Establishment of a Type I Diabetic Model of Renal Nephropathy in Rats

Dale E. Mais, PhD
MPI Research, Mattawan, MI, USA

INTRODUCTION

Streptozotocin (STZ)-induced pancreatic injury is commonly used for creating rodent models of type 1 diabetes which develop renal injury with similarities to human diabetic nephropathy[1]. A recent review is available that talks about this model and its usefulness in the study of human diabetic nephropathy[2]. Besides STZ, a variety of other chemical entities have been used in rodents to induce nephropathy. These include gentamycin[3], cisplatin[4], cyclosporin A[5], and ochratoxin[6]. The MPI Research staff chose the STZ-induced method in rats as a model of nephropathy to make available for other researchers.

INTRODUCTION

The objective of this work was to establish a Type 1 Diabetic Nephropathy model in the rat at MPI Research using STZ as the inducer of the diabetic state.

METHODOLOGY

A total of 20 male Crl:CD(SD) rats were assigned to groups 1 and 2, a streptozotocin (STZ)-treated group and a vehicle treated group. The induction article, streptozotocin (STZ), was administered once on Day 1 via intravenous injection into the tail vein. The dose level for the induction article was 60 mg/kg at a dose volume of 1 mL/kg.

Observations for clinical signs were conducted on the day of receipt and before randomization. On occasion, clinical observations were recorded at unscheduled intervals.

Body weights were measured and recorded on the day of receipt, before randomization, and weekly beginning on Day 1.

Food and water consumption was measured and recorded for 24-hour periods beginning before induction and on Days 7, 26, and 64.

Glucose and insulin evaluations were conducted on all animals before induction, and on Days 7, 22, 36, 50, and 64. The animals were fasted for at least 4 hours before sample collection. For glucose analysis, a properly calibrated AlphaTrak™ glucometer was used according to MPI Research Standard Operating Procedure (SOP). For insulin analysis, blood samples (approximately 0.6 mL) were taken from the sublingual vein. No anticoagulant was used for the insulin samples. Blood samples were collected from several animals before euthanasia *in extremis*. Animals were not fasted before these unscheduled collections. The data for these unscheduled collections are not reported, but are maintained in the study file.

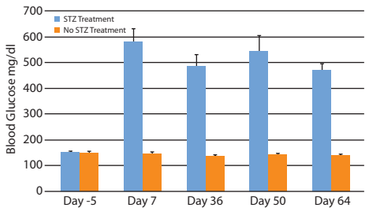
Serum creatinine clearance evaluations were conducted on all animals before induction, and following completion of the corresponding 24-hour urine collection periods on Days 7, 36, and 64. The animals were not fasted before sample collection. For serum creatinine clearance analysis, blood samples (approximately 0.6 mL) were taken from the sublingual vein. A serum separator was used for the serum creatinine clearance samples. Blood samples were collected from several animals before euthanasia *in extremis*. The data for these unscheduled collections are not reported, but are maintained in the study file.

Urinalysis evaluations were conducted on all animals for 24-hour periods beginning before induction and on Days 7, 36, and 64. The animals had access to food and water during the sample collection periods. For urinalysis, the animals were housed in stainless steel metabolism cages and urine was collected. Whenever possible, the urine samples were divided into five aliquots as follows, and stored frozen at -50° to -90°C. Different tests could be performed on these aliquots.

Immunohistochemistry evaluations were performed at MPI Research on designated tissues for a variety of biomarkers, which included Nrf-2, NQO1, Gamma-GCS, Beta-actin, FN, Collagen IV, P21, 8-oxo-dG, TGF-B1, and Nitrotyrosine. Electron microscopy was performed by Experimental Pathology Laboratories, Inc. (EPL) on designated tissues collected at termination.

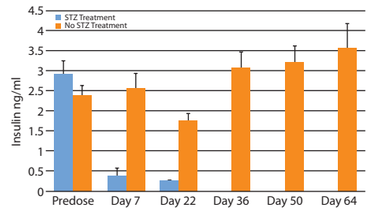
RESULTS

Time Course of STZ-Induced Glucose Changes in Rat



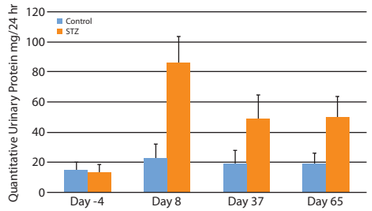
Time course of increased blood glucose during the course of the study in STZ-treated rats compared to control or Day -5 pre-STZ treatment

