

Development of Protocols to Optimize Treatment and Prediction of Type 1 Diabetes in Rodent Models



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Introduction

Clinical trials of agents to prevent or reverse type 1 diabetes (T1D) depend on robust pre-clinical data using animal models of the disease. Development of agents for preventing and treating T1D is hampered by the difficulty of transitioning small scale basic research findings to clinical development. The cost of pre-clinical *in vivo* evaluation in animals, in particular the cost of maintaining and supplying necessary model systems, can be prohibitive. To address the need for preclinical screening of new therapeutics, the National Institute for Diabetes, Digestive and Kidney Diseases (NIDDK) has developed the Type 1 Diabetes Preclinical Testing Program (T1D PTP). Under this program, a contract testing facility bridges the gap between discovery and clinical testing.

In people with T1D, the goal is to maintain nearly normal glucose levels, accomplished by injection of insulin several times per day based upon rigorous monitoring of glycemic levels. Current blood glucose control in diabetic rodent models focuses on maintaining the diabetic animal in a state of moderate glycosuria, with normal weight gain in the absence of severe ketonuria. This is achieved by once daily injections of titrated insulin doses or implantation of continuous release insulin pellets. The T1D PTP plans to standardize insulin treatment methods in NOD mice and BBDP rats, and to achieve more stringent control of blood glucose levels to more closely resemble the human condition. It is expected that the lessening of hyperglycemic stress will provide the best chance for beta cells to function properly while interventions are being tested.

Ongoing research in the diabetes field suggests that the best time to begin treatment to prevent/reverse diabetes may be during the pre-diabetic stage prior to overt hyperglycemia. This type of interventional treatment would allow a compound to have an effect while some beta cells are still remaining. Glucose tolerance tests (GTT) can be used to identify animals likely to convert to hyperglycemia with a few weeks time. T1D PTP also plans to standardize GTT testing in rodent models of disease to identify pre-diabetic animals for testing of agents.

Methods

Animal Models

- *NOD/LtJ Mice
- *BBDP/Wor Rats

Diagnosis of Diabetes

- *Mice were bled 2x/week beginning at 10 weeks of age
- *Animals with BG>250mg/dL for 2 consecutive days were considered diabetic
- *Rats were screened 2x/week for glycosuria
- *Animals with glycosuria and plasma glucose level >250mg/dL were considered diabetic

Insulin Treatment Protocols

- *Insulins were injected subcutaneously (sc) delivered sc continuously
- *Blood glucose levels were measured at various times relative to insulin administration
- *Factors considered in development of protocol
 - *Type of insulin
 - *Protamine zinc insulin (PZI)
 - *Humulin R, 50/50 and 70/30
 - *Dose
 - *Timing of dose relative to fed state
 - *Frequency of dosing
 - *Delivery method (injection vs continuous delivery via Alzet osmotic pumps)

Glucose Tolerance Testing (GTT)

- *NOD mice tested at 12, 14 or 16 weeks of age
- *Injected intraperitoneally (ip) with 2g/kg glucose
- *Blood glucose levels measured at 0, 30, 60, 90 or 120 minutes relative to glucose administration
- *Blood glucose vs time was plotted and the area under the curve (AUC) was calculated

Results

Insulin Treatment of NOD Mice

Figure 1: Injection of Insulin in NOD Mice but is not Sufficient to Maintain 24 Hour Control

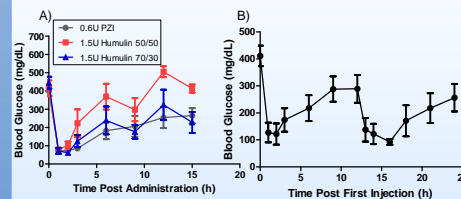


Figure 1: A) 15 diabetic female NOD mice (duration of diabetes ranging from 1-6 days) were randomized into 3 treatment groups based upon duration of diabetes. Insulin was diluted 1:2 with the appropriate diluent prior to injection. Animals were given a single injection of the indicated dose of insulin 1 hour after the lights turned off. Blood glucose measurements were taken at 0, 1, 2, 3, 6, 9, 12 and 15 hours relative to insulin administration. B) Diabetic NOD mice were administered 0.6U of diluted PZI at time 0 (diluted 1:2 with diluent) and 0.2U of diluted PZI 12 hours later. Administration of insulin corresponded to 1 hour after lights off and 1 hour after lights on. Blood glucose measurements were taken at 0, 1, 2, 4, 6, 9 and 12 hours relative to insulin administration. Average blood glucose levels +/- SEM are shown for 5 animals per group.

