Expression of cyclophilin D and its effect on diabetes in the Akita mouse

Katherine H. Smith¹, David Rometo, MD¹, Kenneth Polonsky, MD¹, Burton Wice, PhD¹

¹Washington University School of Medicine, Department of Medicine
Division of Endocrinology, Metabolism, and Lipid Research

ABSTRACT

Objective: To determine if diabetes can be genetically ameliorated in the Akita mouse

Introduction: The Akita mouse is a model of diabetes in which there is a spontaneous mutation in the insulin gene that leads to increased beta-cell death. Previous studies in another mouse model, Pdx1 deficient mice, have shown that beta-cell death can be decreased by the ablation of cyclophilin D, a component of the mitochondrial permeability transition pore. This effectively cures diabetes in the mice. The purpose of this study is to determine whether decreased expression of the Ppif gene, the gene that encodes cyclophilin D, will ameliorate diabetes in the Akita mouse, as well.

Methods: Akita mice were crossed with mice that lack the Ppif gene, i.e., Ppif-/- mice to create an Akita model with decreased cyclophilin D expression. At eight weeks of age, the Akita and the Akita Ppif-/- mice underwent intraperitoneal glucose tolerance tests. Also, the pancreata from the mice were harvested, mounted on slides, stained for insulin, and examined under a microscope to determine if beta-cell mass was restored by the change in expression of cyclophilin D.

Results: The ablation of the Ppif gene showed no effect on the intraperitoneal glucose tolerance test of the mice. Likewise, the Akita Ppif-/- mice showed no increase in beta-cell mass when compared to the beta-cell mass of the Akita mice.

Conclusion: Unlike in previous studies, decreased cyclophilin D seems to have no alleviating effects on diabetes in the Akita mouse model. This implies that the beta-cell death in the Akita mouse and in the Pdx1 deficient mouse occur via different pathways. Further research is needed to investigate these pathways and to apply the findings to genetically treat diabetes in humans.

METHODS

All animal studies used protocols approved by the Washington University Animal Studies Committee.

Genetic Ablation of Ppif gene in the Akita mice

The previously described Akita-/- and Ppif-/- mice were bred to obtain Akita-/-/Ppif-/- mice.

The pups were genotyped to determine their expression of the Akita and Ppif genes using PCR and restriction enzyme digestion.

Glucose Tolerance

The mice were placed on standard chow (Harlan Laboratories) at weaning.

Ambient glucose levels were measured periodically throughout the study.

Intraperitoneal glucose tolerance tests (IPGTTs) were performed after a 16-h fast by administering 2 g dextrose/kg body weight and serially monitoring blood glucose concentrations every 30 minutes intervals for two hours.

Beta-cell Mass

Pancreata were harvested from the mice at 12 weeks of age.

Formalin-fixed pancreas sections were stained for insulin to identify the insulin-producing cells, found in the islets, in the pancreas of the mice.

The sections were also counterstained to identify all the pancreas tissue.

The areas of both the insulin-producing cells and the whole pancreas were measured and the ratio of these areas was recorded.

RESULTS (cont’d)

- The ablation of the Ppif gene had no ameliorating effect on either the diabetic phenotype of the Akita mice or the beta-cell mass.
- This implies that beta-cell death (and/or lack of proliferation) occurs via a different pathway in the Akita mice, compared to the Pdx1 deficient mice from the previous study.
- This conclusion is logical as the Pdx1 deficient mice more closely resemble type 2 diabetes in humans, while the Akita mice mirror type 1 diabetes.
- While these findings cannot be directly applied to diabetes in humans, the results of this study lead us to believe that different approaches must be taken to cure the two types of diabetes in humans. However, further research is needed to investigate these beta-cell death pathways further and to apply the findings to genetically treat diabetes in humans.

REFERENCES