ORIGINAL RESEARCH—BASIC SCIENCE

Prolonged Therapy with the Soluble Guanylyl Cyclase Activator BAY 60-2770 Restores the Erectile Function in Obese Mice

Fábio H. Silva, PhD,* Luiz O. Leiria, PhD,* Eduardo C. Alexandre, MSc,* Ana Paula C. Davel, PhD,† Fabíola Z. Mónica, PhD,* Gilberto De Nucci, PhD,* and Edson Antunes, PhD*

*Department of Pharmacology, Faculty of Medical Sciences, University of Campinas, Campinas, Brazil; †Department of Anatomy, Cellular Biology, Physiology and Biophysics, Institute of Biology, University of Campinas, Campinas, Brazil

DOI: 10.1111/jsm.12682

ABSTRACT

Introduction. Cardiovascular and endocrine-metabolic diseases associated with increased oxidative stress such as obesity lead to erectile dysfunction (ED). Activators of soluble guanylyl cyclase (sGC) such as BAY 60-2770 reactivate the heme-oxidized sGC in vascular diseases.

Aim. This study aimed to evaluate the effects of 2-week oral intake with BAY 60-2270 on a murine model of obesity-associated ED.

Methods. C57BL/6 male mice were fed for 12 weeks with standard chow or high-fat diet. Lean and obese mice were treated with BAY 60-2770 (1 mg/kg/day, 2 weeks).

Main Outcome Measures. Measurements of intracavernosal pressure (ICP), along with acetylcholine (10^{-9} to 10^{-5} M) and electrical field stimulation (EFS; 4–10 Hz)-induced corpus cavernosum relaxations in vitro, were obtained. Levels of cyclic guanosine monophosphate (cGMP), reactive oxygen species (ROS), and sGC protein expressions in cavernosal tissues were measured.

Results. Cavernous nerve stimulation caused frequency-dependent ICP increases, which were significantly lower in obese compared with lean mice ($P < 0.05$). Two-week therapy with BAY 60-2770 fully reversed the decreased ICP in obese group. Acetylcholine-induced cavernosal relaxations were 45% lower ($P < 0.001$) in obese mice, which were fully restored by BAY 60-2770 treatment. Likewise, the EFS-induced relaxations in obese mice were restored by BAY 60-2770. Basal cGMP content in erectile tissue was 68% lower ($P < 0.05$) in obese mice, an effect normalized by BAY 60-2770. Levels of ROS were 52% higher ($P < 0.05$) whereas protein expression of α1 sGC subunit was reduced in cavernosal tissue of obese mice, both of which were normalized by BAY 60-2770. In lean group, BAY 60-2770 did not significantly affect any functional, biochemical, or molecular parameter analyzed.

Conclusions. Two-week therapy with BAY 60-2770 restores the erectile function in obese mice that is associated with reduced ROS levels, up-regulation of α1 sGC subunit, and increased cGMP levels in the erectile tissue. **Silva FH, Leiria LO, Alexandre EC, Davel APC, Mónica FZ, De Nucci G, and Antunes E. Prolonged therapy with the soluble guanylyl cyclase activator BAY 60-2770 restores the erectile function in obese mice. J Sex Med 2014;11:2661–2670.**

Key Words. Reactive Oxygen Species; Obesity; Cyclic GMP; Corpus Cavernosum; Intracavernous Pressure; Erectile Dysfunction; Oxidative Stress

Introduction

Erectile dysfunction (ED) is characterized by a persistent inability to achieve and/or maintain an erection sufficient for satisfactory sexual performance [1]. A significant decrease in the quality of erectile function was described in obese individuals presenting vascular risk factors

[2]. An increased risk of ED among obese men was later reported, as determined by follow-up questionnaires [3,4]. Lifestyle changes aimed at reducing body weight and increasing physical activity in obese men were shown to ameliorate ED [5]. Animal models of ED in rodents have provided additional support for understanding the ED physiopathology. Obese Zucker rats [6] and leptin receptor-deficient (db/db) obese mice exhibit ED [7], as demonstrated by reductions of intracavernous pressure (ICP) by cavernosal nerve electrostimulation. Evaluation of in vitro functional responses of cavernous tissue to electrical field stimulation (EFS) and exogenous vasoactive agents in db/db [8] and high-fat-fed obese mice [9] also shows impaired cavernosal relaxations.

