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CLINICAL SCIENCE

Acute effect of phosphodiesterase type 5 inhibitor on serum oxidative status and prolidase activities in men with erectile dysfunction

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OBJECTIVES: To investigate the acute effect of phosphodiesterase type 5 (PDE5) inhibitor on erectile dysfunction by evaluating serum oxidative status and prolidase activity.

METHODS: Serum samples of 36 patients with erectile dysfunction and 30 control cases were analyzed for total antioxidant status, total oxidant status, and prolidase activity, before and after the administration of tadalafil citrate.

RESULTS: Before and after tadalafil citrate administration, serum total antioxidant status, total oxidant status, and prolidase were 1.1 ± 0.0 vs. 1.6 ± 0.0 μmol H_2O_2 Eq/L, 10.3 ± 1.1 vs. 6.9 ± 1.2 μmol H_2O_2 Eq/L, and 236.4 ± 19.5 vs. 228.2 ± 19.2 U/L, respectively ($p<0.0001$ for all).

CONCLUSIONS: Evaluation of serum oxidative status and prolidase activity confirmed the beneficial acute effects of PDE5 inhibitor in patients with erectile dysfunction.

KEYWORDS: Phosphodiesterase type 5 inhibitors; Tadalafil citrate; Total antioxidant status; Total oxidant status; Prolidase.

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INTRODUCTION

Although phosphodiesterase type 5 (PDE5) inhibitors were initially approved for the treatment of male erectile dysfunction (ED), their clinical spectrum has expanded owing to the enormous advances that have been made during the past decade. In preliminary studies, this progress in clinical practice was not restricted to human sexuality, either male or female, other than male ED, but extended to systemic disorders such as cardiovascular conditions and diabetes mellitus, owing to the beneficial effects of PDE5 inhibitors on endothelial functions, depression, pulmonary hypertension, pre-eclampsia, inflammation, chronic heart failure and renal insufficiency, hypertensive disorders, and even to cancer treatment, owing to induction of an apoptotic mechanism.[1-5]

It is well known that oxidative stress leads to impaired vasodilatation of the coronary, pulmonary, and peripheral vascular beds.[6] There is limited information about the effect of PDE5 inhibitors on the serum oxidative mechanism, although its effect in restoring endothelial nitric oxide, which results in vasodilatation and inhibition of chemotaxis and platelet aggregation, is well known.[7] In some studies sildenafil citrate has been shown to have protective effects against oxidative stress by inhibiting free radical formation and supporting antioxidant redox systems, and it is suggested that this reduced oxidative stress results in improvement of endothelial function.[8,9] We have shown that serum prolidase activity is significantly associated with the presence and severity of vasculogenic ED, and elevated serum prolidase activity may be an independent predictor of ED.[10] However, to our knowledge, the effect of the PD5 inhibitors on serum prolidase enzymes, which are closely related to serum oxidative status and endothelial functions, has not yet been documented.

Prolidase is a cytosolic enzyme, necessary for specific splitting of imidodipeptides with proline or hydroxyproline at their C-terminals, and has a major role in collagen turnover and cell growth.[10,11] The relationship between collagen and prolidase activity was observed during a study of fibrotic processes, where an increase in prolidase activity was accompanied by an increase in tissue collagen

deposition.[11] The negative effect of free radicals is mediated by degradative agents such as proteolytic enzymes and the final step of collagen degradation is mediated by prolidase.[12] Surazynski et al. noted that prolidase may also have a role in angiogenesis since prolidase deficiency is associated with angiopathy.[13]

In this study, we investigated the acute effects of PD5 inhibitor (tadalafil citrate 20 mg) as an on-demand use PDE5 inhibitor at peak serum level on serum oxidative status and prolidase enzyme activities in patients with ED.

MATERIAL AND METHODS

Subjects

The study included 36 patients with clinically documented ED (aged 37–59 years, mean±SD 49.5±6.5) who had been followed up at the department of urology, had a stable monogamous relationship with a female partner, and for whom PDE5 inhibitor treatment had been recently proposed. Controls comprised 30 people with no ED (aged 35–58 years, mean±SD 50.1±6.7). The study was approved by Harran University's Institutional Review Board and was performed in accordance with the ethical standards laid down in the 1975 and 1983 Declaration of Helsinki. The participants were informed about the study protocol and written consent was obtained from all participants before the start of the study.

