Effect of Angipars on Neuropathic Pain in Streptozotocin-Induced Diabetic Rats

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Original Article

Abstract

Introduction: Diabetes is the most common cause of peripheral nerve involvement. Evaluating the effect of antioxidants on diabetic neuropathic pain is important. This study aimed at evaluating the effects of Angipars medicine in the treatment of neuropathic hyperalgesia in single dose streptozotocin-induced diabetic rats.

Methods: The study was performed on 50 Spraque dawley rats of 250-300 grams weight. The rats were divided into four groups of control, sham, Angipars-receiving diabetic, and vehicle-receiving diabetic, with at least 8 rats in each group. Diabetes was induced by intraperitoneal injection of 45 mg/kg streptozotocin dissolved in a 0.05 M citrate buffer. After confirming the diabetes, the diabetic rats received (5, 10, 20 mg/kg, intraperitoneal) Angipars and vehicle for 2 weeks. At the end of the eighth week, the control and treated rats were examined through the hot plate and tail flick tests. ANOVA was used to evaluate the statistical difference and pvalue<0.05 was considered as significant.

Results: At the end of the eighth week, the response time to thermal hyperalgesia decreased in the vehicle and sham groups compared with the control group. Angipars at doses of 5 and 10 mg increased the response time to thermal hyperalgesia compared to the vehicle and sham groups in hot plate test. In the tail flick test, 10mg Angipars increased the response time to pain similar to the control group.

Conclusion: This study showed that, as an antioxidant, Angipars is capable of reducing neuropathic hyperalgesia in animals with diabetes.

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Introduction:

Pain is one of the first signs of illness and the most prominent sense among sensory experiences through which humans realize their sickness. In fact, pain is the most common symptom of a disease (1). Experience and research in the past decades have shown that novel medicines are associated with side effects despite their appropriate and undeniable therapeutic impacts (2). Consequently, referral to medicinal plants is considered again nowadays and many attempts were made to use them.

Diabetes-induced neuropathy is often associated with hyperalgesia and allodynia (3). Although many studies have reported that neurodegeneration or changes in the neurotransmitter system is responsible for change in pain perception in patients with diabetes (4,5), its exact mechanism is not yet known.

Some studies have shown that diabetes is often associated with chronic neuropathic pain and oxidative stress plays an important role in the development of many neurological and behavioral changes in diabetic patients (6,7). Therefore, evaluation of the effects of antioxidants on pain relief in diabetic neuropathy is important.

Angipars (semelil) is a medicine containing the extract of *Melilotus officinalis* and can improve blood flow, reduce inflammation, lead to angiogenesis, and decrease diabetic foot ulcers. Despite its short production time, many studies have investigated the therapeutic effects of this drug and most of them were carried out in diabetes-induced wounds.

Accordingly, recent studies have suggested that Angipars (semelil) can exert therapeutic effect on diabetic foot wounds (8) and does not show acute and chronic toxic effects (9-11).

Therefore, based on the mechanisms involved in the physiopathology of diabetic neuropathy and anti-oxidative and anti-inflammatory effects of Angipars, this study investigated its possible effect on prevention of neuropathic hyperalgesia in diabetic model of rats.

Methods:

This experimental study was performed on 50 Spraque dawley rats of 250-300 grams weight. The rats were kept at standard conditions of 22±2°C temperature and 12-hour periods of light and darkness during the course of treatment, except for testing, while food and water were freely available.

The rats were divided into four groups of control, sham (diabetic with any compound received), Angipars-receiving diabetic, and vehicle-receiving diabetic (distilled water-receiving diabetic), with at least 8 rats in each group.

Diabetes was induced by intraperitoneal injection of 45mg/kg (12,13) streptozotocin (STZ) dissolved in 0.05 M citrate buffer (14). To detect diabetes, the glucose levels of blood collected from the tail vein was measured one week after STZ injection by a glucometer kit (Accu-Chek). The rats were fasted from 8 pm to 8 am before measuring blood sugar.

