The Development of an Inquiry-Based Laboratory Module Exploring the Pathophysiology of Diabetes

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Abstract

Histotechnology is a commonly used tool in medical research, pathological testing, and pharmaceutical development. Given this, we designed an inquiry-based laboratory module that equips our students with knowledge of tissue sampling, processing and imaging so that they are ready for professional careers in the biomedical sciences. Treated rats were injected with streptozotocin (a known diabetagen that destroys pancreatic beta cells) while control rats were injected with buffer solution. Rats were sacrificed one week following treatment. Pre- and post-injection weights were compared following one week of treatment, as well as final blood samples for glucose analysis and insulin determinations using an enzyme-linked immunosorbent assay (ELISA). Additionally, pancreatic tissue was collected and fixed in Bouin's fixative. Paraffin embedded tissue was sectioned using a microtome, and hematoxylin/phloxine staining was performed by the students. The number of islet beta cells were compared between control and treated rats. Blood glucose measurements demonstrated that streptozotocin-treated rats had significantly higher blood glucose levels and lower beta cells numbers, while the ELISA tests indicated that treated rats had lower blood insulin concentrations. Following this three-week laboratory module, students scored higher on competency tests and presented an individual poster with images and quantitative data analysis that included insulin concentrations, blood glucose levels, and histological images of pancreatic islets, in addition to beta cell quantification. Overall, students gained hands-on experience with hypothesis testing and an understanding of the pathology of diabetes.

Introduction

- There is a need for an inquiry-based an inquiry based scientific education where students learn to acquire knowledge on their own, giving them an increase in understanding of the scientific method, an increase scientific literacy, and direct practice in the processes of hypothesis testing (Riga et al., 2017).
- We developed a three week inquiry-based laboratory module that was designed to introduce students to methods in histotechnology, ELISA interpretation, and data analysis.

Learning Objectives:

- Connect the pathophysiology of diabetes with the clinical manifestations of diabetes (e.g. changes in lives histology, decreased weight and insulin levels, and increased blood glucose levels).
  - Understand how streptozotocin causes diabetes.
  - Discuss how β cells produce insulin.
  - Demonstrate how insulin controls blood glucose and affects weight and causes other pathologies.
  - Explain why histological examination of tissues is important for understanding pathophysiology.
  - Classify cells of the liver and relate changes from normal to the onset of diabetes.
  - Explain the relationship between histology and the pathogenesis of diabetes.
  - Explain what a competitive ELISA is and how it is used to determine insulin levels.

Materials & Methods

Rats were:

- Weighed and injected with 65 mg/kg Streptozotocin (a known diabetogen)
- Provided food and water *ad lib* for one week
- Sacrificed using a CO_2 overdose and weighed
- Blood was collected, measured for blood glucose levels and serum was isolated and stored at -20°C
- Tissues (e.g. pancreas, liver, kidney) were removed and placed in Bouin's fixative.

Tissue Analysis:

- Pancreatic tissue was embedded in paraffin and sectioned using a microtome.
- Tissue was stained using Hematoxylin/Phloxine Histology (Bancroft and Stevens, 1990).
- The percentage of β cells between control and treated animals was compared.

Body Weight:

- Weights were obtained from control and streptozotocin-treated rats pre- and post treatment.
- Weights were compared to determine change in weight for each group.

Blood Glucose Levels:

- A drop of blood was placed on a glucose monitor test strip
- Blood glucose levels were recorded and compared.

ELISA:

- Plasma was obtained from each animal.
- An ELISA was performed according to the instructions provided by Fisher Scientific (2015).
- A standard curve was created using excel.
- Insulin concentrations (μIU/mL) of all rats were obtained using the equation of the line (see Figure 3).

Assessment:

- Students were assessed using a pre- and posttest that was based on the learning objectives of the module.
- Additionally, students prepared lab reports in the form of posters for summative assessment.

Figure 1: Students in BIO 4640 processing and imaging tissue samples.

