Effect of statil on kidney structure, function and polyol accumulation in diabetes mellitus

Gregg Faiman,¹ Paul Ganguly,¹ Adi Mehta² and James A. Thliveris¹

Departments of Anatomy ¹ and Medicine, ² University of Manitoba, Winnipeg, Manitoba, Canada

Received 19 January 1993; accepted 6 May 1993

Abstract

We examined the effects of an aldose-reductase inhibitor, statil, which blocks the conversion of glucose to sorbitol, in rats rendered diabetic with streptozotocin in order to determine whether the anticipated changes in sorbitol content was associated with beneficial lack of changes in renal morphology and function. Groups of diabetic, insulin-treated and untreated rats were fed statil daily for a period of five months; each group was paired with a non-drug-treatment control group. At the conclusion of the study period, statil was not found to affect renal sorbitol concentrations nor did it effect functional or structural changes seen in the kidney. We conclude that further study, using other doses of statil and longer duration over which data is collected, must be undertaken in order to implicate the polyol pathway in the renal complications of diabetes mellitus. (Mol Cell Biochem 125: 27–33, 1993)

Key words: diabetes mellitus, sorbitol, polyol pathway, kidney

Introduction

The polyol pathway utilizes glucose through a noninsulin-dependent system. Glucose is converted to sorbitol, via the enzyme aldose reductase, and subsequently oxidized to fructose by sorbitol dehydrogenase [1]. In an insulin-deplete state, the primary glucose utilization pathway, which uses the enzyme hexokinase, is saturated leading to increased shunting into and utilization of the polyol pathway. Excess sorbitol formation is thought to cause a hyperosmotic effect resulting in an influx of fluid, cellular swelling and derangement of intracellular organelles and function [2]. As well, the accumulation of intracellular sorbitol causes a diminution of intracellular myoinositol which is a necessary fuel for such cells. At the same time, sodium, potassium-ATPase activity is impaired [3]. The sum effect of all of these events leads to the onset of the characteristic cellular pathology formed in early diabetes mellitus [4].

The use of aldose reductase inhibitors to block the polyol pathway has been shown to reduce some of the complications in tissues which utilize this pathway like the lens and peripheral nerves. Inhibiting the function of aldose reductase in peripheral nerves has normalized or ameliorated various abnormalities, such as decreased axonal transport, nerve conduction velocity and diminution of myoinositol content, seen in experimental diabetes [5].

The kidney also utilizes the polyol pathway as an insulin-independent route for glucose metabolism. There-
fore, it is possible that blocking the polyol pathway, by means of an aldose reductase inhibitor such as statil, would minimize the complications found in the diabetic kidney. Accordingly, the effect of statil on kidney structure, function, and polyol accumulation was examined in streptozotocin-induced diabetes mellitus in the rat.

**Methods and materials**

**Animals**
Sixty male Sprague-Dawley rats (180–200 g) were used in this study. Diabetes mellitus (DM) was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg) dissolved in cold citrate buffer (pH 4.5). Hyperglycemia (i.e. blood glucose greater than 20 mmol/l) and glucosuria were present 3 days after injection of streptozotocin. Controls consisted of animals injected with only the citrate buffer. Statil, an aldose reductase inhibitor (Stuart Pharmaceuticals, Wilmington, DE) dissolved in water was administered via oral gavage at a dose of 25 mg/kg daily; the concentration shown to prevent development of cataracts and reduce sorbitol in kidney cortex [10]. A number of diabetic animals (DM) received daily subcutaneous injections of protamine-zinc insulin (I) (Connaught Laboratories, Willowdale, ON) to maintain euglycemia. The dosage was approximately 2 μg/day depending upon the animal and adjusted according to biweekly blood glucose monitoring. Based on the aforementioned regime, diabetic and control animals were thus divided into the following groups of 10 animals each: Group A (DM), Group B (DM + Statil), Group C (DM + Statil + I), Group D (DM + I), Group E (Control + Statil), Group F (Control). All animals were housed in individual cages with food and water ad libitum. The duration of the study was for five months in order to assess chronic effects of diabetes on renal structure and function.

Twenty-four hour urine collections and blood samples were obtained for assessment of creatinine clearance and glycated hemoglobin at 5 months of study. Body weights of all animals were recorded weekly for the duration of the study and kidney weights measured when the animals were killed.

**Functional studies**
Blood glucose was monitored biweekly in whole blood obtained by tail bleed and quantified using Dextrostix read on a glucometer (Ames Division, Miles Laboratory Ltd, Rexdale, ON). Prior to use, the instrument was calibrated and tested using standard glucose solutions according to manufacturer’s specifications. Glycated hemoglobin was measured by affinity chromatography using a commercially available kit (Glyco-Test, Pierce Chemical Co, Rockford, ILL [7]). Renal function was evaluated by creatinine clearance expressed in relation to kidney weight at 5 months of study. Serum and urine creatinine and urea were measured by an autoanalyzer (Beckman Astra, Beckman Inc, Bea, CA) using an alkaline picrate [8] and rate conductivity [9] methods, respectively.

**Procurement of tissues**
After 5 months of study, animals were anesthetized with sodium pentobarbital and blood drawn by cardiac puncture for subsequent analysis of creatinine, urea, glycated hemoglobin, and blood glucose. Both kidneys were rapidly removed one snap frozen for sorbitol determinations and the other placed in 10% buffered formalin and processed for light microscopy. Sorbitol content was determined in whole kidney homogenate according to the standard methods described by Bergmeyer [10], using a Bausch and Lomb 2000 spectrophotometer to measure the changes in optical density of the NAD and NADP-linked reactions. For histology, tissue samples were obtained from four different regions of each kidney. Sections (5 μ) were stained with hematoxylin and eosin (H & E) and periodic-acid Schiff (PAS) and scored semi-quantitatively on a scale of 0–4+ for the presence of mononuclear infiltrates, tubular atrophy, interstitial fibrosis and enhanced deposition of mesangial matrix. Quantification of renal corpuscle and glomerular tuft area and volume density and urinary space area was performed using a ZIDAS (Zeiss Interactive Digital Analysis System, Carl Zeiss, Oberkochen, Germany) image analysis system. Measurements were performed on 100 renal corpuscles randomly selected from each of the four regions of the kidney per animal. Blood, urine and tissues were analyzed without foreknowledge of their source.

**Statistics**
Statistical assessment of the data was carried out by using analysis of variance and Tukey’s procedure, and the Kruskal-Wallis rank-sum test [11], where appropriate. Values less than 0.05 were considered statistically significant.