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Blockade of Toll-Like Receptor 4 Attenuates Erectile Dysfunction in Diabetic Rats

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ABSTRACT

Introduction: While increased toll-like receptor (TLR)4 activity may contribute to the pathophysiology of vascular diseases, the molecular mechanisms disrupted by this receptor in the vasculature are still poorly understood. Additionally, it is unknown if TLR4 mediates erectile dysfunction (ED) during diabetes.

Aim: To investigate whether pharmacological blockade of TLR4 affects erectile function in a murine model of diabetes.

Methods: Sprague Dawley rats (Charles River Laboratory, Wilmington, MA, USA) received a single streptozotocin injection (65 mg/kg, 28 days) and were treated with an anti-TLR4 antibody (1 μg/d, intraperitoneally) for the last 14 days of the treatment. Additionally, cavernosal strips were acutely incubated for 30 minutes with CLI-095 (10^{-5} mol/L), a TLR4 inhibitor. Functional studies, Western blotting, erectile function, immunohistochemistry, and biochemical analyses were performed.

Main Outcome Measures: Oxidative stress, cyclic guanosine monophosphate (cGMP) levels, and functional studies were evaluated in treated and nontreated cavernosal strips from control and diabetic animals. Additionally, in vivo erectile function was assessed.

Results: Enhanced TLR4 expression was observed in corpus cavernosum from diabetic rats compared with control animals. Long-term blockade of TLR4 slightly improved diabetes—induced ED in rats due to attenuation of oxidative stress and increased cGMP levels in penile tissue, which ameliorated cavernosal relaxation. Functional experiments revealed that acute or chronic inhibition of TLR4 decreased hypercontractility in response to phenylephrine and improved nitrergic relaxation in corpus cavernosum from diabetic rats.

Clinical Implications: TLR4 blockade may be a novel therapeutic strategy to assist in ED management.

Strengths & Limitations: The strength of this article stems from the fact that we showed that TLR4 blockade partly improves erectile function in vivo in diabetic rats. Its limitations mainly include that messenger RNA analysis for the nitric oxide/cGMP pathway were not performed.

Conclusion: In summary, TLR4 participates in the mechanisms of diabetes-associated ED and blockade of this receptor positively affects penile vascular function. **Nunes KP, de Oliveira AA, Szasz T, et al. Blockade of Toll-Like Receptor 4 Attenuates Erectile Dysfunction in Diabetic Rats. J Sex Med 2018;XX:XXX—XXX.**

Key Words: Toll-Like Receptor 4; Vascular and Erectile Dysfunction; Diabetes; Cyclic Guanosine Monophosphate

INTRODUCTION

30 million U.S. men have erectile dysfunction (ED), and its prevalence is expected to increase with obesity, diabetes, and aging.[1—3] Apart from ED's direct physiological effects, it has additional consequences as it negatively affects psychological well-being, reproductive health, and satisfaction in intimate relationships.[4] Vascular dysfunction is a hallmark of ED.[5] Thus its onset may be a warning of systemic vascular disease.[6—8] Also, ED is considered a common complication in diabetic patients.[9]

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Although diabetes and ED are different diseases, they tend to be associated, mainly because of overlapping pathways, leading to vascular damage in both conditions. The current literature highlights that the main mechanisms impaired in these diseases include abnormal levels of pro-inflammatory cytokines (eg, TNF-α), augmented oxidative stress, increased mitogen-activated protein kinases activity, and dysregulation in nitric oxide (NO) signaling.

Recently, an important component of the innate immunity, toll-like receptor (TLR)4, has been shown to be associated with disruption of vascular homeostasis and, consequently, vascular dysfunction in chronic diseases.[10] Beyond immune cells, TLR4 has been demonstrated to be expressed in a variety of other cells including endothelial and smooth muscle cells (SMCs).[11] While molecular mechanisms underlying TLR4 activity in chronic diseases are yet to be defined, mounting evidence supports that TLR4 partially mediates vascular diseases such as diabetes,[12] hypertension,[13] and vascular ED.[14] Downstream TLR4, not only the transcriptional factor nuclear factor-κB is activated, but also other critical inflammatory mediators.[15] Additionally, increased TLR4 expression has been suggested to be involved in the modulation of the nicotinamide adenine dinucleotide phosphate oxidase enzyme during pathological conditions.[16] Indeed, increased TLR4 expression contributes to oxidative stress and the release of pro-inflammatory cytokines in damaged vessels.[17]

