A Protein Tyrosine Kinase Inhibitor, Imatinib Mesylate (Gleevec), Improves Erectile and Vascular Function Secondary to a Reduction of Hyperglycemia in Diabetic Rats

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ABSTRACT

Introduction. Erectile dysfunction (ED) afflicts 50% of diabetic men, many of whom experience poor results with phosphodiesterase type 5 inhibitors. The protein tyrosine kinase (PTK) inhibitor imatinib (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) has therapeutic potential in diabetic men by maintaining β-cell function.
Aim. To determine if imatinib has a beneficial effect on erectile and vascular function in diabetic rats.
Methods. Male Sprague-Dawley rats were divided into six groups: (i) control; (ii) imatinib (50 mg/kg, daily gavage)-treated control; (iii) diabetic; (iv) preventive imatinib (8 weeks); (v) reversal imatinib (4 weeks untreated diabetes and 4 weeks of treatment); and (vi) insulin (8 weeks)-treated diabetic rats.
Main Outcome Measures. After 8 weeks, all groups underwent cavernosal nerve stimulation and measurements of intracavernosal pressure (ICP) and mean arterial pressure (MAP). Contractile and relaxation responses were evaluated using isolated strips of corpus cavernosum smooth muscle (CCSM) and aorta.
Results. Diabetic rats exhibited a 32% decrease in weight and fivefold increase in blood glucose levels. Imatinib-treated diabetic rats gained weight and partially improved blood glucose levels. Diabetic rats displayed a decrease in ICP/MAP. While maximum electrical field stimulation- and acetylcholine (ACh)-induced relaxations in CCSM strips from the diabetics were reduced, preventive imatinib or insulin treatment normalized ICP/MAP ratios and improved relaxation responses. ACh responses in diabetic aortas were diminished by 50.1% and restored by imatinib. While contractile responses to phenylephrine in diabetic CCSM were not altered, there was a significant enhancement (59.4 %) in the aortic contractile response in diabetic rats, which was restored by imatinib and insulin treatment.
Conclusions. In diabetic rats, prolonged therapy with imatinib improves diabetes-related ED and vascular function, which may involve normalization of high glucose levels and restoration of PTK activation. Future studies are needed to elaborate on the actions of imatinib on diabetic vascular complications. **Gur S, Kadowitz PJ, and Hellstrom WJG. A protein tyrosine kinase inhibitor, imatinib mesylate (Gleevec), improves erectile and vascular function secondary to a reduction of hyperglycemia in diabetic rats. J Sex Med 2010;7:3341–3350.**

Key Words. Diabetic Rat; Erectile Function; Imatinib Mesylate; Platelet-Derived Growth Factor; Protein Tyrosine Kinase; Hyperglycemia; Bcr-Abl

Introduction

The control of cellular processes, such as cell growth, division, and death, involves signal transduction, which commonly involves the transfer of the terminal phosphate moiety of adenosine triphosphate to tyrosine residues on substrate proteins, catalyzed by the protein tyrosine kinase (PTK) family of enzymes [1,2]. Some crucial PTKs are associated with platelet-derived growth factor receptors (PDGF-R), Bcr-Abl protein, c-Kit protein (stem cell factor receptor), and protein kinase AI [1,2].

The first PTK inhibitor, imatinib mesylate (Gleevec), is a rationally designed oral signal transduction-blocking agent that has clinical effectiveness in patients with chronic myeloid leukemia (CML) [3,4]. Tumor cells in CML express

abnormally active forms of Bcr-Abl, c-Kit, or the PDGF-R that are effectively killed or growth arrested by imatinib. PDGF is an important activator of smooth muscle migration and proliferation [5,6]. PDGF can stimulate the activation and protein levels of Rho [7]. However, nitric oxide (NO) inhibits human smooth muscle cell migration via blockade of the RhoA/Rho-kinase pathway [8].