All patients provided a detailed sexual history, and underwent a physical examination, blood chemistry and endocrine assay, and color Doppler ultrasonography during pharmacologically induced and sexually stimulated erection. Patients with angina during intercourse, unstable angina or any other evidence of recently diagnosed coronary artery disease, poorly controlled blood pressure or orthostatic hypotension, congestive heart failure, arrhythmia, significant renal or hepatic dysfunction, anemia, and patients aged >60 years were excluded. Additionally, patients who had been receiving any medication that had an effect on serum oxidative status (vitamin E, vitamin C, L-arginine, etc) within the past 4 weeks or who had undergone pelvic surgery were also excluded. All blood samples for determination of total antioxidant status (TAS), total oxidant status (TOS), and prolidase activities were collected from the subjects just before and 2 h after 20 mg oral tadalafil citrate (Cialis®, Lilly Inc, Istanbul, Turkey) administration—that is, at the peak plasma level, which occurs at approximately 2 h after administration. During these 2 h, subjects remained at rest under clinic conditions and were not allowed to consume anything except water. Blood sample collections were performed in an air-conditioned consulting room at a temperature of 23–25℃.

Measurement Total Oxidant–Antioxidant Status

An automated colorimetric measurement method was used for the analysis of TOS.[14] The results were expressed as μmol H_2O_2 Eq/L. TAS was measured by an automated measurement method in which hydroxyl radicals are produced via the Fenton reaction and the colored dianisidinyl radical cations consequently produced in the reaction medium of the assay can be measured by absorbance.[15] The results were expressed as mmol Trolox Eq/L.

Measurement of Prolidase Activity

Serum was diluted 40-fold with 25 mmol/L Mn^{2+}, 40 mmol/L Trizma HCl buffer (pH 8.0) and preincubated at 37℃ for 2 h. The reaction mixture containing 30 mmol/L Gly-Pro, 40 mmol/L Trizma HCl buffer (pH 8.0), and 100 μL of preincubation serum in 1 mL was incubated at 37℃ for 30 min. The reaction was stopped by adding 0.5 mL 20% trichloroacetic acid solution. The supernatant was used for measurement of proline by the method proposed by Myara et al.,[16] which is a modification of Chinard's method.[17]

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA) version 11.0 for Windows. Results were expressed as mean±SD for all continuous variables. Differences between control, pre- and post-medication groups were assessed using an independent samples t-test. Differences between pre- and post-medication groups were assessed using a paired sample t-test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Demographic characteristics and risk factors for ED of the patients are given in Table 1. The serum samples of 30 control cases and 36 patients with ED (body mass index; range 22.0–29.8 kg/m^2, mean±SD 27.0±2.2 kg/m^2), before and after tadalafil citrate administration (120 min), were analyzed. For controls, serum levels of TOS, TAS, and prolidase were 4.37±0.9 μmol H_2O_2 Eq/L, 1.83±0.2 mmol Trolox Eq/L, and 218.09±17.5 U/L, respectively. The mean basal and post tadalafil citrate administration serum parameters (TOS, TAS, and prolidase) of the patients are summarized in Table 2. While the mean basal serum level of TAS and TOS were 1.1±0.0 and 10.3±1.1 μmol H_2O_2 Eq/L, the post tadalafil citrate values were 1.6±0.0 and 6.9±1.2 mmol Trolox Eq/L, respectively. Both reductions in TOS and increases in TAS serum values were significant ($p<0.0001$). The mean prolidase activity under the influence of the drug was 228.2±19.2 U/L, in comparison with the basal value of 236.4±19.5 U/L—a significant reduction ($p<0.0001$).

DISCUSSION

This study aimed to document the acute effects of tadalafil citrate at peak serum levels on the vascular system by evaluating serum oxidative–antioxidative status, and prolidase enzyme activities. Tadalafil citrate was chosen for this study, because it has the longest half-life of the PDE5

Table 1 - Demographic characteristics and risk factors for erectile dysfunction of the patients and control cases.

	Control (n = 30)	Patients (n = 36)	p Value
Demographic characteristics			
Age (years) (mean ±SD)	50.1±6.7	49.5±6.5	>0.05
BMI (kg/m^2)	28.0±2.9	27.0±2.2	>0.05
Risk factors n (%)			
Smoking	12 (40)	14 (39)	
Depression	-	2 (6)	
Diabetes mellitus	8 (27)	12 (33)	
Hypertension	13 (43)	12 (33)	
Chronic heart failure	5 (17)	7 (19)	
Hypercholesterolemia	4 (13)	7 (19)	

BMI, body mass index.

