Tadalafil Effect on Metabolic Syndrome-Associated Bladder Alterations: An Experimental Study in a Rabbit Model

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ABSTRACT

Introduction. Metabolic syndrome (MetS) and lower urinary tract symptoms (LUTS) are often associated. Bladder detrusor hyper-contractility—a major LUTS determinant—is characterized by increased Ras homolog gene family, member A/Rho-associated protein kinase (RhoA/ROCK) signaling, which is often upregulated in MetS.

Aim. This study investigated the effects of tadalafil dosing on RhoA/ROCK signaling in bladder, in a rabbit model of high-fat diet (HFD)-induced MetS.

Methods. Adult male rabbits feeding a HFD for 12 weeks. A subset of HFD animals was treated with tadalafil (2 mg/kg/day, 1 week: the last of the 12 weeks) and compared with HFD and control (feeding a regular diet) rabbits.

Main Outcome Measures. In vitro contractility studies to evaluate the relaxant effect of the selective ROCK inhibitor, Y-27632, in carbachol precontracted bladder strips. Evaluation of RhoA activation by its membrane translocation. Immunohistochemistry for ROCK expression has been performed to evaluate ROCK expression in bladder from the different experimental groups. mRNA expression of inflammation, pro-fibrotic markers by quantitative RT-PCR has been performed to evaluate the effect of tadalafil on MetS-induced inflammation and fibrosis within the bladder. The in vitro effect of tadalafil on RhoA/ROCK signaling in bladder smooth muscle cells was evaluated by using chemotaxis assay.

Results. Bladder strips from HFD rabbits showed hyper-responsiveness to Y-27632, indicating RhoA/ROCK overactivity in HFD bladder compared with matched controls. Accordingly, the fraction of activated (translocated to the membrane) RhoA as well as ROCK expression are increased in HFD bladder. Tadalafil dosing normalized HFD-induced bladder hypersensitivity to Y-27632, by reducing RhoA membrane translocation and ROCK overexpression. Tadalafil dosing reduced mRNA expression of inflammatory, pro-fibrotic, and hypoxia markers. A direct inhibitory effect of tadalafil on RhoA/ROCK signaling in bladder smooth muscle cell was demonstrated by using chemotaxis assay. Pre-treatment with tadalafil inhibited both basal and PDGF-induced migration of bladder smooth muscle cells.

Conclusions. Tadalafil dosing reduced RhoA/ROCK signaling and smooth muscle overactivity in an animal model of MetS-associated bladder alterations. Our findings suggest a novel mechanism of action of tadalafil in alleviating LUTS in MetS patients. **Vignozzi L, Filippi S, Comeglio P, Cellai I, Morelli A, Maneschi E, Sarchielli E, Gacci M, Carini M, Vannelli GB, and Maggi M. Tadalafil effect on metabolic syndrome-associated bladder alterations: An experimental study in a rabbit model. J Sex Med 2014;11:1159–1172.**

Key Words. LUTS; Metabolic Syndrome; Tadalafil; RhoA/ROCK Signaling; OAB; Rabbit Bladder; Prostatic Enlargement

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Introduction

The historical, simplistic, causal relationship among prostatic enlargement and lower urinary tract symptoms (LUTS) has been challenged nowadays by the recognition of an incomplete overlap between voiding symptoms and urodynamic markers of prostatic conditions [1]. Indeed, many men with benign prostatic hyperplasia (BPH) do not develop LUTS, and LUTS may occur independently of BPH [2]. Additionally, epidemiological evidence indicates that these bothersome urinary symptoms are not gender-specific, having a high prevalence also in women [3]. Hence, although an enlarged prostate can contribute to the onset of LUTS, other extra-prostatic factors are supposed to be equally important in LUTS determinism [2]. Bladder dysfunction—characterized by detrusor overactivity and/or overactive bladder (OAB)—is considered one of the main extra-prostatic LUTS determinants in both genders [4].

The pathogenesis of LUTS, including OAB, is complex and largely unknown. Increasing evidence links a number of age-related disorders, including metabolic and cardiovascular disorders, with the pathogenesis of LUTS [5]. In particular, it is epidemiologically well-established that metabolic syndrome (MetS) is not only frequently associated with, but also poses an increased risk for, the development and progression of LUTS in men. Several MetS features, including abdominal obesity [6], hypertension [7–9], hyperglycemia and type 2 diabetes mellitus (T2DM) [8,10], low high-density lipoprotein cholesterol and increased triglycerides plasma level [10–12], have all been related to LUTS. Therefore, recent investigations were aimed at understanding the potential pathogenic mechanisms underlying the association between MetS and LUTS. Animal models have provided a great deal of information. In a genetic mouse model of T2DM/obesity, both bladder dysfunction and prostatitis were described [13]. However, monogenic alterations related to metabolic derangements have rarely associated to human LUTS [14]. Bladder dysfunctions, including overactivity [15] or fibrosis [16], were often observed in hyperlipidemic rats and were associated with pelvic ischemia and smooth muscle atrophy. In male rats fed a fructose enriched diet, unstable bladder contractions and increased concentrations of $M_{2,3}$-muscarinic receptors, suggestive of bladder overactivity [17], were reported. Similar results were observed in rats with streptozotocin-induced diabetes [18,19].

We recently developed a non-genomic animal model of MetS, feeding adult male rabbits a high-fat diet (HFD) for 12 weeks [20–25]. This animal model essentially recapitulates the human phenotype, showing, along with the classical features of MetS (hyperglycemia, impaired glucose tolerance, dyslipidemia, hypertension, increased visceral obesity, and hypogonadism), also severe alterations at the low urinary tract (LUT) level. In MetS rabbits, we characterized a prostatitis-like syndrome [22] and peculiar bladder alterations, including fibrosis, hypoxia, and inflammation [23], confirming that MetS could affect the whole of the LUT. More important, bladder from MetS animals showed increased signaling of Ras homolog gene family, member A (RhoA)/Rho-associated protein kinase (ROCK) [23], a pathway deeply involved in detrusor hyper-contractility and bladder dysfunction, as often observed in human LUTS [4]. We previously tested the effect of chronic (2 mg/kg/day, for 12 weeks; preventive) or acute (2 mg/kg/day, for the last of the 12 weeks; curative) tadalafil dosing in HFD animals, focusing on prostate alterations [25]. Interestingly, we found that not only chronic (i.e., preventive), but also acute (i.e., curative), tadalafil dosing in HFD animals was effective in reducing MetS-associated prostatic abnormalities, including fibrosis and inflammation [25].

Aims

This study was aimed at investigating the effect of acute (curative) tadalafil dosing on MetS-associated increased signaling of RhoA/ROCK in bladder.

Materials and Methods

MetS Rabbit Model

The HFD-induced rabbit model of MetS has been obtained as previously described [20]. Male New Zealand White rabbits (Charles River, Calco, Lecco, Italy), weighing about 3 kg, were individually caged, under standard conditions, in a temperature- and humidity-controlled room, on a 12-hour light/dark cycle. Water and food were unrestricted throughout the study. After 1 week of standard rabbit diet, animals were randomly numbered and assigned to different groups: control (N = 43) or treatment group