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Study of a painful diabetic peripheral neuropathy model induced by streptozotocin: conclusions before investigating non-paralytic botulinum molecules

Painful diabetic peripheral neuropathy **(**PDPN) is a common complication of diabetes characterized by peripheral nerve dysfunction and debilitating pain symptoms. This article investigates the streptozotocin (STZ)-induced model of PDPN (45 mg/kg i.p.) to evaluate whether it can be further used to study the efficacy of non-paralytic botulinum molecules in pain control. In the study changes in relative weight, glucose levels, and mechanical and temperature sensitivity in the experimental group compared to the control group of rats were assessed. The obtained data indicate the reliability of the model. The results showed a significant decrease in relative weight and alterations in glucose levels in the experimental group, highlighting the metabolic impact of PDPN. Moreover, the rats in the experimental group exhibited heightened mechanical and temperature sensitivity, mirroring the neuropathic pain experienced by patients with PDPN. These findings support the suitability of the STZ-induced PDPN model for preclinical studies investigating non-paralytic botulinum molecules as analgesics. In conclusion, this model provides a valuable platform for future research aimed at understanding the underlying mechanisms and developing effective interventions for PDPN.

Keywords: painful diabetic peripheral neuropathy, streptozotocin-induced model, preclinical study, non-paralytic botulinum neurotoxin, mechanical sensitivity, temperature sensitivity.

Introduction

Painful diabetic peripheral neuropathy (PDPN) is a common complication that can occur with diabetes. This disease affects about 25 % of people with diabetes mellitus [1], of whom there are currently more than 537 million worldwide [2]. PDPN has been described as a manifestation of peripheral nerve dysfunction in people with diabetes after other potential causes have been ruled out. This condition is usually defined by unpleasant sensations such as burning or stabbing pain, tingling, increased sensitivity to touch, or an electric shock-like sensation [3].

PDPN has a significant impact on the quality of life of people with diabetes and contributes to increased healthcare costs associated with diabetes care. Patients with PDPN experience significant physical and mental changes in quality of life. According to a study by Davies, *et al.*, about 80 % of patients with PDPN experience moderate to severe pain [4]. This chronic pain interferes with various aspects of daily life, impairing physical functioning, mobility, and the ability to perform routine activities. Patients with PDPN may experience decreased work productivity, inability to participate in social and recreational activities, and loss of independence. In a survey conducted among working people with painful diabetic neuropathy, it was found that 53 % of them reported a decrease in performance at work. In addition, the average number of missed workdays in the four weeks prior to the survey was more than five [5]. The debilitating nature of pain in PDPN also contributes to the development of comorbidities and psychological distress. Approximately 43 % of people with PDPN report associated symptoms of anxiety, depression, and sleep disturbances [6]. The presence of severe pain and its detrimental effect on daily life can lead to social isolation and deterioration in overall mental well-being.

Although PDPN is a complication of diabetes mellitus, it is essential to recognize that it is a distinct entity pathophysiology of which is still poorly understood. While various studies have investigated the relationship between glucose control and neuropathic pain-related complications in patients with diabetes, glucose control's effectiveness in treating PDPN remains unclear. The mainstay of treatment is symptom control with pharmacotherapy, which has limited efficacy [7].

Numerous drugs are used to treat PDPN, including carbamazepine, antidepressants, gabapentin, and opioids [8]. Recently, treatments such as lidocaine patches and high doses of capsaicin have been proposed [9]. However, due to the lack of long-term analgesic action and side effects, these drugs are often inef-

fective or poorly tolerated [10]. The most frequent adverse effects include sedation, anticholinergic effects (e.g., dry mouth, constipation, and urinary retention), and orthostatic hypotension. Other dose-limiting side effects are drowsiness, dizziness, and peripheral edema. In some patients, especially the elderly, therapy may cause or exacerbate cognitive or gait impairment [11].

According to earlier published research by Meyer-Rosberg, *et al.* [12], patients suffering from peripheral neuropathic pain usually experience limited pain relief. The effectiveness of existing pharmacotherapeutic agents in painful diabetic neuropathy is limited: satisfactory pain reduction is observed in less than one-third of patients [13]. These results indicate that there is a significant need to improve pain management in patients with PDPN. The growing prevalence of patients with diabetes further emphasizes the need to develop practical therapeutic approaches.

Research has shown promising results regarding the ability of botulinum toxin (BoNT) to induce long-lasting analgesic effects in various chronic pain conditions. A systematic review by Wang, *et al.* [14] confirms the efficacy and safety of botulinum toxin A (BoNT/A) treatment in patients with PDPN. BoNT/A treatment significantly reduced the sensation of warmth and deep and superficial pain compared to placebo treatment [15, 16]. Recently, new variants of non-paralytic botulinum toxin molecules have been proposed as potential treatment options for chronic pain [17–20]. To evaluate the safety and efficacy of non-paralytic botulinum molecules in the context of painful diabetic peripheral neuropathy, preclinical studies using an appropriate model of diabetes are needed.