Animals with blood glucose levels of more than 200 mg/dL were considered as diabetic (13,15). In order to treat neuropathic hyperalgesia, (5,10,20 mg/kg, intraperitoneal) Angipars was used in this study for 2 weeks, 6 weeks after the diagnosis of rats diabetes. The control group (vehicle) received distilled water for 2 weeks from the end of the sixth week (16). The medications were obtained from Pars Pharmaceutical Company.

Pain was assessed through the two methods of tail flick and hot plate.

The tail flick test is a standard test for measurement of hyperalgesia. In this test, thermal light with an intensity of 5 is focused on the tail end by a tail flick device (LE7406) and the tail flick latency is measured from the beginning of radiating heat to flickering of the tail, in seconds. To prevent tissue damage to the tail, the maximum time of heat radiation was considered 10 seconds. The tail flick latency was measured 3 times for each animal and the mean of these measurements was reported as the latency time (TFL). There were 5 minutes intervals between measurements (17).

The hot plate device (LE7106) was the other tool used to measure the sensitivity to pain. The device includes a plate of 19 cm diameter and a Plexiglas wall of 30 cm height. The device is heated via electrical resistance connected to a timer and a thermostat. The degree was set to 52 °C. The thermal pain response time was measured since the start of the test until the animal began to lick his front legs or jump. The cut-off for animal reaction to thermal pain was considered 60 seconds (17).

The data were presented as mean±SEM. Statistical difference was assessed by one-way ANOVA, and in case of significance, the differences between groups were analyzed by Tukey Post Hoc. pvalue<0.05 was considered as the significance level.

Results:

Metabolic profile

Plasma glucose concentrations in diabetic rats at the end of the first week was 287.24% more than the control group (pvalue<0.0001). As can be seen in Table 1, the diabetic rats weighted lower than the controls after 8 weeks. Treatment with Angipars (5, 10, 20 mg/kg) for 2 weeks had no significant effect on blood glucose levels in diabetic rats (Table 1).

Table 1- Body Weight and Blood Glucose Levels Before and After Intervention in all Groups

Group (n=8)	Weight before injection of STZ (g)	Weight after intervention (g)	Blood sugar before intervention (mg/dl)	Blood sugar after intervention (mg/dl)
Control	270±4.62	276.25±4.70	125±9.87	132.87±12.59
Sham	260±8.16	215.57±9.54	447.14±29.97	512.85±28.92
Vehicle	262.85±5.65	222.57±5.42	423.71±41.14	491.42±33.19
Angipars 5mg	257.14±8.08	210±17.32	457.14±43.31	492.85±51.94
Angipars 10mg	275.55±4.74	263.33±10.54	391.33±22.21	459.22±31.66
Angipars 20mg	266.25±4.97	231.25±5.80	395.87±23.05	471.25±20.73

* Data are in mean±SD

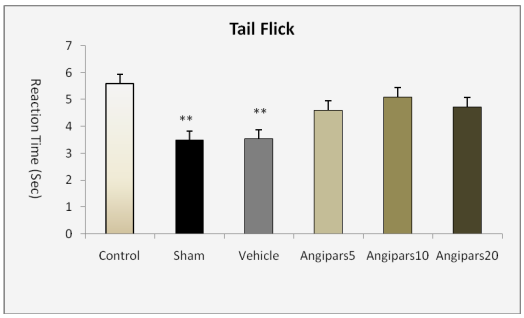

Figure 1- Effect of Angipars on the reaction time to pain in the tail flick test

** Pvalue <0.01 significant difference of the vehicle and sham groups with the controls.
††† pvalue <.001 significant difference of the 10 mg Angipars-receiving group with the vehicle and sham groups.