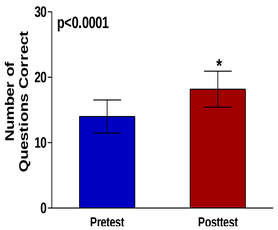

Figure 2: Students in BIO 4640 answered 25 questions (prepared by the PI's) before and after this laboratory module. Following the completion of the laboratory module and preparation of the lab reports, students scored significantly higher on the posttest when compared to the pretest. (Paired t-test; p<0.0001; N=22)

Results

Tissue Analysis:

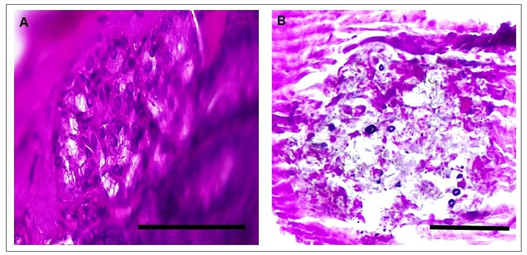

Figure 3: Following sectioning, students stained and imaged pancreatic islets from control (A) and streptozotocin-treated (B) rats.

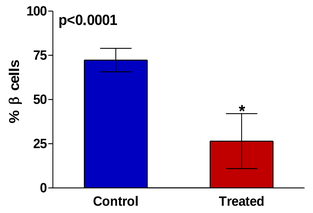

Figure 4: Students analyzed images of the pancreas for control and treated rats and calculated the percentage of β cells present in the pancreas. There were significantly more β cells in control rats, when compared to treated rats. (t-test, p<0.0001)

Body Weight and Blood Glucose Levels:

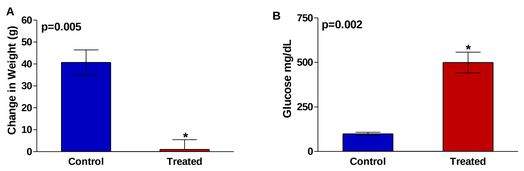

Figure 5: Following treatment with streptozotocin, rats gained significantly less weight (A) and had significantly higher blood glucose levels (B). (t-test)

ELISA:

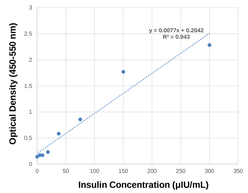

Figure 6: Students prepared a standard curve of known insulin concentrations and calculated the equation of the line in order to determine the insulin concentrations (μIU/mL) for control and treated rats.

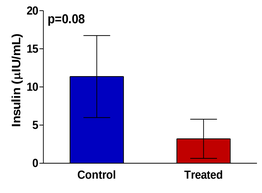

Figure 7: Following the completion of the ELISA, students determined that control rats have slightly higher insulin levels than streptozotocin-treated rats. (t-test; p=0.08)

Conclusions

- The National Science Foundation (2011) Vision and Change document calls on scientific educators to actively involve students in their learning process, rather than make them passive learners.
- We have developed an inquiry-based laboratory module that allows students to link changes in tissue morphology, blood glucose and insulin levels and body weight with the destruction of pancreatic β cells.
- Students found that streptozotocin causes toxicity to β cells, indicated by the disrupted β cell islets in images of pancreatic tissue of streptozotocin-treated rats compared to the control rats, this was additionally confirmed by comparing the percentages of β cells in the pancreatic tissue of these rats.
- Further, they found that β cell disruption lead to malfunctioning of insulin release and glucose metabolism as indicated by increased blood glucose levels and weight loss in streptozotocin-treated rats.
- Overall, our students were able to integrate the information regarding the causes and effects of diabetes by performing various laboratory experiments and presenting the information in a cohesive laboratory report.

Future Directions

- Design and develop a histotechnology course using this module as a foundation for teaching preparation, sectioning and staining.
- Modify this a module for use in the freshman Anatomy and Physiology Laboratory I (Nursing A&P) course.
- Have students perform other staining procedures (e.g. immunocytochemistry and hematoxylin and eosin) on other tissues (e.g. skeletal muscle, liver and kidney) to determine if other morphological changes are present.
- Compliment this module with a Course-based Undergraduate Research Experience (CURE) using histotechnology in order to introduce students to hypothesis testing and data analysis.

References

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