fewer side effects, for abnormal sensation in painful diabetic neuropathy.

1. Introduction

Painful diabetic neuropathy is a common complication of diabetes mellitus (Ziegler and Fonseca, 2015). The patients often suffer from severe hyperalgesia and allodynia, which can be disabling and devastating (Didangelos et al., 2014). However, as the mechanism of painful diabetic neuropathy is incompletely understood, the treatment of painful diabetic neuropathy remains unsatisfactory (Greig et al., 2014). Therefore, it is imperative to look for effective drugs, especially with fewer side effects, for the management of painful diabetic neuropathy.

Metformin, a classic and widely used anti-diabetic drug (Kirpichnikov et al., 2002), has been proved to possess beneficial effects on nervous system in several studies. Recent studies have shown that metformin might inhibit injury-induced neuropathic pain through adenosine monophosphate-activated kinase (AMPK) pathway (Melemedjian et al., 2011). Metformin has also been shown to reverse mechanical allodynia in lumbar radiculopathy pain induced by spinal nerve ligation in rats and nerve injury in mice (Taylor et al., 2013). In addition, metformin is capable of attenuating hyperexcitability in sensory neurons that were exposed to cytokines and growth factors which has been associated with chemotherapy-induced neuropathic pain. In an *in vivo* study, metformin protects against chemotherapy induced neuropathic pain in a mouse model (Mao-Ying et al., 2014). All those findings show the neuroprotective effect of metformin on sensation and raise its potential in alleviating neuropathic pain in diabetes mellitus. However, such a possibility has not been investigated *in vivo* in previous studies. Therefore, the present study was designed to investigate the potential beneficial effect of metformin

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on hyperalgesia and allodynia in rats with painful diabetic neuropathy.

2. Materials and methods

2.1. Animal model of painful peripheral diabetic neuropathy

All experimental procedures were performed under a protocol which was approved by the Institutional Ethical Committee of General Hospital of Shenyang Military Area Command of PLA. Adult male Sprague–Dawley rats (Laboratory Animal Center of the General Hospital of Shenyang Military Area Command of PLA, Shengyang, China), weighing from 200 g to 240 g, were used for the experiment. Streptozotocin (STZ, Sigma, St. Louis, MO) was intraperitoneally injected (50 mg/kg) to induce painful diabetic neuropathy ($n=60$). Normal control rats ($n=60$) received the same volume/kg saline. Three weeks after administration, severe hyperglycemia and neuropathy with hyperalgesia and allodynia was found in STZ-treated animals (Messinger et al., 2009). Then the STZ-treated rats were randomly divided into 5 groups (STZ control group, low-dose metformin group, mid-dose metformin group, high-dose metformin group, AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribonucleoside) group, $n=12$ in each group). The normal control rats were also randomly divided into 5 groups (normal control group, low-dose metformin group, mid-dose metformin group, high-dose metformin group, AICAR group, $n=12$ in each group). On the 21st day, metformin/AICAR or saline was given to the rats. For the metformin groups, the animals were intraperitoneally injected metformin at 30 mg/kg, 200 mg/kg or 500 mg/kg, which were dissolved in 10 ml of saline. For the AICAR group, the animals were intraperitoneally injected AICAR at 160 mg/kg, which was dissolved in 10 ml of saline. For the STZ control group and normal control group, the animals received normal saline (10 ml). The metformin, AICAR and saline were given for seven consecutive days. On the 5th day of the metformin/AICAR treatment, behavioral assessment of mechanical sensitivity was performed at 6 time points following drug treatment (0, 1, 2, 3, 4 and 5 h after metformin). On the 6th and 7th day, behavioral assessments of heat nociception (6th day) and cold allodynia (7th day) were performed to assess hyperalgesia and allodynia at the same time points as the mechanical assessments. Immediately after behavioral assessments on the 7th day, body weight, blood glucose, levels of malondialdehyde, superoxide dismutase, and advanced glycation end-products in the blood were measured. The sciatic nerves of the animals were harvested for further investigation of AMPK-associated mechanisms.