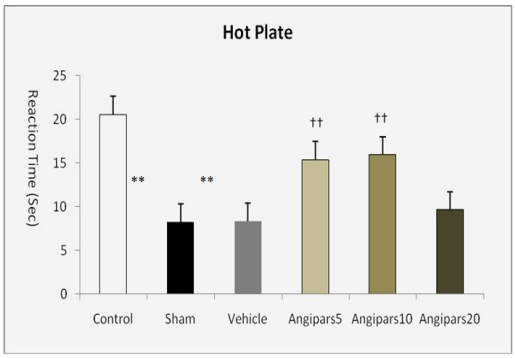

Figure 2- Effect of Angipars on the reaction time to pain in the hot plate test

** Pvalue <0.01 significant difference of the vehicle and sham groups with the controls.
††† pvalue <.001 significant difference of the 5 mg and 10 mg Angipars group with the vehicle and sham groups.

According to the effect of Angipars on treatment of diabetic neuropathic hyperalgesia assessed with the hot plate test, it was shown that diabetes and high blood sugar for long time can lead to neuropathic hyperalgesia and reduce the response time to thermal pain; so that eight weeks after induction of diabetes in this study, the reaction time to thermal pain in the vehicle-receiving diabetic group and the sham group was significantly decreased compared to the control group (pvalue<0.01).

Intraperitoneal administration of Angipars at a dose of 5 and 10 mg for 2 weeks resulted in a significant increase in the reaction time to pain in STZ-induced diabetic rats compared to the vehicle-receiving diabetic and sham groups in hot plate test (pvalue<0.01).

The reaction to thermal pain in the 20mg Angipars group had no significant increase compared to the vehicle-receiving diabetic and sham groups (Fig. 2).

According to the effect of Angipars on treatment of diabetic neuropathic hyperalgesia assessed with the tail flick test, it was shown that the reaction time to pain was significantly lower in the sham and vehicle groups than the controls (pvalue<0.01). The reaction time to pain was significantly increased in the 10 mg Angipars group compared to the sham (pvalue<0.01) and vehicle (pvalue<0.001) groups, although the reaction time to pain in the 5mg and 20mg Angipars groups had no significant difference compared to the sham and vehicle groups, the reaction to pain was close to that of the control group (Fig. 1)

Conclusion:

In the present study, it was shown that the use of Angipars, after induction and proving of diabetic neuropathy, can reduce hyperalgesia in the test of

response to thermal pain in diabetic animals during a period similar to the control condition.

Peripheral damage of neurons caused by oxidative stress can be diagnosed in more than 50% of people with diabetes (18). Peripheral neuropathy is associated with increased activity of nerve fibers at an early stage which leads to impairment in natural sensitivity of nervous system to painful stimuli and development of diabetic hyperalgesia (19). Therefore, modification of pain in these patients is important. Accordingly, it seems necessary to evaluate the treatment strategies which can reduce the progression of neuronal injury and prevent the development of hyperalgesia through decreasing oxidative stress and exerting anti-oxidative effects.

Many studies have been performed regarding the beneficial effects of antioxidants on performance and structure of peripheral nerve tissues. Podratz J *et al*. showed that antioxidants are essential for myelination of the dorsal root of neuronal axons (19). It was shown in another study that consumption of palm extracts and melatonin, both as antioxidant, can reduce neuropathic hyperalgesia in animals with diabetes (20).

