Animal Models of Diabetic Complications Consortium
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Part A:

Principal Investigator's Summary

Diabetic Uropathy

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1. Program Accomplishments:

The overall goals of the diabetic uropathy core within the AMDCC are:

a) To establish assays for phenotyping characterization of the lower urinary tract complications of diabetes, and
b) To perform phenotyping assays on the developed animal models in collaboration with other AMDCC laboratories.

Our main strategy is to:

1) Define the elements of diabetic uropathy in small animals that are representative of the humanoid condition. The challenge for diabetic uropathy is not so much finding the animals in which complications of diabetic uropathy occur, as it appears that the majority of animals that become diabetic develop diabetic uropathy regardless of their inter-species differences. The challenge, therefore, is to discover what really constitutes the changes of diabetic uropathy that are accurate representations of the humanoid condition. No single theory explains the variation in the clinical presentation of diabetic cystopathy. From the reported literature, it appears that diabetic cystopathy can present as a range of filling or voiding bladder dysfunction. Portions of such presentations are shared with other conditions such as diuresis induced bladder dysfunction. During the last year, we have confirmed the presence of diabetic cystopathy in a number of mice used in the AMDCC, including male C57BL/6, Glut-4 knock out, and KLS.
2) Continue to systematically explore, in active communication with the EAC, the findings in small animals until the full extent of diabetes induced specific pathologies representative of human conditions are identified in the animal models.
3) Continuously search and expand the development of assays that would identify diabetic cystopathy beyond the existing methods. An example of this strategy is the exploration of the assessment of afferent autonomic neuropathy in diabetic animals by measurement of the Current Perception Threshold (see project #4 below).
4) Expand the collaboration between our laboratory and other laboratories of the AMDCC by conducting in-vitro assays on the bladder tissues of animals sacrificed in other laboratories and in-vivo assays on animals that are tested or developed in other laboratories.

Major achievements have been:

Project 1: **Daneshgari F, Huang X, Bena J, Saffore L: Temporal Differences in Bladder Dysfunction Caused by Diabetes, Diuresis, and Treated Diabetes in Mice. Submitted to Diabetes Feb 2004.**

Abstract- Diabetic Bladder Dysfunction (DBD) is a common complication of diabetes mellitus (DM) with a poorly understood natural history. This study examined the specific role of DM on temporal changes in bladder function in streptozotocin (STZ)-induced diabetes 3, 9, 12 and 20 weeks after DM in male C57BL/6 mice in comparison to age-matched DM treated with insulin, 5% sucrose-induced diuretic and normal control mice. Conscious cystometrograms (CMG) of

mice were examined in addition to the measurement of micturition and 24 hour urine volumes, post void volumes, and body and bladder weight of the animals. Diabetes resulted in decreased body weight, which reversed with treatment. Bladder weight and urine output increased in DM and diuretic mice. Bladder capacity and compliance increased in the DM and diuretic groups, correlating with increased urine production. Peak micturition pressure (PMP) increased initially in both DM and diuretic mice. However, in DM mice, PMP dropped dramatically at and after 12 weeks to a lower level compared with all other groups. These data suggest that similar changes in bladder capacity, compliance and voiding ability are seen only during the first 9 weeks of diabetes or diuresis, whereas significant decline in the voiding ability of the bladder is seen in diabetes only after 12 weeks of disease in the mice. This data suggest that that bladder undergoes a transition from a compensated to a decompensated dysfunction 9-12 weeks after STZ-induced DM in mice.

Project 2: Liu G, Daneshgari F: Alterations in Neurogenically Mediated Contraction Caused by Diabetes and Diuresis. American Journal of Physiology – Renal Physiology. 2005. Feb 1.