2.2. Behavioral assessment of mechanical sensitivity

The mechanical sensation was assessed before STZ treatment, 21 days after STZ treatment and 5 days after metformin treatment. The mechanical sensitivity was measured using a series of von Frey filaments (Vogelaar et al., 2004; Chaplan et al., 1994). Briefly, the animals were placed in a cage with wire-mesh-bottom. Five hairs with forces of 0.38 g, 2.3 g, 4.1 g, 6.7 g, 8.2 g, 10.7 g, 13.2 g and 15.1 g were applied 10 times to the foot pads in the plantar aspect of the hind paw in ascending order of force. The forces exerted by the von Frey hairs were checked before testing using a micromanipulator to advance them towards a sensitive balance. The hair was applied for 3–5 s, with an inter-stimulus interval of 30 s. Care was taken not to stimulate the same point twice in succession. Abrupt paw withdrawal, licking, and shaking were taken to be positive responses. The threshold was the minimal force which elicited more than 5 positive responses. Before STZ treatment, every 3 days after STZ treatment and on the 5th day of metformin treatment, the number of positive responses for the Von Frey filament (diameter: 0.5 mm, bending force: 13.2 g), which has been shown to elicit nociceptive responses, was recorded and counted at 0, 1, 2, 3, 4 and 5 h after drug administration. A significant decrease in mechanical threshold and increase in the frequency of foot withdrawals in response to mechanical stimulation was interpreted as mechanical hyperalgesia.

2.3. Behavioral assessment of heat nociception

The latency to heat stimuli was used to evaluate heat nociception, which was recorded before and every 3 days after STZ treatment. On the 6th day of metformin treatment, behavioral assessment of heat sensitivity was performed at 6 time points following metformin/AICAR treatment (0, 1, 2, 3, 4 and 5 h after drug administration). The heat nociception was measured according to the procedure described in previous study (Hargreaves et al., 1998). Briefly, a paw thermal stimulation system was used to measure the nociceptive response to heat. A radiant heat source was used to stimulate the plantar side of the hind paw (46–48 °C). Then an automatic timer was used to record the paw withdrawal latency in seconds when the rats withdraw the paw. If the animals do not withdraw the paw for 20 s, the heat stimulation would be stopped to prevent thermal injury. All the tests were repeated for three times at intervals of 5 min between each application.

2.4. Behavioral assessment of cold allodynia

The latency to cold stimuli was used to evaluate cold allodynia, which was recorded before and every 3 days after STZ treatment. On the 7th day of metformin treatment, behavioral assessment of cold allodynia was performed at 6 time points following metformin treatment (0, 1, 2, 3, 4 and 5 h after drug administration). The cold allodynia was assessed according to the procedure described in previous study (Chen et al., 2014). Before the formal experiment, a series of temperatures were tested in the preliminary studies. It was found that the diabetic rats were sensitive to cold simulation, and 21 °C was the threshold which could elicit

Table 1
The metabolic parameters and oxidative status immediately after metformin treatment.

Groups	Body weight (g)	Blood glucose (mmol/l)	MDA (nmol/l)	SOD (U/ml)	AGEs (μg/ml)
Normal	280.3 ± 18.2	6.8 ± 1.6	13.7 ± 2.4	156.2 ± 32.6	9.7 ± 0.5
STZ	226.7 ± 23.5[a]	16.7 ± 0.8[a]	28.1 ± 1.9[a]	87.3 ± 16.0a	18.2 ± 1.4[a]
STZ+metformin (30 mg/kg)	237.4 ± 18.1[a]	15.2 ± 1.2[a]	19.2 ± 1.5[a,b]	108.7 ± 28.6[a,b]	15.3 ± 1.5[a,b]
STZ+metformin (200 mg/kg)	245.2 ± 25.4[a]	14.5 ± 1.3[a]	14.3 ± 2.1[b,c]	129.5 ± 25.1a[b,c]	12.3 ± 0.8a[b,c]
STZ+metformin (500 mg/kg)	248.8 ± 32.0[a]	14.2 ± 0.9[a]	14.1 ± 1.3[b,c]	132.8 ± 22.4a[b,c]	11.5 ± 0.7a[b,c]
STZ+AICAR (500 mg/kg)	239.5 ± 19.7[a]	15.8 ± 0.6[a]	13.5 ± 1.1[b,c]	130.4 ± 13.7a[b,c]	11.7 ± 0.6a[b,c]

[a] $P<0.05$ for the comparison with normal rats.
[b] $P<0.05$ for the comparison with STZ-treated rats.
[c] $P<0.05$ for the comparison with STZ-treated rats received low dose metformin (30 mg/kg).