Models of pain diabetic peripheral neuropathy. In preclinical studies, the most preferred animal models for diabetes research are rats for various reasons, including human-like physiology, the size of the animal as a whole and the proportionality of necessary substructures in organs, and a large amount of accumulated data. In models of diabetes, the rat model is more similar to the human model in many respects, including the ability of environmental factors to influence the disease [21]. Models of diabetes in rats can be divided into genetic or experimentally induced, the latter being more cost-effective and easier to induce, so they are widely used for research purposes. Experimentally induced models include surgical, dietary, chemical, or combined methods. In particular, streptozotocin (STZ) is the most widely used diabetogenic chemical to create animal models of diabetes [22]. STZ is a compound that selectively damages pancreatic β-cells, resulting in reduced insulin synthesis and increased blood glucose levels. Subsequent hyperglycemia over time leads to the development of painful diabetic peripheral neuropathy. To induce PDPN in rats, it is possible to use both a single relatively high dose of STZ (35–65 mg/kg intravenously or intraperitoneally) and multiple injections of a low dose of STZ (15–20 mg/kg for five consecutive days intravenously or intraperitoneally) [23]. It is critical to use a model in which the animals exhibit disease-related traits and maintain a relative activity level, as immobile animals are not suitable for behavioral testing.

Experimental

The object of study. All animal experiments were carried out by the design of the study (Fig. 1), approved by the local ethical commission (conclusion № IRB-377 dated February 24, 2022, supplement № IRB-A377 dated November 18, 2022). Mature male white laboratory outbred rats born and raised in the conditions of the educational and scientific laboratory base of the Kazakh National University named after al-Farabi (Almaty, Republic of Kazakhstan) were used. All animals were kept under natural light conditions at 21-22 °C. Food and water were provided *ad libitum.*

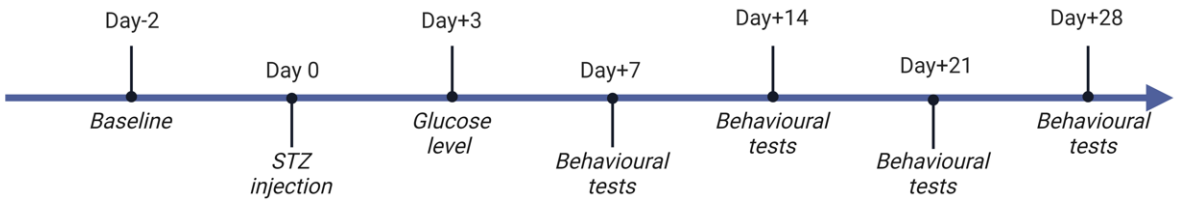


Figure 1. Timeline of the study

Induction and control of hyperglycemia. Hyperglycemia was induced by a single dose (45 mg/kg) of STZ dissolved in sodium citrate buffer (pH 5.5) by intraperitoneal injection. To facilitate intraperitoneal absorption of the drug, access to food was limited to 4–6 hours before drug administration. During the first 48 hours after injection, 10 % sucrose solution was provided as the only source of water. Blood glucose levels were measured 72 hours after STZ injection to verify the development of a hyperglycemic state. If the

blood glucose level did not exceed 13.8 mmol/L, the rat was excluded from the study. Further testing for hyperglycemia was carried out with periodicity one time per week.

Behavioral testing. Before conducting any behavioral tests, the animals were acclimatized for a week. They were carefully accustomed to the test equipment and procedures, which were strictly from 8:00 am to 2:00 pm. Preference was given to procedures that result in less long-term damage or pain, namely mechanical allodynia evaluation and thermal hyperalgesia evaluation. In these tests, a reduction of up to 80 % is considered sufficient to confirm the development of PDPN.

Assessment of mechanical allodynia — electronic won Frey. For testing, the rat was placed in a hanging cage with a mesh floor. The von Frey filament is applied to the plantar surface of the hind paw. The pressure at which the paw withdrawal occurred was recorded. The procedure was repeated five times with 1-minute intervals between stimuli. The average value for each rat was calculated by averaging three of the five measurements, excluding the highest and lowest values.

Assessment of thermal hyperalgesia — Hargreaves test. To quantify temperature sensitivity, rats were placed in a chamber located on a raised transparent floor. A radiant heat source under the floor delivered a thermal stimulus to the plantar surface of the hind paw. The time between the start of the stimulus and withdrawal was recorded. If the paw was not withdrawn within 25 seconds, the stimulus was terminated to avoid tissue damage. Sequential stimuli were applied three times with a 3-minute break between stimulations. The average withdrawal delay was calculated by averaging the three measures.