Diabetic bladder dysfunction (DBD) is among the most common and bothersome complications of diabetes mellitus (DM). Autonomic neuropathy has been counted as the cause of DBD. In this study, we compared the alterations in the neurogenically-mediated contractile responses of the urinary bladder in rats with streptozotocin -induced diabetes, 5% sucrose-induced diuresis, and age-matched controls. Male Sprague-Dawley rats were divided into 3 groups: 9-week diabetics, diuretics and age-matched controls. Micturition and morphometric characteristics were evaluated using metabolic cage and gross examination of the bladder. Bladder detrusor muscle strips were exposed to either periodic electrical field stimulation (EFS) or to EFS in the presence of atropine, alpha,beta-methylene adrenasine 5'-triphosphate, or tetrodotoxin. The proportions of cholinergic, purinergic and residual nonadrenergic-noncholinergic (NANC) components of the contractile response were compared among the three groups of animals. Diabetes caused significant reduction of body weight compared to diuresis and controls, although the bladders of diabetic and diuretic rats weighed more than the controls. Both diabetes and diuresis caused significant increase in fluid intake, urine output, and bladder size. Diabetes and diuresis caused similarly increased response to EFS, and reduced response to the cholinergic component compared to controls. However, the purinergic response was significantly smaller in diuretic bladder strips compared with controls, but not in diabetics. A residual NANC of unknown origin increased significantly, but differently in diabetics and diuretics compared with controls. In conclusion, neurogenically-mediated bladder contraction is altered in the diabetic rat. Diabetic-related changes do not parallel diuretic induced changes, indicating that the pathogenesis of DBD needs further exploration.

Project 3: NIH-NIDDK- R41 HD050684-01- Daneshgari (PI): Assessment of Afferent Autonomic Neuropathy in the Diabetic Animal.

Diabetic bladder dysfunction (DBD) or diabetic cystopathy is one of the most common and debilitating complications of diabetes mellitus (DM) affecting well over 50% of patients with

either type I or type II diabetes. DBD could affect both the filling and voiding function of the bladder leading to urinary incontinence, poor emptying, high post void residual, and urinary tract infection. Dysfunction of the autonomic nervous system (ANS) innervating the bladder is suspected to play a major role in the pathogenesis of DBD. Yet, no reliable diagnostic test exists to assist in the detection of disturbances in the ANS of the bladder. We aim to complete the development and testing of a product that will allow laboratory scientists and clinicians to test the status of the afferent ANS of the bladder in animals or human subjects.

The Transurethral Afferent Autonomic Nerve Conduction Threshold (TUR-ACT) Device will incorporate use of a newly developed probe with a commercially available product (Neurometer, Neurotron, Inc.) in measuring the afferent Autonomic Nerve function in the bladder of animals and humans. In Phase I of this STTR application, we will: a) test the feasibility of the use of the TUR-ACT Device to assess the autonomic nerve conduction threshold of the small fiber afferent pathways in the bladders of diabetic and normal rats; and b) test the possible change of the CPT value in a normal rat after placement of a suprapubic tube (SPT) to conduct a cystometrogram (CMG), after intravesical administration of capsaicin, a C-fiber neuro-selective inhibiting agent, and Ice-water. In phase II of this STTR application, we will conduct a pilot clinical study to assess pelvic autonomic neuropathy in diabetic humans in comparison to non-diabetic controls in regard to: a) type of diabetes (Type I or Type II); b) duration of diabetes (time course factor); and c) urodynamic and clinical characteristics of diabetic cystopathy.

The long-term goal of this proposal is to develop a user friendly measurement tool to enable laboratory scientists and clinicians to assess the disturbances of the ANS affecting the lower urinary tract of animals and humans with DBD. The scope and experiments of this proposal are fully responsive to RFA HD-04-018 because they will further the development of a device that will help investigators and clinicians in the detection and/or assessment of the early signs of autonomic dysfunction in bladder dysfunction, and ensure the availability of a quantitative test of autonomic dysfunction that can readily be used in an outpatient setting – all of which are the stated goals of the RFA.

Project 4: Glut-4 Deficient Protects Mice Against Diabetic Bladder Dysfunction. **2005 Annual Meeting of the American Urological Association.**

Introduction and Objectives – Glut-4, a muscle cell-membrane protein involved in the transmembrane transportation of glucose has been used as the therapeutic target for treatment of the complications of diabetic mellitus. Our laboratory has reported the failure of bladder contractility after 9 weeks of diabetes in C57black (C57Bl/6) mice. Our aim was to study the effects of diabetes duration on bladder function in the Glut-4 knock-out mouse.