Figure 2: Continuous Delivery of Insulin Maintains Long-term Glycemic Control in NOD Mice

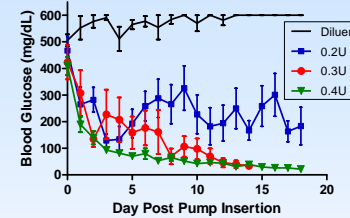


Figure 2: Diabetic female NOD mice with a duration of diabetes <10 days were implanted with 14 day Alzet pumps. Pumps contained either diluent (black line) or Humulin R at a concentration to provide for release of either 0.2U (blue squares), 0.3U (red triangles) or 0.4U (green circles) of insulin over a 24 hour time period. Daily blood glucose levels were measured approximately 8-9 hours after lights on. Two mice in the 0.4U group had to be taken out of study due to low blood sugar levels. N=3/group for diluent; 6/group for 0.2 and 0.3; and 12-15/group for 0.4.

Insulin Treatment of BBDP Rats

Figure 3: Two Daily Doses of PZI Maintains Glucose Control in BBDP Rats

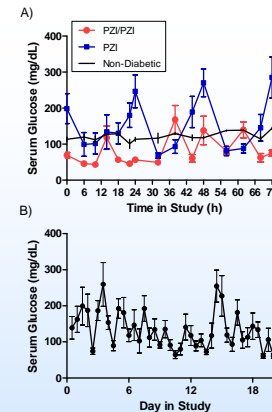


Figure 3: (A) Groups of 12 diabetic male BBDP rats were treated with either a single daily titrated dose of PZI (blue squares) or 2 daily titrated doses of PZI (red circles) while age-matched, nondiabetic male BBDP rats were left untreated (N=6; black line). Serum glucose levels were measured every 6-8 hours. (B) A group of 11 diabetic male BBDP rats were treated with 2 daily titrated doses of PZI for three weeks. Serum glucose levels were measured every 12 hours. Averages +/- SEM are shown.

GTT Testing in NOD Mice

Figure 4: GTT Testing at 14 Weeks of Age Identifies Pre-Diabetic NOD Mice

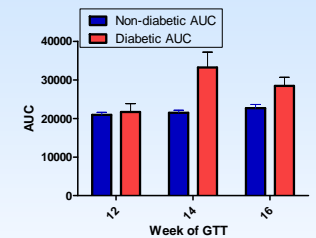


Figure 3: Groups of 50 non-diabetic NOD mice underwent GTT testing at either 12, 14 or 16 weeks of age. A single dose of glucose (2g/kg) was injected intraperitoneally. Blood glucose levels were measured at 0, 30, 60, 120 and 180 minutes relative to glucose administration. Blood glucose levels over time were plotted for each mouse and the area under the curve was calculated. Mice were held for 2 weeks post GTT testing to determine which animals became diabetic. The average AUCs of mice that turned diabetic versus those that did not turn diabetic were compared for each week of testing. Differences in the average AUC between diabetic and nondiabetic animals was highly significant when animals were tested at 14 weeks of age (p<0.001) and significant when animals were tested at 16 weeks of age (p<0.05).

Conclusions

- *Injection of PZI, Humulin 50/50, Humulin 70/30 insulin is not sufficient to maintain 24 hour blood glucose control in NOD mice
- *Subcutaneous delivery of Humulin R insulin in NOD mice results in 24 hour blood glucose control
- *Blood glucose levels are dependent upon dose of insulin delivered
- *In contrast to NOD mice, blood glucose levels are well controlled in BBDP rats with two daily doses of PZI
- *Preliminary data suggests that GTT testing identifies prediabetic mice when conducted at 14 weeks of age

Acknowledgements

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