Time Course of STZ-Induced Insulin Changes in Rat



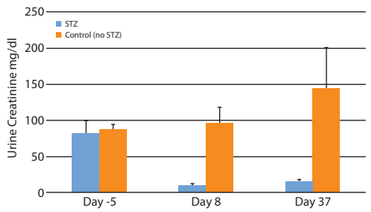
Time course of decreased blood insulin during the course of the study in STZ-treated rats compared to control or Day -5 pre-STZ treatment

Quantitative Urinary Protein

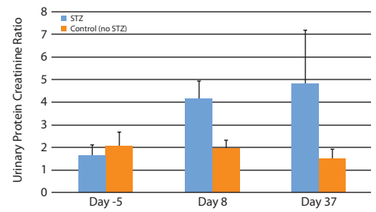


Time course of quantitative urinary protein excretion over a 24-hour period during the course of the study in STZ-treated rats compared to control or Day -4 pre-STZ treatment

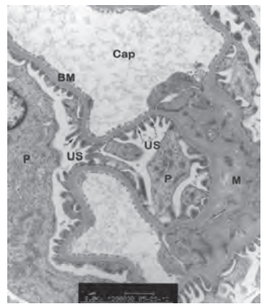
Urine Creatinine



Urinary Protein Creatinine Ratio



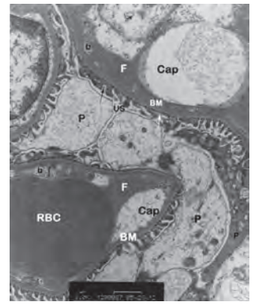
Urinary creatinine and urinary protein creatinine are shown above before STZ treatment (Day -5) and at Days 8 and 37; indicates by Day 8 and beyond kidney dysfunction



Control Kidney EM of glomerular structure

- Part of a glomerulus was shown in greater detail. It shows part of a well-formed podocyte (P) body and nucleus.
- The numerous foot processes and their filtration slits (arrows) were well-formed.
- Abundant urinary space (US) is shown between podocyte bodies and their foot processes.
- Part of a mesangial cell and adjacent mesangial matrix (M) was along right side of the image.
- Basement membrane (BM) had relatively uniform thickness.
- All structures were considered normal.

RESULTS CONTINUED



STZ Kidney EM of glomerular structure

- Marked podocyte (P) swelling and multifocal foot process fusion (arrows)
- Focal podocyte cytosol condensation (degeneration) [see white P]
- Reduced urinary space (US) adjacent to podocytes, capillary endothelium swelling and blebs (b),
- Fibrin (F) deposits in capillary lumens
- Consistent with diabetic nephropathy

DISCUSSIONS & CONCLUSIONS

STZ induced a rapid state of type 1 diabetes in the rat as shown with the glucose/insulin data during the time course of the study. In addition, a variety of markers measured in the blood and urine indicated pathological response of the kidney function during the study.

Differential immunohistochemical staining was present in 8-oxo-dG, TGF-B1, and Nitrotyrosine stains (see table on right).

The glomerular (GL) and tubulo-interstitial (TI) scoring values for 8-oxo-dG staining were highest in the Uninduced Controls with the Vehicle Control scoring values being the next highest.

GL and TI scoring values for TGF-B1 were lower in all STZ-induced groups than in the Uninduced Control group; however, there was a slight increase in scoring value in the 25 mg/kg dh404 treated group when compared to the Vehicle Control group.

GL and TI scoring values for Nitrotyrosine were similar in the Uninduced and Vehicle Control groups and the 5 mg/kg dh404 group. Nitrotyrosine GL and TI scoring values decreased in the 10 and 25 mg/kg dh404 treated groups.

There were no remarkable differences in GL and TI scoring values between groups for Nrf-2, NQO1, Gamma-GCS, Beta-actin, FN, Collagen IV, and P21.

In conclusion, STZ induces type 1 diabetes and over time results in a nephropathy which is measurable with a variety of techniques and biomarkers.

Dose Level	Vehicle Control	Uninduced Control
Number Examined	7	10
Kidney, right		
Vacuolation, tubular	7	0
-minimal	1	0
-mild	6	0
Regeneration, tubular	1	0
-minimal	1	0
-mild	0	0
Necrosis, tubular	5	0
-minimal	5	0
-mild	0	0
Mineralization, tubular		
-minimal	2	0

Summary of Immunohistochemistry Scoring Values (Mean) - Terminal Male

Stain	8-oxo-dG		TGF-B1		Nitrotyrosine	
Group/ Animal	GL	TI	GL	TI	GL	TI
Vehicle Control	0.6	1.2	1.0	1.0	1.4	2.6
Uninduced Control	1.5	2.4	1.5	1.8	1.5	2.7

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