Pharmacology Discovery Services

White Paper:

Streptozotocin (STZ)-Induced Diabetic Pain Model – Techniques to Successfully Evaluate Drug Candidates

Introduction

It is currently estimated that diabetes mellitus impacts over 450 million people worldwide and that number is forecast to increase over 50% in next 25-30 years.[1] Estimates on the growth rates for diabetes, the number of negative impacts on vascular health and other complications presents a serious challenge to global health.

The number of people with diabetes mellitus is expected to increase substantially in coming decades. Diabetic neuropathy is a common complication with limited therapeutic options. Reliable, and reproducible, *in vivo* models are needed for the development of novel treatment options for diabetic neuropathy.

Diabetic neuropathy, specifically diabetic distal symmetric polyneuropathy (DSPN), is a common complication of the disease. Estimates vary, but it's believed up to 50% of diabetics will develop neuropathic pain over the duration of their disease.[2] Pharmacological intervention is especially important for the management of DSPN for Type II diabetics and there are limited therapeutic options.[3] The need for additional pharmacological options requires the availability of reliable, and reproducible, animal models to conduct *In vivo* efficacy studies during drug development.

The Challenge

The streptozotocin (STZ)-induced diabetes mellitus rat model is commonly used to test drug candidates. Two key challenges with the STZ model are acute toxicity and animal husbandry. Animal age plays a key role in minimizing acute toxicity, the lowest mortality rates are achieved with rats that are 6-11 weeks old at induction.[4] High levels of urine and diarrhea make animal husbandry critical to assure that desired pain endpoints are reproducibly met. Animal cleanliness is critical for endpoints such as thermal hyperalgesia and mechanical allodynia to assure that reproducible data is generated.

This white paper provides information on the STZ-induced diabetic pain rat model from Pharmacology Discovery Services (PDS). Robust, and highly reproducible, pain endpoints are achieved by precise induction of diabetes and thorough animal husbandry techniques throughout the course of a study. Information on the techniques used, and data generated with this model, for the evaluation of drug candidates that target DSPN is provided.

STZ-Induction of Diabetes in Rats

Groups of 10 male Sprague Dawley rats weighing 225 - 250 grams that are 7-8 weeks old are employed. The rats are provided by BioLasco Taiwan. Space allocation for 2 - 3 animals is 45 x 23 x 21 cm with frequent bedding changes (3 times per week). All animals are maintained in a well-controlled temperature (20 - 24°C) and humidity (30% - 70%) environment with 12 hours light/dark cycles. Free access to standard lab diet and autoclaved tap water were granted.

Streptozotocin (STZ; 50 mg/kg) freshly dissolved in 0.1 M citrate buffer (pH 4.5) is injected intraperitoneally (IP) on Day 0. Three days later, diabetes is confirmed by existence of blood glucose >350 mg/dL as measured by glucometer. STZ (50 mg/kg, IP) significantly increased blood glucose levels when compared to sham rats with a success rate of 85 % (Figure 1). Any animals with the following criteria would be euthanized prior to conducting a neuropathic pain study:

- Body weight loss exceeding 20% of their pre-treatment weight
- Body weight is less than 80% of the control group's mean body weight
- Severe autonomic syndromes, such as morbidity, severe dehydration (more than 8%), severe diarrhea, limited mobility and /or activity, failure to eat and/or drink

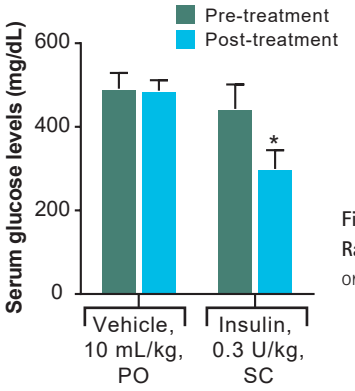

Figure 1. STZ-Induction of Diabetes in Rats. *p <0.05, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

Neuropathic Pain Study Endpoints

Animal health is monitored biweekly for 14 days, at which point the animals are tested prior to study inclusion to confirm the induction of mechanical allodynia and thermal hyperalgesia following STZ treatment. Mechanical allodynia is assessed using the Chaplan up/down method employing von Frey filaments on the plantar surface of the hind paw. Thermal hyperalgesia is assessed by the Hargreaves method, using a focused light beam on the plantar surface of the paw. Test substance and vehicle are administered systemically by any route (pre-treatment) to groups of 10 animals at pre-selected post dose time points before the level of mechanical allodynia and thermal hyperalgesia are determined (post-treatment).Highly reproducible data is achieved for both endpoints which enables an accurate assessment of test articles. In Figure 2; Gabapentin (100 mg/kg, IP) significantly reversed STZ (50 mg/kg, IP) induced diabetic neuropathic pain when compared to vehicle treated rats at 0.5 and 2 h post dose as measured by mechanical allodynia. In Figure 3; Gabapentin(100 mg/kg, IP) significantly reversed STZ (50 mg/kg, IP) induced diabetic neuropathic pain when compared to vehicle treated rats at 1 and 2.5 h post dose as measured by thermal hyperalgesia.

