Serum Biomarker Measurements of Endothelial Function and Oxidative Stress After Daily Dosing of Sildenafil in Type 2 Diabetic Men With Erectile Dysfunction

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Purpose: We investigated changes in serum biomarkers of vascular function after short-term, continuous sildenafil dosing in men with type 2 diabetes with erectile dysfunction.

Materials and Methods: Men with erectile dysfunction associated with type 2 diabetes mellitus were randomized to receive continuous, daily sildenafil (50 mg for 1 week run-in and 100 mg for 3 weeks) (148), or placebo (144) for 4 weeks (phase I) and then sildenafil (25, 50 or 100 mg) on demand for 12 weeks (phase II). Blood draws at baseline and after phases I and II were analyzed for cyclic guanosine monophosphate (endothelial function marker), 8-isoprostane (oxidative stress marker), and interleukin-6 and interleukin-8 (inflammatory cytokines). Primary and secondary erectile function outcome variables were affirmative responses on Sexual Encounter Profile question 3 (ability to maintain erection sufficient for sexual intercourse) and Erection Hardness Score, respectively.

Results: Serum cyclic guanosine monophosphate levels were increased in the sildenafil group relative to the placebo group at 4 ($p < 0.01$) and 16 ($p < 0.05$) weeks, correlating with affirmative responses to Sexual Encounter Profile question 3 at the 4-week interval only ($p < 0.05$). Serum 8-isoprostane levels were decreased to a nonsignificant degree in the sildenafil group at 4 weeks with no further change at 16 weeks, whereas interleukin-6 and interleukin-8 levels were unchanged at either interval, and these levels were unassociated with erectile function outcomes.

Conclusions: These data suggest that short-term, continuous sildenafil treatment causes systemic endothelial function to be enhanced and remain so for a duration after its discontinuation. However, they do not indicate any influence of this treatment on systemic oxidative stress or inflammation, or an effect on long-term erectile function improvement.

Key Words: cyclic nucleotide phosphodiesterases, type 5; penile erection; rehabilitation; cardiovascular diseases

Abbreviations and Acronyms

cGMP = cyclic guanosine monophosphate

ED = erectile dysfunction

EF = erectile function

EIA = enzyme-linked immunoassay

IL = interleukin

PDE5 = phosphodiesterase type 5

SEP = sexual encounter profile

SHIM = Sexual Health Inventory for Men

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ERECTILE dysfunction, which is defined as the consistent inability to attain or maintain a penile erection of sufficient quality to perform satisfactory sexual intercourse, has been increasingly associated with serious health comorbidities.[1,2] Numerous investigations in recent years have sup-

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ported its association with cardiovascular diseases including diabetes mellitus, neurological illnesses, hormonal disturbances and psychosocial conflicts as well as lifestyle conditions such as adverse dietary habits, physical inactivity and cigarette smoking.

The association between cardiovascular diseases and ED is not surprising in view of the known requirement of the vascular system for physiological penile erection. By corollary, recently elucidated mechanisms responsible for the vasculopathic changes of the systemic circulation may apply equally to the penile vasculature. Current opinion in sexual medicine research suggests that the pathogenic mechanisms underlying vascular disease in the penis (eg endothelial dysfunction, vascular inflammation) resemble that occurring in other locations of the cardiovascular system.[2] Furthermore, it is believed that ED is linked with systemic cardiovascular disease and, in fact, it may serve as an early manifestation of a global vasculopathy.[2]

Major interest has been given recently to the application of pharmacologic therapies to improve vascular biological function in the penis, which would suggest an approach to reverse or prevent ED. Several studies have suggested that continuous use of oral vasoactive pharmacotherapy such as PDE5 inhibitor drugs (eg sildenafil, tadalafil, vardenafil) may exert sustained vasculoprotective benefits under circumstances of cardiovascular disease as well as vasculogenic ED.[3,4] However, scientific support for this proposal remains limited and further study of the molecular mechanisms involved in such pharmacotherapeutic regimens is necessary.

Using the vasculogenic ED disease model of type 2 diabetes mellitus, we evaluated EF responses to short-term, continuous sildenafil therapy, correlating serum measurements referable to endothelial function and the vascular inflammatory response.

MATERIALS AND METHODS

Study Design

This study represented a retrospective, side analysis of a principal study designed to evaluate the effect of sildenafil on EF in a population of type 2 diabetic men with ED, which was conducted from December 2002 to January 2004.[5] The principal study was a multicenter, double-blind, randomized, placebo controlled, parallel group study with a daily dosing phase (phase I) and an on demand (prn) flexible dose, open label phase (phase II). Phase I consisted of a 1-week washout/run-in period during which the subjects took sildenafil 50 mg or placebo daily followed by a 3-week period in which subjects taking sildenafil were escalated to sildenafil 100 mg and subjects taking placebo continued this treatment (4 weeks total). Phase II consisted of a 2-week period in which all subjects received sildenafil 50 mg prn for sexual activity after which they were able to dose adjust to sildenafil 25, 50 or 100 mg prn based on investigator assessment of efficacy and toleration for an additional 10 weeks (12 weeks total). The principal and side studies received institutional review board regulatory approvals, and written informed consent was obtained from all subjects.

Study Population

Subjects were type 2 diabetic men age 35 to 70 years with clinically documented ED, as confirmed by a SHIM score of 21 or less.[6] Type 2 diabetes mellitus was established to be at least 1 year in duration and fulfill the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.[7]

Biomarker Assays

Blood samples were drawn from an antecubital vein at least 24 hours after drug dosing at (baseline), and at 4 and 16 weeks. Samples were collected in ethylenediaminetetraacetic acid tubes, stored immediately on ice and centrifuged at 4C 3,000 rpm for 10 minutes. The serum was separated and stored at −80C until analysis. Analyses were performed on a cohort basis applying a selection of approximately 30 random subjects from each study group. A larger cohort number was restricted primarily because of insufficient serum samples for complete measurements on a per subject basis at all study intervals.

Serum measurements of cGMP, serving as an indicator of endothelial function,[8] and 8-isoprostane, serving as a major oxidative stress marker,[9] were obtained using competitive enzyme-linked immunoassay kits in accordance with manufacturer instructions (Cayman Chemical, Ann Arbor, Michigan). Serum concentrations of representative cytokines IL-6 and IL-8 were quantified using the Bio-Plex™ bead suspension array system according to the manufacturer instructions (multiplex kit #171A11080, Bio-Rad, Hercules, California).[10]

EF Assessments

Data were retrieved from subject event logs, which documented EF, and were entered into a database before statistical analysis. These logs, which required entries after each sexual encounter, included the 5-item SEP[11] and an appraisal of erection hardness (which preceded the now validated Erection Hardness Score).[12] EF was reported subjectively as a binary outcome (ie yes or no). Data were collected at baseline, at 4 weeks (last available entry within this interval) and at 16 weeks (last available entry within this interval). A successful intercourse attempt (an affirmative response to SEP-3, "Did your erection last long enough for you to have successful intercourse?") was used as the primary efficacy outcome. Erection hardness, which designated a penile erection sufficiently rigid for vaginal penetration, served as the secondary efficacy outcome.

Statistical Analysis

Univariate descriptive statistics included chi-square tests of binary erection outcomes vs treatment group at each point and t tests of biomarker measurements vs treatment group at each point. While accounting for repeated measures for each subject, multivariable analyses of the treatment effect on EF outcome were performed using logistic regression in a generalized estimating equations framework, with an exchangeable correlation structure. Bio-