Methods – Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) in wild type (WT) and age matched male Glut-4k/o/ C57Bl/6 mice. An equal number of age, sex, and weight matched C57Bl/6 and Glut-4k/o/ C57Bl/6 mice were used as controls. One pair from each diabetic and non-diabetic group was housed together with free access to water and food. 34, 36 and 42 weeks after the induction of diabetes, the animals underwent anesthesia by urethane (1.5 g/kg, i.p.) and cystometrogram through a P50 tube placed transurethraly. After sacrifice by decapitation, the whole bladder was removed and weighed. Distribution, mean±SE

of the data on animal weight, glucose level, bladder weight, capacity, compliance and CMG pressure parameters were compared among the three aged groups using a two sided t-test, with a p value of <0.05 considered significant.

Results – A total of 12 animals were used (n=4 in each group). Diabetes caused a significant reduction of the body weight and an increase in the bladder weight of 34 week-WT animals. The changes in the body and bladder weight of the 36 and 42 week age groups of diabetic Glut-4k/o animals were not statistically different from their non-diabetic controls. Although all diabetic animals showed a significant increase in their bladder capacity compared to non-diabetic controls, the diabetic 34 week-WT mice's increase in bladder capacity was twice as much as either age group of Glut-4k/o animals. Most importantly, none of the diabetic Glut-4k/o groups showed reduction in their peak micturition pressure as seen in 34 week old WT mice.

Conclusions – These findings suggest that Glut-4 deficiency has protective effects on the decay of bladder contractility that is seen in STZ-induced diabetes in C57Bl/6 mice. This data supports that notion that diabetic bladder dysfunction could be prevented by manipulation of a glucose transport system.

Project 5: Daneshgari F, Liu G, Imrey P: Time-Dependent Functional Changes of the Urinary Bladder in the Diabetic Rat (submitted to AJP Feb 2005)

Diabetic bladder dysfunction (DBD) is among the most common and bothersome complications of this disease. While both filling and voiding problems have been reported, the precise functional changes in the diabetic bladder remain unclear. To examine the role of diabetes duration on bladder function, 60 male Sprague-Dawley rats were compared to age-matched controls at 3, 6, 9, 12 and 20 weeks after diabetes induction with streptozotocin. In cystometrograms, under urethane anesthesia, peak leak pressure was elevated in diabetes and increased gradually during weeks 3-9 in both diabetic and control animals. However, at 12-20 weeks, the diabetic rats deviated strongly from this trend, with peak leak pressures decreasing and resting pressures after 45 minutes post-void increasing from 9-week levels and compared to controls (p<0.0001 for interactions). In organ bath studies, elevated contractile force responses of diabetic animals to stimulation by carbamylcholine chloride (carbachol, CCh), potassium chloride (KCl), adenosine 5'- triphosphate (ATP), and electric field stimulation (EFS) peaked at 6 or 9 weeks, but at 12-20 weeks generally reverted towards those of controls (interaction p=0.0022 for CCh, p=0.01 for EFS). In combination, these findings suggest that diabetic bladders may undergo a transition from a compensated to a decompensated state, and that transition in the streptozotocin rat model may begin 9-12 weeks after induction.

2. Collaboration within Your Group:

AMDCC investigators – Over the last year, we have shared diabetic animals and experiments with Dr. Tim Kern from Case Western Reserve University and Dr. Eva Feldman, from the University of Michigan. From this collaboration, we have studied CMG in Aminoguanidine treated rats and Glut-4 KO (project #4). A trend is emerging that indicates that due to the geographical proximity of three core laboratories of the AMDCC (Dr. Feldman in Ann Arbor, Michigan; Drs. Kern and Daneshgari, in Cleveland, Ohio), phenotyping assays for diabetic

neuropathy, uropathy, and retinopathy can be performed in the same animal by all three core laboratories with relative logistical ease. If this trend is fruitful, it would be a significant achievement for the core laboratories of the AMDCC.

3. Collaboration with Other AMDCC Groups:

During 2004, the members of our laboratory have regularly traveled to Michigan and conducted in-vivo cystometrogram on a variety of animals that the Michigan group has made available to us. Among those are Glute-4 knock out mice and KLS mice. In addition, we have received bladder material from Duke laboratory on pig models of diabetes. Studies on these specimens are ongoing.