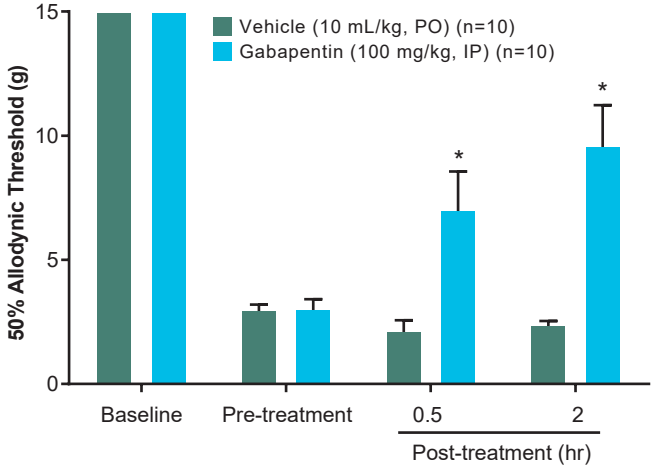

Figure 2. The Effects of Gabapentin on STZ induced Mechanical Allodynia. *p <0.05, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

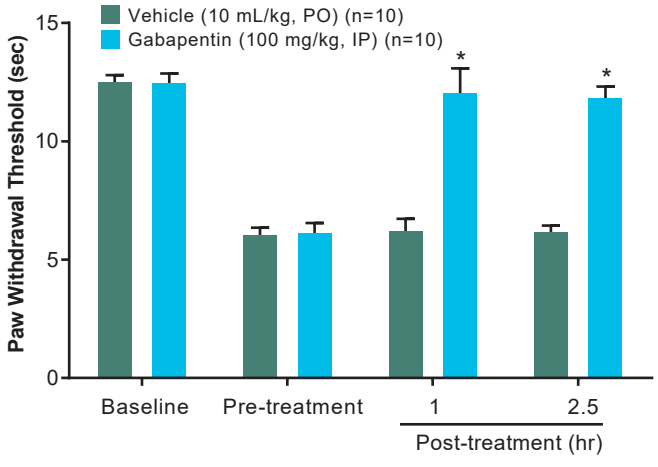

Figure 3. The Effects of Gabapentin on STZ induced Thermal Hyperalgesia. *p <0.05, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

Conclusion

Successful induction of diabetes and animal husbandry throughout the duration of a study are both critical to achieve reproducible mechanical allodynia and thermal hyperalgesia study endpoints in the STZ-induced pain model. To induce diabetes PDS uses rats that are 7-8 weeks old. With respect to animal husbandry the previously described procedures are followed throughout the duration of a study. These husbandry procedures allow the rats to be maintained in standard bedding with frequent bedding changes (3 times per week). Following these procedures, PDS has not had to euthanize any animals after the induction of diabetes in previous STZ-induced diabetic pain studies.

Housing, experimentation, and animal disposal are performed in general accordance with the "Guide for the Care and Use of Laboratory Animals: Eighth Edition" (National Academies Press, Washington, D.C., 2011) in our AAALAC-accredited laboratory animal facility. In addition, the animal care and use protocol was reviewed and approved by the IACUC at Pharmacology Discovery Services Taiwan, Ltd.

References

1. Cho NH *et al.*, IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018 Apr; 138:271-281.
2. Callaghan BC *et al.*, Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol.* 2012 Jun; 11(6):521-34.
3. Pop-Busui R *et al.*, Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care.* 2017 Jan;40(1):136-154.
4. Wang-Fischer Y *et al.*, Improving the Reliability and Utility of Streptozotocin-Induced Rat Diabetic Model. *J Diabetes Res.* 2018 Sep 23; 2018:8054073.

For more information visit: PharmacologyDiscoveryServices.com

Pharmacology Discovery Services is a partner lab of Eurofins Discovery