Penile erection is primarily initiated by nitric oxide (NO) released from nitrergic nerves and endothelial cells that binds to soluble guanylyl cyclase (sGC) in cavernosal smooth muscle, resulting in production of cyclic guanosine monophosphate (cGMP), which leads to relaxation of corpus cavernosum smooth muscle and penile erection [10]. The increased oxidative stress associated with obesity is well described to interfere with the NO-sGC-cGMP signaling pathway through scavenging of NO, reducing its availability for target cells [11]. In addition, increased oxidative stress decreases the activity and protein expression levels of sGC, reducing the cGMP-induced responses [12]. S-nitrosylation of sGC leading to enzyme desensitization is suggested to be the link between blunted response to NO and oxidative stress [13]. Generation of reactive oxygen species (ROS) has been implicated in both neurogenic and vasculogenic ED [14]. Low-density lipoprotein receptor-null mice fed with cholesterol-enriched diet display ED associated with increased protein expressions of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits and endothelial nitric oxide synthase uncoupling [15].

Recent progress in understanding of the NO and cGMP signaling pathway has identified novel targets for drug development [16], including non-responsive patients to classical phosphodiesterase-5 (PDE5) inhibitors [17]. For instance, BAY 41-2272 (NO-independent, heme-dependent sGC stimulator [18]) directly stimulates sGC and increases the sensitivity of the enzyme toward NO, elevating the cGMP levels by stimulating the sGC mostly via NO-independent mechanisms [19,20]. BAY 41-2272 induces penile erection in rats [21] and promotes in vitro rabbit and human cavernosal relaxations [22].

More recently, sGC activators emerged as superior therapeutic drugs because they activate sGC when heme iron is found in its oxidized state (Fe^{3+} instead of Fe^{2+}) or when heme group is missing [12,23,24]. These compounds comprise BAY 58-2667 and its chemical analog BAY 60-2770 [25,26], as well as HMR-1766 [27]. It is accepted that oxidation of heme moiety (Fe^{3+}) of sGC may render the enzyme insensitive to endogenous NO, thus preventing the downstream cGMP-PKG signaling pathway in different tissues [12]. Recently, BAY 60-2770 was shown to act as a heme mimetic, binding to the heme pocket of the H-NOX domain via carboxylate-mediated interaction [28]. BAY 60-2770 exerts potent erectile activity in rats [29] and ameliorates the urethral dysfunction in obesity-associated overactive bladder [30], both of which are enhanced by heme-oxidation with ODQ. sGC activators may be of potential interest for the treatment of ED, but little is known about the actions of such compounds in the erectile tissue [29]. Moreover, considering that obesity increases ROS production that in turn may oxidize the sGC leading to impairment of cavernosal relaxations, it is plausible to speculate that BAY 60-2770 amplifies the enzyme activity regaining the erectile function. Therefore, in the present study, we have used a murine model of obesity-associated ED to evaluate the effects of a 2-week oral intake of BAY 60-2270 on the impaired cavernosal relaxations.

Materials and Methods

Animals

All animal procedures and the experimental protocols were according to the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation and approved by the institutional Committee for Ethics in Animal Research/University of Campinas (UNICAMP). Four-week-old male C57BL6/J mice were provided by the Central Animal House Services of UNICAMP. The animals were housed three per cage on a 12-hour light–dark cycle.

Oral Treatment with BAY 60-2770

Mice were fed for 12 weeks with either a standard chow diet (carbohydrate: 70%; protein: 20%; fat: 10%) or a high-fat diet that induces obesity (carbohydrate: 29%; protein: 16%; fat: 55%). Lean and