ORIGINAL RESEARCH—BASIC SCIENCE

Superoxide Anion Production in the Rat Penis Impairs Erectile Function in Diabetes: Influence of In Vivo Extracellular Superoxide Dismutase Gene Therapy

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ABSTRACT

Introduction. Superoxide anion may contribute to erectile dysfunction (ED) in diabetes mellitus by reducing cavernosal nitric oxide (NO) bioavailability. The purpose of this study was to determine if gene transfer of extracellular superoxide dismutase (EC-SOD) can reduce superoxide anion formation and determine if this reactive oxygen species may contribute to diabetes-related ED in an experimental model of diabetes.

Methods. Three groups of animals were utilized: (1) control; (2) streptozotocin (STZ)-diabetic rats [60 mg/kg intraperitoneally (ip)] intracavernosally injected with AdCMVβgal (negative control); and (3) STZ-rats intracavernosally injected with AdCMVEC-SOD. Two months after ip injection of STZ, groups 2 and 3 were transfected with the adenoviruses and 2 days after transfection, all animals underwent cavernosal nerve stimulation (CNS) to assess erectile function. Confocal microscopy for superoxide anion and von Willebrand Factor (vWF) was performed in the STZ-diabetic rat. Superoxide anion production, total SOD activity, and cyclic guanosine monophosphate (cGMP) levels were measured in each experimental group of rats.

Results. Confocal microscopy demonstrated superoxide in smooth muscle and endothelial cells of the STZ-rat cavernosum and colocalized with vWF in the endothelium. Higher superoxide anion levels and decreased cGMP levels were found in the penis of STZ-rats at a time when erectile function was reduced. Two days after administration of AdCMVEC-SOD, superoxide anion levels were significantly lower in the penis of STZ-rats. Total SOD activity and cavernosal cGMP was increased in the penis of EC-SOD-transfected rats. STZ-rats transfected with AdCMVEC-SOD had a peak intracavernosal pressure (ICP) and total ICP to CNS that was similar to control rats.

Conclusions. These data demonstrate that in vivo adenoviral gene transfer of EC-SOD can reduce corporal superoxide anion levels and raise cavernosal cGMP levels by increasing NO bioavailability thus restoring erectile function in the STZ-diabetic rat.

Key Words. Erectile Dysfunction; Gene Therapy; Diabetes; Nitric Oxide; Superoxide Anion

Introduction

A frequent vascular complication of diabetes mellitus is erectile dysfunction (ED), with an estimated prevalence in diabetic men to be as high as 50–75% [1,2]. The exact mechanism of ED in diabetic patients is complex and can be caused by several mechanisms including autonomic neuropathy, endothelial dysfunction, and hormonal imbalance. Penile erection is a complex neurovascular event that is dependent on the vascular tone of the corpus cavernosum. Relaxation of corporal smooth muscle is essential for normal erectile function and substantial evidence exists to implicate neuronal and endothelial nitric oxide (NO) as the principal mediator of cavernosal smooth muscle relaxation and penile erection [3–6]. Impairments in both the neurogenic and endothelium-dependent cavernosal smooth muscle relaxation exist in diabetes mellitus [7,8].

Substantial evidence supports the involvement of reactive oxygen species (ROS), in particular superoxide anion, in diabetic vascular dysfunction [9,10]. Both acute and chronic elevations in blood glucose induce superoxide anion production in the vascular endothelium and nerve terminals as a result of increased ROS [11–13]. ROS such as superoxide anion can influence the biological activity of NO by reacting with NO, thus reducing its bioavailability. Of interest, impaired NO bioavailability as a result of increased ROS and advanced glycation end-products (AGEs) cause autonomic neuropathy and endothelial dysfunction in diabetic animal models of ED [14–23]. Conceptually, the increased levels of superoxide anion in the penile vasculature may cause the decrease in NO bioavailability observed in the diabetic penis. Thus, reducing superoxide levels may be an important method to preserve penile NO bioactivity. Therefore, the aims of our study were to investigate the levels of expression of superoxide anion in the penises of diabetic rats, and to examine the effects of adenoviral gene transfer of extracellular superoxide dismutase (EC-SOD) to the penis in order to determine the consequence of overexpression of EC-SOD on superoxide anion production, cavernosal cyclic guanosine monophosphate (cGMP) levels, and erectile function in the penile vascular bed of the diabetic rat.

Methods

Development of Diabetes

Adult male CD rats (Harlan Sprague-Dawley, San Diego, CA) were divided into three groups: (1) age-matched control rats receiving intraperitoneal (ip) injection of citrate buffer (100 mM citric acid, 200 mM disodium phosphate, pH 7.0); (2) rats receiving ip injection of streptozotocin (STZ; Sigma Chemical Company, St. Louis, MO) in a dose of 60 mg/kg and subsequently transfected with AdCMVβgal; and (3) rats receiving ip injection of STZ (60 mg/kg) and subsequently transfected with AdCMVEC-SOD. STZ-diabetic rats (groups 2 and 3) received one intracavernosal injection of the adenoviruses 2 months after ip injection of STZ. Total body weight and blood glucose levels were determined before and after ip injection of STZ with blood glucose levels determined with an Accu-check blood glucose meter (Roche Diagonistics, Indianapolis, IN). Animals were considered diabetic if their blood glucose levels were greater than 200 mg/dL. The blood glucose levels and total body weight of the control and STZ-diabetic rats are summarized in Table 1. These procedures have been previously described [24,25]. All procedures were performed in accordance with the National Institute of Health (NIH) regulations and rats were maintained under controlled temperature and lighting.

Oxidative Fluorescent Microtopography

Hydroethidine (Molecular Probes, Eugene, OR), an oxidative fluorescent dye, and von Willebrand Factor (vWF) were used to evaluate superoxide anion levels in situ and endothelial cells, respectively. These methods have been described previously [25,26]. Hydroethidine is freely permeable to cells and, in the presence of superoxide anion, is oxidized to red-fluorescent ethidium bromide (EtBr), where it is trapped by intercalation with DNA. EtBr is excited at 488 nm and has an emission spectrum of 610 nm. This method provides sensitive detection of superoxide anion levels in situ [26]. Penises were removed 2 months after

Table 1 Weight and blood glucose levels in control and STZ-diabetic rats

	Control rats	STZ-diabetic rats
Initial weight (g)	332 ± 21	341 ± 26
Final weight (g)	447 ± 37*	278 ± 15*
Initial blood glucose (mg/dL)	101 ± 15	96 ± 19
Final blood glucose (mg/dL)	111 ± 28	483 ± 47*

* ($P < 0.05$) value is significantly different from that obtained at the start of the study.