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FTY720 Supplementation Partially Improves Erectile Dysfunction in Rats With Streptozotocin-Induced Type 1 Diabetes Through Inhibition of Endothelial Dysfunction and Corporal Fibrosis

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ABSTRACT

Introduction: Erectile dysfunction (ED) in patients with diabetes mellitus (DM) seriously affects their quality of life. However, these patients show a poor effect rate for oral phosphodiesterase type 5 inhibitors. Thus, new treatment methods are urgently needed. Fingolimod hydrochloride (FTY720) was approved in 2010 for the treatment of patients with the relapsing-remitting form of multiple sclerosis.

Aim: To investigate whether FTY720 supplementation could ameliorate ED induced by DM (DMED).

Methods: Forty male Sprague-Dawley rats (8 weeks old) were used for the experiment. Thirty-two had type 1 DM induced by streptozotocin and the other eight rats constituted the control group. Eight weeks later, the erectile function of rats was assessed with an apomorphine test. Only some rats with DMED were treated with FTY720 orally every day for 4 weeks; the other rats remained in the same condition for 4 weeks.

Main Outcome Measure: Metabolic parameters; erectile function; sphingosine-1-phosphate receptor 3 (S1P3), protein kinase B (Akt), nitric oxide (NO), and cyclic guanosine monophosphate (cGMP) signaling pathway; corporal fibrosis; apoptosis level; and Smad and non-Smad signaling pathways.

Results: There were no significant differences in the initial body weights and fasting glucose concentrations among the three groups. Erectile function in the DMED group was significantly impaired compared with the control group and was partly, but significantly, improved in the DMED + FTY720 group. The DMED group showed inhibited activity of the S1P3-Akt-NO-cGMP signaling pathway, and the inhibition was partly reversed in the DMED + FTY720 group. The DMED group showed serious corporal fibrosis, higher apoptosis level, higher ratio of Bax to Bcl-2, and higher expressions of the Smad pathway (transforming growth factor-β1, Smad, and connective tissue growth factor) and the non-Smad pathway (transforming growth factor-β1, rho-associated protein kinase, LIM domain kinase 2, and cofilin). However, FTY720 supplementation partly increased the ratio of smooth muscle to collagen, decreased the ratio of Bax to Bcl-2, and inhibited activity of the Smad and non-Smad pathways.

Conclusion: FTY720 supplementation inhibited endothelial dysfunction and corporal fibrosis, ultimately leading to partial improvement of DMED in rats. This finding provides evidence for a potential treatment method for DMED. **Cui K, Ruan Y, Wang T, et al. FTY720 Supplementation Partially Improves Erectile Dysfunction in Rats With Streptozotocin-Induced Type 1 Diabetes Through Inhibition of Endothelial Dysfunction and Corporal Fibrosis. J Sex Med 2017;XX:XXX–XXX.**

Key Words: FTY720; Diabetes Mellitus; Erectile Dysfunction; Endothelial Dysfunction; Corporal Fibrosis

INTRODUCTION

Erectile dysfunction (ED), defined as the inability to attain or maintain a penile erectile sufficient for successful vaginal intercourse, has drawn worldwide attention as a common disorder.[1] ED has several potential causes, including certain vascular risk factors, hormonal disorders, and neurologic disturbances.[2] Diabetes mellitus (DM) is one of the most common related factors, with approximately 30% to 80% patients with DM also having ED, a sixfold increase over its incidence in men without DM.[3]

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Table 1. Primers used in conventional PCR and real-time reverse transcription PCR

Genes	Primer sequences	Usage (PCR)
eNOS	forward: 5′-GATCCTAACTTGCCTTGCATCCT-3′	real time
	reverse: 5′-TGTAATCGGTCTTGCCAGAATCC-3′	
TGF-β1	forward: 5′-CATTGCTGTCCCGTGCAGA-3′	real time
	reverse: 5′-AGGTAACGCCAGGAATTGTTGCTA-3′	
β-actin	forward: 5′-AAGAGCTATGAGCTGCCTGA-3′	real time
	reverse: 5′-TACGGATGTCAACGTCACAC-3′	

eNOS = endothelial nitric oxide synthase; PCR = polymerase chain reaction; TGF-β1 = transforming growth factor-β1.