Results and Discussion

In this study, we aimed to explore the characteristics of a local rat model of PDPN by comparing two groups: a control group (n = 6) and an experimental group (n = 22). The experimental group is planned to be divided into three subgroups with different treatment plans in future studies.

Based on the observations, it was noted that the rats in the experimental group showed distinct behavioral changes compared to the control group. PDPN rats showed inactivity, as well as increased fluid intake (about 70 ml per day) and food (about 12 g of food per 100 g of body weight per day), decreased physical activity, self-care, and exploratory behavior. PDPN rats had an increased frequency of urination, which may be due to elevated glucose levels leading to polyuria. It was also observed that rats from the experimental group experienced episodes of diarrhea.

We analyzed the percentage change in weight over a 4-week period. The control group, consisting of healthy rats, showed a mean percentage weight gain of 3.68 g (±0.91) over a 4-week period. In contrast, the experimental group showed a different pattern of weight change. Rats in this group experienced a mean percentage weight loss of 15.31 g (±2.16). The observed weight loss in the experimental group is consistent with the metabolic changes associated with hyperglycemia.

Glucose levels were measured at multiple time points in the control group and the experimental group (Fig. 2). Throughout the study, the control group maintained a relatively stable glucose level. On the contrary, in the experimental group, a noticeable increase in glucose levels was observed after the administration of STZ on the third day after the injection. This hyperglycemic state persisted for all four weeks, indicating persistent hyperglycemia.

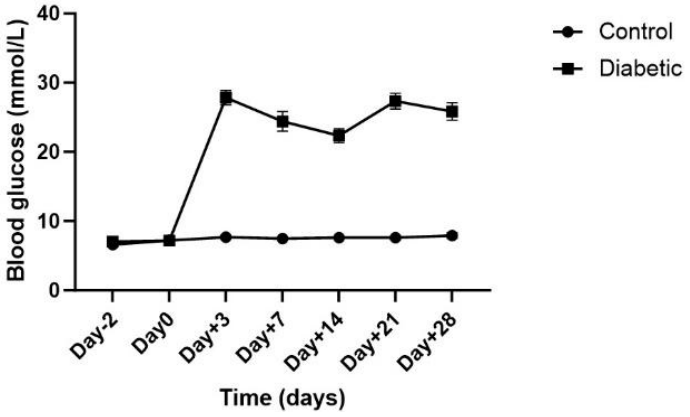
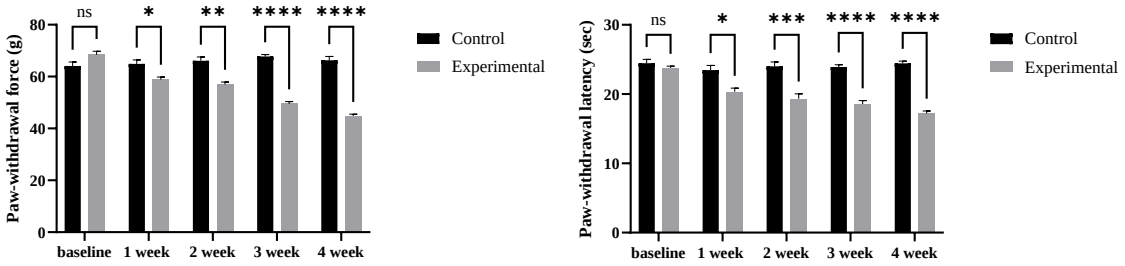


Figure 2. Rats treated with STZ develop a stable level of hyperglycemia

The thresholds of mechanical sensitivity of rats of both groups were assessed using electronic von Frey at different time points (Fig. 3*a*). At baseline, both the control and experimental groups showed similar von Frey responses with mean values of 64.07 and 68.41, respectively. At week 4 (D+28), the control group maintained a relatively stable response with a mean value of 66.31. In contrast, the diabetic rat group showed a significant decrease in mechanical sensitivity with a mean value of 44.82, in line with the development of PDPN.



a *b*

Figure 3. Rats treated with STZ develop a neuropathic pain response to mechanical (*a*) and temperature (*b*) stimuli. Control animals treated with vehicles did not show significant changes in behavioral response

Temperature sensitivity thresholds were also evaluated (Fig. 3*b*). The control group showed consistent temperature sensitivity throughout the study period. However, in the experimental group, there was a marked decrease in temperature sensitivity starting from the 1st week (D+7). At week 4 (D+28), this group showed a significant decrease in temperature sensitivity with a mean value of 17.20. These data suggest that the STZ model of diabetic neuropathy has led to a progressive decrease in heat sensitivity over time.

