DIABETES INDUCED NEPHROPATHY IN THE T2DN RAT

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Abstract

A unique rat model of type 2 diabetes has been created that develops diabetic nephropathy and mimics the progression of the disease seen in humans. To produce this model, the diabetic GK was crossed with FHH, a hypertension-induced end stage renal disease model, to produce the new T2DN rat. Previous characterization of this normotensive model shows that these rats exhibit thicken of basement membranes, focal segmental glomerulosclerosis, interstitial fibrosis, and formation of protein casts. The damage increases with age and by 18 months has advanced to severe global glomerulosclerosis with acellular nodules resembling Kimmelstiel-Wilson nodules characteristic of human diabetic nephropathy. To evaluate whether the development and progression of nephropathy is due to diabetes or influenced by the genetic background from FHH, 12 month old T2DN were uninephrectomized to accelerate renal damage and treated with either exogenous insulin (4 U/day) or glyburide (2, 5, or 7 mg/kg/day). Fasting blood glucose was reduced from 174.0 13.8 mg/dL to 75.0 7.0 mg/dL in insulin treated animals, while glyburide had no effect on fasting glucose levels. After 4 months, animals treated with insulin had a significant reduction in renal damage with decreased interstitial fibrosis, glomerulosclerosis and protein casts and demonstrate the nephropathy in the T2DN is a dependent on hyperglycemia. The T2DN has become the new model of choice for the identification of new targets for the treatment of diabetic nephropathy and proof-of-therapeutic-concept studies.

Background

Diabetes-Associated Renal Disease

Diabetes-associated renal disease remains the major cause of ESRD in the United States. Health Care Costs for dialysis alone exceed 25 Billion dollars a year.

Reason for lack of progress in preventing diabetic nephropathy

Lack of models for screening compounds for new drug therapies. No rodent model of diabetes exhibits progressive renal disease and lesions resembling those seen in man. Cell models not particularly informative.

Solution: We combined the genome of the FHH rat that develops renal disease but not diabetes with that of the GK rat that develops type II diabetes but not renal disease.

Characteristics of Human Diabetic Nephropathy

Progressive proteinuria and declining GFR (elevated Pcr)

Glomerulosclerosis with thickening of glomerular basement membranes, increase in mesangial matrix deposition and formation of Kimmelstiel Wilson nodules, vascular sclerosis and hylanosis, renal tubular necrosis, renal interstitial fibrosis.

Model Characteristics

Non-obese model of type 2 diabetes
Elevated fasting glucose levels (~200 mg/dL compared to <100 mg/dL for control)
Elevated insulin levels (1.75-2.5 ng/dl compared to <1.0 ng/dL for control)
Elevated glycosylated hemoglobin levels (7-9%)
Retinopathy and peripheral neuropathy, vascular dysfunction
Exhibits lipid abnormalities
- Cholesterol ~200 mg/dL compared to <100 mg/dL for control
- Triglycerides ~350 mg/dL compared to <75 mg/dL for control

Progressive proteinuria increasing to ~500 mg/day by 15 months of age compared to ~25 mg/day for age matched controls
Useful for studying drugs that
- Enhance β cell function (young animals)
- Preserve β cell function
- Improve insulin sensitivity (adults)

Methods

Twelve month old T2DN were uninephrectomized to accelerate renal disease.
Intraperitoneal glucose tolerance test (IPGTT)
Exogenous insulin (4 U/24 hours) was delivered by implanted insulin pellet (Linshin Canada, Inc.).
After 4 months of treatment with insulin or vehicle the kidneys were collected fixed in 10% formalin, sectioned and stained with Masson's Trichrome stain.

Results

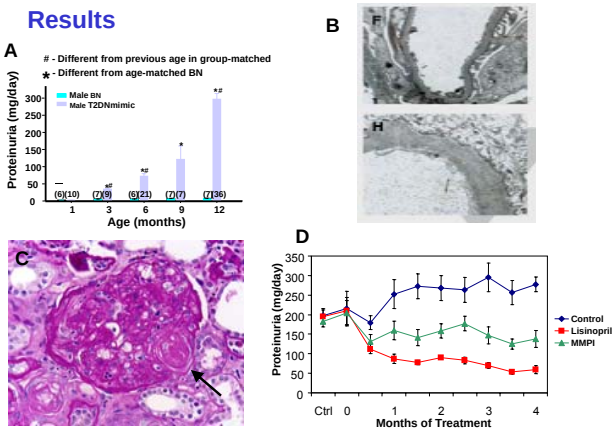

Figure 1. Characteristics of the T2DN rat include (A) progressive proteinuria, (B) thickening of the basement membrane and podocyte damage, and (C) nodular glomerulosclerosis. The renal disease progression can be reversed with ACE inhibitors (D).