DM-induced ED (DMED) has been described as a complex disorder involving many pathologic processes such as endothelial dysfunction, fibrosis, atherosclerosis, and the formation of advanced glycation end products.[4,5] Because of the complexities of DMED, phosphodiesterase type 5 (PDE5) inhibitors, the first-line drug treatment for ED, which enhances the nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) pathway, shows no strong benefits in patients with DM.[6,7] Thus, new treatment strategies for DMED are urgently needed.

Sphingosine-1-phosphate (S1P), a bioactive sphingolipid metabolite derived from platelets, has comprehensive effects on physiologic and pathologic processes through its binding to five S1P receptors (S1P1−S1P5).[8] FTY720 (fingolimod; 2-amino-[2-{2-(4-octylphenyl) ethyl}-1,3-propanediol hydrochloride) is fingolimod hydrochloride. Fingolimod was approved in 2010 for the treatment of patients with the relapsing-remitting form of multiple sclerosis. Also, FTY720, as a synthesized S1P receptor agonist, is a synthetic structural analog of myriocin (ISP-1), a metabolite of *Isaria sinclairii* that acts through S1P receptors.[9,10] Increasing evidence has shown that FTY720 might serve as a potential therapeutic approach for cardiac vascular dysfunction in DM based on its protective effects, including ameliorating cardiac microvascular barrier impairment and pathologic angiogenesis and restoring coronary flow reserve.[11−14] Tölle et al[15] reported that FTY720 could stimulate NO production and protect against cardiac vascular dysfunction in DM through its effects on S1P3. Moreover, recent studies have shown FTY720 treatment is therapeutically effective in various animal models of fibrosis, including the liver,[16] kidney,[17] and heart.[18] Therefore, we explored whether FTY720 supplementation could ameliorate DMED through the promotion of NO production and the inhibition of corporal fibrosis.

In this study, we investigated the effects of FTY720 in an in vivo rat model of DMED and explored its underlying mechanisms. Our results showed that FTY720 ameliorated ED in rats with DM, supporting a potential new treatment strategy for DMED.

METHODS

Drugs

Streptozotocin (STZ), apomorphine (APO), FTY720, and pentobarbital were purchased from Sigma-Aldrich (St Louis, MO, USA).

Animal Treatment

This study was approved by the animal care and use committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China. Forty male Sprague-Dawley rats (8 weeks old) were used for this study. Of these, 32 were injected intraperitoneally with STZ, and the others, used as the control (C) group, were injected with vehicle (citrate phosphate buffer 0.1 mol/L; pH 4.2). After 72 hours, blood glucose levels were measured with a blood glucose meter (ACCU-CHEK Performa; Roche Diagnostics, Shanghai, China) and only rats with fasting glucose concentrations higher than 16.7 mmol/L were considered to have DM.

After 8 weeks, 30 rats with DM had survived. DMED status was assessed with an APO test. Of the rats with DM, 17 had APO-negative results, confirming DMED, and were used in subsequent experiments.[19,20] One group of rats with DMED was treated with oral FTY720 1 mg/mL dissolved in doubly distilled water (1 mg/kg; DMED + FTY720 group, n = 9) every day for 4 weeks,[21] and the other group was treated with saline on the same schedule (DMED group, n = 8). The C group (n = 8) was composed of age-matched rats without DM as described earlier.

Table 2. Metabolic parameters*

Variable	Control	DMED	DMED + FTY720
Initial weight (g)	234.72 ± 12.29	236.25 ± 11.07	230.44 ± 10.48
Final weight (g)	474.80 ± 35.47	216.30 ± 30.22†	218.26 ± 41.43†
Initial fasting glucose (mmol/L)	6.41 ± 0.57	6.33 ± 0.84	6.29 ± 0.46
Final fasting glucose (mmol/L)	6.46 ± 0.43	30.04 ± 2.36†	29.47 ± 3.27†
Mean arterial pressure (mm Hg)	109.27 ± 10.04	112.54 ± 9.2	111.6 ± 11.3

DMED = diabetes mellitus−induced erectile dysfunction.
*Data are presented as mean ± SD.
†$P < 0.001$ vs control group.