Erectile dysfunction (ED) is a complication of diabetes mellitus, and it has been estimated that 50% of men with diabetes will develop ED within 6 years after initial diagnosis and most will have a poor response to phosphodiesterase type 5 inhibitors [9,10]. Numerous studies suggest that diabetic-related ED is a consequence of impaired neurogenic and endothelial function of the corpus cavernosum smooth muscle (CCSM) [9,11]. Recent studies demonstrate an overexpression of the RhoA/Rho-kinase pathway in diabetes as a contributor to ED [12]. PDGF-R protein expression was increased under high glucose conditions concomitant with increased protein levels and activation of Rho [7]. Imatinib has been shown to induce regression of type 2 diabetes mellitus [13]. Hagerkvist et al. [14] reported that imatinib ameliorates type 2 diabetes in rats by decreasing insulin resistance by inhibition of mitogen-activated protein kinase (MAPK) such as c-jun NH2-terminal kinase (JNK). Treatment of prediabetic and new onset diabetic mice with imatinib prevented type 1 diabetes, suggesting Abl-dependent mechanisms [15]. In a study by Lassila et al. [16], imatinib treatment prevented the development of atherosclerotic lesions and diabetes-induced increases in aortic PDGF-R beta expression and PDGF-R beta phosphorylation as well as other prosclerotic and proinflammatory cytokines. In these studies, the effect of imatinib on preventing or reversing diabetes appears to be directed toward several PTKs, e.g., cAbl, PDGF-R, and MAP kinase JNK.

We hypothesized that the PTK inhibitor imatinib could reverse the endothelial and erectile function changes observed in the Streptozotocin (STZ) diabetic rat. Preliminary results from this investigation are presented [17].

Materials and Methods

Animals and Treatment

A total of 100 male Sprague-Dawley rats were divided into six groups: (i) Control; (ii) imatinib-treated control; (iii) diabetic; (iv) imatinib (preventive)-treated diabetic; (v) imatinib (reversal)-treated diabetic; and (vi) insulin-treated diabetic rats. Diabetes was induced by STZ (45 mg/kg, i.v.). Three days after injection, rats with blood glucose levels of ≥250 mg/dL with glycosuria were considered to be diabetic. Diabetes duration was 8 weeks. The preventive, reversal imatinib, and insulin treatment studies were then undertaken. In the prevention study, imatinib (50 mg/kg of body weight daily by gavage) treatment was started within 3 days of STZ injection and continued for 8 weeks. In the reversal study, a group of diabetic rats were untreated for the first 4 weeks and then given imatinib treatment for the last 4 weeks. The insulin-treated diabetic rats received a daily evening injection (at 17:00 hours) of insulin (Humulin N; Eli Lilly and Company Indianapolis, IN, USA) in doses of 5–30 U/kg/day individually for 8 weeks.

The body weight and blood glucose levels were monitored weekly. All experiments were conducted under the standard procedure guidelines of the Tulane University Animal Care and Use Committee.

In Vivo Evaluation of Erectile Function

For intracavernosal pressure (ICP) (mm Hg) measurement, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p), the trachea was cannulated (polyethylene [PE]-240 tubing) to maintain a patent airway, and a carotid artery was cannulated (PE-50 tubing) for measurement of mean arterial pressure (MAP) (mm Hg) using a transducer (Statham, Oxnard, CA, USA) attached to a data acquisition system (Biopac MP 100 System, Santa Barbara, CA, CA). A 25 G needle filled with 250 U/mL of heparin and connected to the PE tubing was inserted into the right crura of the penis and connected to the pressure transducer to continuously measure ICP. The right major pelvic ganglion and cavernosal nerve were identified. A stainless steel bipolar hook stimulating electrode was placed around the cavernosal nerve posterolateral to the prostate on one side. MAP and ICP were continuously measured and the cavernosal nerve was stimulated (2.5, 5 and 7.5 volts, 15 Hz, 30 seconds pulse width) with a square wave stimulator (Grass Instruments, Quincy, MA, USA).

Measurement of Isometric Tension in CCSM Strips

After anesthesia, the penis was removed and placed in a Krebs bicarbonate solution (containing in mM: NaCl: 118.1, KCl: 4.7, KH_2PO_4: 1.0, $MgSO_4$: 1.0, $NaHCO_3$: 25.0, $CaCl_2$: 2.5, and glucose: 11.1 pH) and gassed with 95% O_2 and 5% CO_2. The dis-