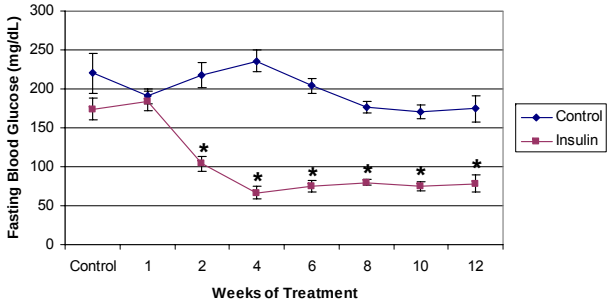

Figure 2. Fasting glucose levels in uninephrectomized of male T2DN rats treated with vehicle or insulin. Animals treated with insulin had significantly lower fasting glucose levels compared to control animals ($p<0.05$).

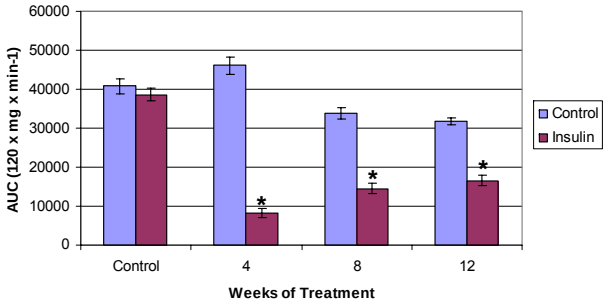

Figure 3. AUC from control and insulin treated T2DN rats following an intraperitoneal glucose challenge. Insulin significantly reduced the AUC ($p<0.05$).

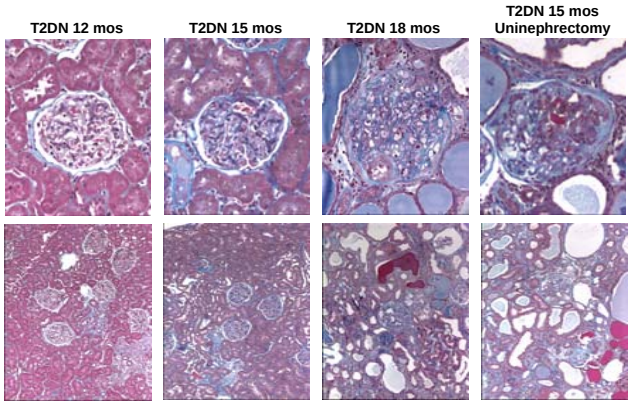

Figure 4. Histological changes in the kidney of the T2DN rat at 12, 15 and 18 months of age. T2DN renal damage includes glomerular hypertrophy, thickening of glomerular and tubular basement membranes, expansion of the mesangial matrix, glomerulosclerosis, and the formation of glomerular nodules by 18 months of age. Uninephrectomizing 12 month old T2DN reduces the time needed for robust development of renal lesions from 6 to 3 months.

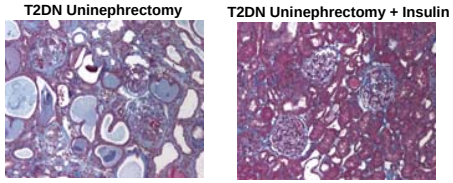

Figure 5. Histological changes in the kidney of the uninephrectomized T2DN rat with and without insulin treatment. Insulin substantially reduced the degree of glomerulosclerosis, renal interstitial fibrosis and tubular necrosis compared to that seen in the control animals.

Conclusions

T2DN is the only diabetic rodent model that develops progressive proteinuria and diabetic nephropathy. It is ideal for the study of new drug therapies for the treatment of diabetes, diabetic nephropathy and related end organ damage.

PhysioGenix provides the model and has 12 month old rats in stock. It offers in vivo phenotyping services for the treatment of diabetes and prevention or reversal of diabetic nephropathy.

PhysioGenix has also identified the regions of the genome that influence diabetic nephropathy and diabetes in this model and can identify the genetic basis of diabetes and renal disease (new drug targets) in an 18 months period.