Conclusions

In our study, we examined a streptozotocin-induced PDPN model (45 mg/kg i.p.) to assess whether it could be further used to investigate the efficacy of non-paralytic botulinum molecules. We observed significant changes in weight, glucose levels, and mechanical and temperature sensitivity in the STZ group compared to the control group. Our results support the validity of the STZ-induced model as a suitable tool for the study of PDPN. The observed changes reflect the characteristic symptoms of diabetic neuropathy. This model provides a valuable platform for evaluating potential therapeutic interventions, such as non-paralytic botulinum molecules, for treating painful diabetic peripheral neuropathy.

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А.К. Жантлеуова, А.С. Каримова, Б.А. Давлетов

Стрептозотоцинмен индукцияланған диабеттік ауыру диабеттік перифериялық нейропатияның моделін зерттеу: ботулиннің паралич емес молекулаларының зерттеуге дейінгі нәтижелері

Ауырсынатын диабеттік перифериялық нейропатия (АДПН) — бұл перифериялық нервтердің дисфункциясымен және әлсіретін ауырсыну белгілерімен сипатталатын қант диабетінің жиі кездесетін асқынуы. Мақалада стрептозотоцинмен (СТЗ) индукцияланған АДПН моделі зерттеліп, оны одан әрі ботулиннің паралич емес молекулаларының тиімділігін зерттеу үшін қолдануға болатындығы бағаланған. Авторлар егеуқұйрықтардың бақылау тобымен салыстырғанда эксперименттік топтағы салыстырмалы салмақтың, глюкоза деңгейіндегі, механикалық және термиялық сезімталдықтағы өзгерістерді бағалады. Алынған мәліметтер модельдің сенімділігін көрсетеді. Нәтижелер эксперименттік топтағы салыстырмалы салмақтың айтарлықтай төмендеуін және глюкоза деңгейінің өзгеруін көрсетті, бұл АДПН метаболикалық әсерін көрсетеді. Сонымен қатар, эксперименттік топтағы егеуқұйрықтар механикалық және температураға сезімталдықтың жоғарылауын көрсетті, бұл АДПН пациенттерінің невропатиялық ауырсынуын көрсетеді. Осы нәтижелер СТЗ-индукцияланған модельдің салданбайтын ботулин молекулаларын клиникаға дейінгі зерттеулерге АДПН үшін әлеуетті емдеу ретінде жарамдылығын растайды. Қорытындылай келе, бұл модель негізгі механизмдерді түсінуге және АДПН үшін тиімді араласуларды әзірлеуге бағытталған болашақ зерттеулер үшін құнды платформаны қамтамасыз етеді.

Кілт сөздер: ауырсынатын диабеттік перифериялық нейропатия, стрептозотоцин-индукцияланған модель, клиникаға дейінгі зерттеу, ботулиннің паралич емес нейротоксины, механикалық сезімталдық, температураға сезімталдық.

А.К. Жантлеуова, А.С. Каримова, Б.А. Давлетов

Изучение модели болевой диабетической периферической нейропатии, индуцированной стрептозотоцином: выводы перед исследованием непарализующих молекул ботулина

Болевая диабетическая периферическая нейропатия (БДПН) — частое осложнение сахарного диабета, характеризующееся дисфункцией периферических нервов и изнурительными болевыми симптомами. В статье исследована модель БДПН, индуцированная стрептозотоцином (СТЗ), чтобы оценить, можно ли ее в дальнейшем использовать для изучения эффективности непарализующих молекул ботулина. Авторы оценивали изменения относительного веса, уровня глюкозы, механической и температурной чувствительности в экспериментальной группе по сравнению с контрольной группой крыс. Полученные данные свидетельствуют о надежности модели. Результаты показали значительное снижение относительного веса и изменения уровня глюкозы в экспериментальной группе, что подчеркивает метаболическое влияние БДПН. Более того, крысы в экспериментальной группе проявляли повышенную механическую и температурную чувствительность, отражая невропатическую боль, которую испытывают пациенты с БДПН. Эти результаты подтверждают пригодность СТЗ-индуцированной модели для доклинических исследований непарализующих молекул ботулина в качестве потенциальных средств лечения БДПН. В заключение, эта модель обеспечивает ценную платформу для будущих исследований, направленных на понимание основных механизмов и разработку эффективных вмешательств для БДПН.

Ключевые слова: болевая диабетическая периферическая нейропатия, стрептозотоцин-индуцированная модель, доклиническое исследование, непаралитический ботулинический нейротоксин, механическая чувствительность, температурная чувствительность.

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