Endothelial injury and dysfunction due to oxidative stress reduce NO availability and are critical events in the pathogenesis of vascular diseases. NO is a vaso-protective molecule required for maintaining vascular homeostasis, which is instrumental for a normal erection. Penile erection is a complex physiological mechanism coordinated by the central nervous system and local factors. Upon sexual stimulation, NO is released from neuronal and/or endothelial cells, which in turn stimulates the soluble guanylyl cyclase enzyme. This enzyme activity favors the increase in cyclic guanosine monophosphate (cGMP) concentration, followed by relaxation of SMCs, and erection. On the other hand, decreased NO disrupts penile homeostasis resulting in reduced cavernosal relaxation and, consequently, ED.[18] It is noteworthy that the inactivation of NO by increased oxidative stress is a crucial mechanism in which the endothelium-dependent vasodilation is diminished, not only in ED but also in other vascular pathologies. Specifically, NO is quickly quenched by superoxide, which generates peroxynitrite. Thus, an imbalance between the levels of NO and reactive oxygen species (ROS) promotes endothelial and vascular dysfunction.[19]

Oxidative events downstream TLR4 activation have been extensively discussed in acute conditions, especially upon lipopolysaccharide stimulation. However, the link between TLR4 and the NO signaling pathway as a mechanism affecting the vascular tone in chronic diseases is new and might be a key factor in vasculopathies. Because hyperglycemia leads to NO deficiency,[20,21] many studies report that ED in diabetic patients is partly due to disruption in the NO pathway. High glucose enhances TLR4 expression in human endothelial cells,[22] and TLR4 inhibition reduces oxidative events in diabetic rats. Even though the hypothesis that the TLR4 pathway participates in the development and maintenance of vascular pathologies has gathered wider support, few studies are examining the molecular mechanisms triggered by TLR4 in diabetes. More importantly, the association between TLR4 and NO/cGMP signaling pathway deserves further investigation. Despite advancement in the understanding of TLR4 signaling transduction, the precise model of interaction between this receptor with its ligands is still a debatable issue. Our group has previously demonstrated that TLR4 activation contributes to diabetic bladder dysfunction[23] and hypertension-associated ED.[14] However, it is still unknown if TLR4 mediates ED in diabetic rats. Here, we aimed at determining not only if TLR4 regulates penile vascular tone but also whether this receptor mediates ED in vivo in a rodent model of diabetes. Our findings suggest TLR4 partly contributing to ED in streptozotocin (STZ)—induced diabetic rats. It seems that TLR4 activation increases oxidative stress, which might participate in the mechanisms leading to impairment of NO/cGMP levels in penile tissue.

METHODS

Animals

The animal protocol used in this study was approved by the Animal Use for Research and Education Committee at Augusta University. 12-week-old male Sprague Dawley rats (250—350 g; Charles River Laboratory, Wilmington, MA, USA) were maintained on a 12:12 hours light—dark cycle and fed a standard rat chow diet with water ad libitum. A single intraperitoneal injection of STZ (65 mg/kg) diluted in sterile citrate buffer solution (0.2 mol/L; pH 4.5) was used to induce diabetes. Nondiabetic control animals were injected with an equivalent volume (average 0.7 mL) of citrate buffer solution. At 48 hours after STZ injection, glucose levels were determined using a commercial blood-glucose meter system. Animals considered diabetic (glycemic level $\geq$250 mg/dL) were kept in the animal facility after STZ injection for 28 days.

Chronic Treatment With an Anti-TLR4 Antibody

STZ-induced diabetic rats received a daily intraperitoneal injection of an anti-TLR4 antibody (1 μg/d) diluted in sterile saline solution for the last 14 days of the treatment. The dose was determined based on a previous work.[24] Control animals were injected with vehicle alone. 4 experimental groups were used: (1) CTL, (2) CTL treated with an anti-TLR4 antibody, (3) diabetic, and (4) diabetic treated with an anti-TLR4 antibody; n = 5 animals per group.

Acute Treatment with CLI-095

TLR4 acute inhibition was performed by incubating cavernosal strips with CLI-095 diluted in DMSO (10^{-5} mol/L)