

## **STZ-Induced Diabetes Model – Protocol**

### **OVERALL DESIGN**

Adult male Sprague-Dawley rats are first assayed for their paw-withdrawal thresholds following standard mechanical stimulation (von Frey hairs). The animals then receive injections of streptozotocin to induce insulin-dependent diabetes mellitus. This condition is confirmed by assay of blood glucose levels. Once the condition is confirmed, the animals' paw withdrawal thresholds are re-determined, and only animals with significant decreases in withdrawal threshold compared to their pre-diabetes level are examined further.

In this particular study, animals to be tested further will be randomly assigned to one of 5 groups: three test compound groups (each receiving one of three doses), vehicle only, and positive control (gabapentin). Initial sample size will be 12/group, with final group sizes of at least 10. Each animal will be dosed five times, once daily just prior to testing. The behavior test assays the sensory response of the animals to mechanical stimulation (paw withdrawal threshold), which will then be compared to the animals' pre-diabetes level and to their post-diabetes/pre-drug level of threshold stimulation.

### **ANIMALS**

The rats to be used in this study (male Sprague-Dawley, 250gbw – 300gbw) will be ordered from a commercial supplier and housed in a USDA-approved laboratory. All housing, surgical, and behavior testing facilities, as well as the surgical and behavior testing procedures themselves, have been approved by the relevant Institutional Animal Care and Use Committees.

The total number of animals to be used in this study (12/group x 5 groups = 60) are based on previous findings in this laboratory in which statistically significant differences between experimental and control groups have been found with sample sizes of 10 - 12. Statistical analysis based on an effect size of 0.4 and a standard deviation of 0.5 requires a sample size of 10 subjects per group for a 0.80 level of power.

## **TEST COMPOUNDS**

All test compounds will be supplied by COMPANY, plus instructions for their safe handling and storage. Instructions for preparing the test compounds in vehicle will also be furnished by COMPANY, along with the vehicle itself if the latter is not commercially available. COMPANY will also determine the dosages at which the test compounds will be administered. The positive control (gabapentin) dose will be 100 mg/kg. At the end of this study any remaining test compound will be returned by NDI to COMPANY.

Route of administration will be oral gavage for all compounds, as well as for vehicle-only and gabapentin.

## **INDUCTION OF DIABETES**

Animals will be injected with streptozotocin, dissolved in 0.9% sodium chloride, twice on successive days (75 mg/kg each day, i.p.). Blood glucose level is assayed at one week post-injection, from samples taken from the tail vein, using standard test strips and colorimeter. Only animals with a blood glucose level  $\geq 15$  mM will be considered diabetic.

## **BEHAVIOR TESTING**

Behavioral testing will occur three times: (1) at baseline (pre-diabetes), (2) pre-treatment (post-diabetes), and (3) post-treatment with the test compound.

For all behavior testing, animals will be retrieved from the colony room and habituated to a 30 x 30 x 30-cm Plexiglas test chamber on top of a mesh screen for 15 min. The size of the chamber allows for free movement of the animal and the mesh screen allows for application of calibrated von Frey monofilaments to the plantar surface of each hindpaw.

*Mechanical withdrawal threshold.* The animals are tested to determine mechanical paw withdrawal threshold using the up/down technique (Dixon, 1980; cf. Fuchs et al., 1999). The force applied to each paw by the monofilaments will range from 4 mN to 217 mN. The mechanical withdrawal threshold is calculated by averaging across 3 trials (15 min per trial) of the test session. For each trial, the 50% withdrawal threshold will be calculated using the following formula:  $[X_{th}]_{log} = [vFr]_{log} + ky$  where  $[vFr]$  is the force of the last von Frey used,  $k = 0.2268$  which is the average interval (in log units) between the von Frey monofilaments, and  $y$  is a value that depends upon the pattern of withdrawal responses.

Following induction of diabetes (see Design, above), only animals that have at least a 25% decrease in withdrawal threshold compared to baseline will receive a test compound. These animals will be randomly assigned to one of

the 5 groups described above, and treated with one dose of one test compound, vehicle, or gabapentin. Post-treatment testing of mechanical paw withdrawal threshold will be performed 30 min following dosing.

### **STATISTICAL ANALYSES**

Paw withdrawal thresholds at all four testing time points will be subjected to a repeated-measures ANOVA with Group as the main factor, followed by post-hoc within-group comparisons of the four testing time points using Fischer's HSD test. The results will be presented graphically as group means plus-or-minus standard error. A test compound that reduces mechanical hyperalgesia at either of the two post-treatment times should be significantly different from post-diabetes/pre-treatment testing. A test compound that completely reverses diabetes-induced mechanical hyperalgesia should not be significantly different from baseline.

### **REFERENCES**

Dixon, W.J. Efficient analysis of experimental observations. (1980) **Annual Review of Pharmacology and Toxicology**, 20: 441-462.

Fuchs, P.N., Ringkamp, M., Shiotani, M. and Raja, S.N. (1999) Sympathectomy decreases formalin-induced nociceptive responses independent of changes in peripheral blood flow. **Experimental Neurology**, 155: 95-102.