TNF-α, Erectile Dysfunction, and NADPH Oxidase-Mediated ROS Generation in Corpus Cavernosum in High-Fat Diet/Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Introduction. Patients with diabetes-associated erectile dysfunction (ED) are characterized by an increase in circulating tumor necrosis factor-alpha (TNF-α). However, no study has indicated whether and how TNF-α plays a role in the pathogenesis of ED associated with diabetes.

Aim. We examined the effects and potential mechanism of infliximab (INF), a chimeric monoclonal antibody to TNF-α, on reactive oxygen species (ROS) generation in corpus cavernosum and ED in diabetic rats.

Methods. Four groups of male rats were used: age-matched normal controls; diabetic rats induced by a high-fat diet (HFD) combined with a single streptozotocin (STZ) injection (35 mg/kg body weight, intraperitoneal [i.p.]); nondiabetic rats receiving INF (5 mg/kg body weight/week, i.p.), and diabetic rats receiving INF. Erectile function was assessed with electrical stimulation of the cavernous nerve after 8 weeks. The blood and penile tissues were harvested for plasma biochemical determinations, serum TNF-α measurement, penile ROS detection, and molecular assays of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits, endothelial nitric oxide synthase (eNOS), phospho-eNOS, and neural nitric oxide synthase (nNOS) in the penis.

Main Outcome Measures. The effect of INF on HFD/STZ-induced diabetic ED and NADPH oxidase-mediated ROS generation was studied in diabetic corpus cavernosum.

Results. Untreated diabetic rats displayed significantly decreased erectile parameters, and increased plasma TNF-α levels, penile ROS production, $p47^{phox}$ and $gp91^{phox}$ expression compared with nondiabetic controls. INF neutralized TNF-α and significantly reduced ED in diabetic rats, in which marked decreases in $p47^{phox}$ and $gp91^{phox}$ expression and ROS generation in corpus cavernosum were noted. The ratio of phospho-eNOS to eNOS and expression of nNOS in the penis were significantly increased in INF-treated vs. untreated diabetic rats.

Conclusions. Increased TNF-α expression associated with diabetes contributes to ED by promoting NAPDH oxidase-mediated ROS generation in corpus cavernosum. INF protects against diabetic ED by neutralizing TNF-α.

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Key Words. Diabetes; Erectile Dysfunction; TNF-α; Reactive Oxygen Species; Corpus Cavernosum; Infliximab

Introduction

Erectile dysfunction (ED) is associated with diabetes mellitus (DM). Approximately 75% of men with DM experience ED, which occurs 10–15 years earlier than in age-matched controls [1,2]. Moreover, diabetes-associated ED is more severe [3] and less responsive to oral phosphodiesterase type 5 inhibitors compared with nondiabetic ED [4]. One common denominator for ED and DM is vascular endothelial dysfunction [5]. Impaired cavernosal smooth muscle relaxation mediated by endothelial and neuronal mechanisms has been observed in human and animal diabetic

models [6,7]. Yet the specific role and detailed mechanisms contributing to the development of diabetes-associated ED remain incompletely understood.

Oxidative stress occurs when cells are exposed to excessive levels of reactive oxygen species (ROS), including free radicals such as superoxide and hydroxyl radicals and non-radicals such as hydrogen peroxide. Overproduction of ROS has been strongly implicated in the progression of ED in diabetes, principally through decreasing nitric oxide (NO) bioavailability, resulting not only in acute impairment of cavernosal relaxation but also long-term penile endothelial dysfunction [8]. The link between ROS and ED has been investigated in animal models of diabetes mostly from the perspective of the antioxidant system [9–11]. However, the local mechanism of ROS production in the penis among those with diabetes is rarely examined and largely unknown. Among numerous potential sources of ROS, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase appears to be a major intravascular inducible source in several animal models of vascular disease, including diabetes [12]. Previous experiments in vitro and in vivo have demonstrated that NADPH oxidase is present in the penis and upregulated [9,13–18], which is associated with ROS generation and ED in hypertensive rats [15,16], hypercholesterolemic mice [17], and diabetic rabbits [9] and mice [18]. However, the regulation of NADPH oxidase in penile tissue in vivo and its relation to the pathogenesis of diabetes-associated ED remain largely unknown.

It is now established that cardiovascular diseases (CVDs), including DM, are accompanied by a marked elevated level of circulating and/or local vascular production of tumor necrosis factor-alpha (TNF-α) [19,20], a primary pro-inflammatory cytokine, which has been thought to be an important contributor to endothelial dysfunction and impairment of NO-mediated vasodilation in various vascular beds [21–25]. In addition, clinical studies have reported that increased systemic levels of TNF-α are positively correlated to ED with CVD or without CVD [26–29]. One group reported that anti-TNF-α therapy significantly improved sexual function in male patients with active ankylosing spondylitis [30]. These results suggested that TNF-α is involved in the pathogenesis of ED.

Recently, several animal experiments have evaluated the role of TNF-α in cavernosal tissue for penile erectile function. In a transgenic mice model that overexpresses human TNF-α, decreased induced penile erection was reported [31]. Carneiro et al. showed that the corpora cavernosa from TNF-α knockout mice displayed increased NO-dependent relaxation, which was associated with increased penile endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS) expression [32]. They also demonstrated that TNF-α infusion in normal mice induced decreased nonadrenergic noncholinergic-mediated relaxation associated with decreased eNOS and nNOS expression, and an increased corpora cavernosa response to adrenergic nerve stimulation that would trigger ED [33].

TNF-α stimulates ROS production in tissue by several sources, e.g., mitochondria [34]. Considerable evidence has strongly suggested that in endothelial cells, a major source of ROS is NADPH oxidase [12]. Furthermore, TNF-α treatment significantly increased ROS generation and induced NADPH oxidase subunit $gp91^{phox}$ expression in pulmonary artery endothelial cells, which was blocked by a NADPH oxidase inhibitor, apocynin [35]. These results suggested that NAPDH oxidase serves as a key mediator involved in TNF-α-induced ROS generation. Taken together, this evidence supports a role for TNF-α in penile endothelial dysfunction related to ED development. But so far, no studies have indicated whether and how TNF-α plays a role in the pathogenesis of ED associated with diabetes.

Based on these results and considering recent evidence that TNF-α impaired corpora cavernosa reactivity and erectile function, we hypothesized that increased TNF-α expression associated with diabetes contributes to the development of ED by promoting NADPH oxidase-derived ROS generation in corpus cavernosum. In the current study, we first determined whether ED occurs accompanied by increased circulating TNF-α levels when a high-fat diet (HFD) was combined with streptozotocin (STZ) to induce type 2 diabetes in rats. Then we investigated whether ROS generation is enhanced in the penis from diabetic rats. We also observed alterations of NADPH oxidase subunits (e.g., $p47^{phox}$ and $gp91^{phox}$) expression in the diabetic corpus cavernosum. Infliximab (INF, a chimeric monoclonal antibody to TNF-α) was used to determine whether increased TNF-α is associated with activation of NADPH oxidase and responsible for ROS production in the development of ED. The effect of INF on erectile function of diabetic rats in vivo was also examined.