Currently, we are in the process of working out the logistics of conducting in-vivo CMG on the animals of Hush laboratory at UCLA.

4. Pertinent non-AMDCC Collaboration:

Over the last year, we have established collaboration with the following investigators:

a- George Christ, Ph.D. – Professor of Urology, Wake Forest Institute for Regenerative Medicine – collaboration on time-dependent changes in diabetic cystopathy.

b- Kevin McVary, M.D. – Professor of Urology, Northwestern University – this collaboration entails the establishment of in-vivo and in-vitro measurement of sexual dysfunction in mice.

c- Margot Damaser, Ph.D. – this collaboration has evolved around the development of a model for urinary incontinence in female diabetes. The collaboration has resulted in a number of joint projects, two of which have been submitted as R-21 applications to NIH (R21 DK-071143-01).

d- Osamu Ukimura, M.D. Ph.D. from Kyoto University in Japan – this collaboration has resulted in formation of a research projects and in the submission of a R-41 application for assessment of afferent autonomic neuropathy in diabetic animals (project #3).

e- Timothy Ness, M.D., Ph.D. – Professor of Anesthesiology, the University of Alabama – this collaboration is on the development of a technique to measure the viscomotor response of the bladder in anesthetized animals. This method will be used in the measurement of responses of animals to electrical bladder stimulation (project #3).

5. Address Previous EAC Comments:

- His presentations are important because he is more or less working alone, which is unlike the other groups. It is important for Dr. Daneshgari to continue to demonstrate the significance of uropathic pathology in diabetes, and to clearly communicate how individuals collect bladders for further study. This was an excellent presentation.

Response: Thank you for your encouraging words.

- The human condition of diabetic cystopathy is still imprecisely defined. There are no universally accepted criteria for diagnosing this condition and distinguishing it from other age-related voiding dysfunctions. Without this starting point, it is difficult to assess the validity of any animal model. Continued efforts *must* be made in this area.

Response: Agreed 100%. This relates to defining the humanoid condition of diabetic cystopathy. The PI is actively involved in this area, which is outside the AMDCC objectives and work. We have submitted an application to characterize the bladder dysfunction in the adult diabetic. Our application is currently under review (NIH-NICHD RO1-HD 041162).

- On reviewing the conscious CMG in control and diabetic mice (slide 13), it would appear that the diabetic mice have a later onset of uninhibited contractions and that this increases with duration of diabetes. Is this real? If so, it could point to an alteration in afferent function as well as detrusor overactivity.

Response: Yes, the observation of uninhibited contractions in the early phase of diabetic cystopathy is real and has been reported by us and our collaborator (Dr. George Christ). We have designed additional experiments to explore and define the conditions for this observation.

- Slide 18 clearly suggests that diuresis plays a role in increasing bladder capacity. This is a very important and clinically relevant observation that may tease out one element of the complex picture that is diabetic uropathy.

Response: Detailed statistical analysis of the data shows the presence of similar trends in increasing bladder capacity among diabetic and diuretic animals, although it is at a higher level in DM than in the diuretic group. The results of detailed analysis will be presented at the March 2005 AMDCC meeting.

- There's an interesting divergence in peak pressure (slide 19) in the diabetic and diuretic mice. This suggests that the diabetic condition interferes with the detrusor compensation to the demand of diuresis. This could have real import for the combination of DM and BPH.

Response: The finding of changes in the detrusor compensation is perhaps the most important finding of the two recent studies in our laboratory (project #1-3)). This finding supports the disease specific changes occurring in the diabetic bladder beyond that of the effects of diuresis on bladder function. The results of detailed analysis will be presented at the March 2005 AMDCC meeting.

- The effort to measure and quantitate changes in bladder sensation are an important and clinically relevant addition.

Response: Our effort in the measurement of bladder sensation is continuing. We have submitted a R-41 proposal on further development of methodology of this testing and are actively planning to conduct pilot studies in our lab in collaboration with Drs. Osamu Ukimura and Timothy Ness. We will report progress in the project to the AMDCC meetings accordingly.