In our study, Angipars at low- and medium-dose increased the latency time of response to thermal pain in hot plate test so that the reaction time to pain in these groups approached to the control group. These results were repeated in the other thermal test, *i.e.* the tail flick test. Angipars at a dose of 10 mg/kg increased the latency time of response to thermal pain, and the reaction time to pain in this group was close to that of the control group. Therefore, it seems that the use of 10 mg/kg Angipars for 2 weeks, after induction of diabetic neuropathy, prevents development of diabetic hyperalgesia and increases the response time to pain, similar to the control group. The discussed effects of Angipars can help prevent diabetes-induced hyperalgesia and increase the response time to thermal pain in diabetic rats due to various reasons. Delay in inflammatory reactions and angiogenesis to repair nerve on the other hand, can affect the nerve conduction velocity as well as the time of response to pain induced by diabetes. Several studies have shown that Angipars can completely repair and close wound gap, and some studies reported an enhancement in wound healing. Recent studies using different methods have shown that the extract of this drug, with hydrocortisone-like and anti-inflammatory effects, improves blood circulation in venules of leg ulcers in diabetic patients (9). Larijani *et al*. showed that Angipars can increase the overall blood flow of tissue and hence increase tissue oxygenation due to angiogenesis (21). Angipars is effective through reducing inflammation and oxidative damages on periodontium (22-25). In addition, intravenous administration of Angipars can be an effective treatment for patients with severe gastric wounds (25). Angipars herbal medicine has a positive effect on sperm count in the diabetic animals especially when used in combination with insulin (26).

In summary, it seems that the use of Angipars after proving the neuropathy can be effective in the treatment of diabetes-induced destruction of neurons, and can approximate the reduced threshold of pain in diabetic neuropathy to Norma.

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اثر داروی آنژیپارس بر آستانه درد نوروپاتی در موش‌های صحرایی نر دیابتی شده با استرپتوزوتوسین

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چکیده

مقدمه: دیابت شایع‌ترین علل درگیری اعصاب محیطی می‌باشد. بررسی تأثیر آنتی‌اکسیدان‌ها در تسکین درد نوروپاتیک دیابتی حائز اهمیت است. هدف از انجام این مطالعه، بررسی اثر داروهای آنژیپارس در درمان هیپرآلژزی نوروپاتیک در موش‌های صحرایی نر دیابتی شده با تزریق تک دوز استرپتوزوتوسین (STZ) بود.

روش‌کار: مطالعه بر روی ۵۰ سر موش نژاد Spraque dawley در محدوده وزنی ۳۰۰-۲۵۰ گرمی انجام شد. موش های صحرایی به چهار گروه: کنترل، شم (sham)، دیابتی دریافت‌کننده آنژیپارس و حلال تقسیم شدند (حداقل ۸ موش در هر گروه). القای دیابت با تزریق داخل صفاقی ۴۵ mg به ازای هر کیلوگرم وزن بدن استرپتوزوتوسین حل شده در بافر سیترات 0.05M انجام شد. پس از تأیید دیابتی شدن موش‌ها، موش‌های گروه دیابتی به مدت ۲ هفته آنژیپارس (۵، ۱۰، ۲۰ میلی‌گرم به ازای هر کیلوگرم وزن بدن، داخل صفاقی) و حلال دریافت کردند. در انتهای هفته هشتم، موش‌های کنترل و تیمار شده تحت بررسی آزمون‌های Hot Plate و Tail Flick قرار گرفتند. جهت بررسی تفاوت آماری ازآنالیز واریانس یک طرفه استفاده شد و pvalue<۰/۰۵ به عنوان سطح معنی‌داری در نظر گرفته شد.

نتایج: در انتهای هفته هشتم، زمان پاسخ به هیپرآلژزی حرارتی در گروه حلال و شم نسبت به گروه کنترل کاهش یافت. دوز ۵ mg و ۱۰ آنژیپارس باعث افزایش زمان پاسخ به هیپرآلرژی حرارتی نسبت به گروه حلال و شم در آزمون Hot Plate شد. دوز ۱۰ mg انژیپارس در آزمون Tail Flik باعث افزایش زمان پاسخ به درد در زمانی شبیه به گروه کنترل شد.

نتیجه‌گیری: این مطالعه نشان داد که مصرف انژیپارس به عنوان یک آنتی‌اکسیدان قادر به کاهش هیپرآلژزی نوروپاتیک در حیوانات مبتلا به دیابت می‌باشد.

کلیدواژه‌ها: نوروپاتی دیابتی، موش صحرایی، درد.

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