Table 2 - Mean serum total antioxidant status, total oxidant status and prolidase enzyme activities of the patients (before and after tadalafil citrate) and control cases.

		Patient (n = 36)		p Value		
	Control (n = 30)	BTC	ATC	p_1	p_2	p_3
TOS (μmol H_2O_2 Eq/L)	4.37 ± 0.9	10.3 ± 1.1	6.9 ± 1.2	<0.0001	<0.0001	<0.0001
TAS (mmol Trolox Eq/L)	1.83 ± 0.2	1.1 ± 0.0	1.6 ± 0.0	<0.0001	<0.0001	<0.0001
Prolidase (U/L)	218.09 ± 17.5	236.4 ± 19.5	228.2 ± 19.2	<0.0001	<0.0001	<0.0001

ATC, after tadalafil citrate administration; BTC, before tadalafil citrate administration; TAS, total antioxidant status; TOS, total oxidant status.
p_1, independent samples t-test for control and patient before tadalafil citrate; p_2, independent samples t-test for control and patient after tadalafil citrate; p_3; paired samples t-test for patient before and after tadalafil citrate.

inhibitors, making it an ideal candidate when switching from on-demand use to its use as a prophylactic drug for any indication in the future. The safety of the drug will be important for regular long-term users.

It has been suggested that oral vasoactive pharmacotherapy, such as PDE5 inhibitor drugs, may provide vasculoprotective benefits for cardiovascular disease as well as for vasculogenic ED.[18] Reduced serum oxidative stress is thought to be the foundation of this mechanism. Moreover, in support of this theory from the opposite side, oxidative stress has been shown to have a role in the development and progression of atherosclerosis.[19] It was claimed, in a placebo-controlled study, that 100 mg sildenafil improved the serum oxidative status of subjects without any health problems;[8] this effect lasted for 1 day. A further study reached the same conclusion in an animal model of second-hand smoke-induced ED.[9] Our results for the oxidative status of the patients with ED support these findings for tadalafil citrate and, additionally, we think that the reduced serum oxidative stress may last for more than a day because tadalafil citrate has a longer serum half-life than sildenafil. On the contrary, in a recent study, Burnett et al. found no effect of sildenafil on serum oxidative status in diabetic patients with ED receiving short-term treatment.[18] It should be remembered that all three PDE5 inhibitors have similar oxidative activity.[19] However, to our knowledge, this is the first clinical study that has evaluated the relation between tadalafil citrate and serum oxidative–antioxidative status, showing decreasing TOS and increasing TAS values with treatment.

Prolidase is a highly specific peptidase that has an important functional role in all human tissues and cells. High prolidase activity has been reported in the brain, heart, liver, muscle and, especially, in the kidney. Hereditary prolidase deficiency—a multisystemic disorder—is characterized by a wide spectrum of clinical manifestations, including skin ulcers, mental retardation, and susceptibility to infections. Oxidative stress results in collagen degradation and this process is mediated by prolidase.[12] Moreover, the degree of severity of oxidative stress is directly related to the inhibition of collagen production, and prolidase is thought to be the target enzyme of this process.[20] Our study showed that tadalafil citrate significantly reduced prolidase activity at the peak serum level. This might be due to reduced degradation activity, possibly caused by reduced serum oxidative stress. In addition, reduced prolidase activity also confirms the reduced oxidative stress under the action of tadalafil citrate—a topic still debated in the literature.

Fibrosis of the corpora cavernosa and the media of penile arteries, involving loss of smooth muscle cells, is a common process that underlies most cases of vasculogenic ED.[21] The concept that progressive fibrosis of the smooth muscle tissue within the penile corpora cavernosa is responsible for the vasculogenic ED associated with diabetes, ageing, heavy smoking, and pelvic surgery, has gained support over the past decade.[22] From a functional perspective, this fibrotic process leads to a decrease in the compliance of the corporal tissue after stimulation by the nitric oxide/cyclic guanosine monophosphate system. This inability of the corporal tissue to relax sufficiently to occlude the regressing subtunical veins occurs in most patients with ED[23-26] and is termed venous leakage or corporal veno-occlusive dysfunction. Therefore, the study of fibrosis may provide a unifying view of the vasculogenic disorders affecting the penis. Profibrotic factors, the excessive deposit of collagen fibers and other extracellular matrix, the appearance of a synthetic cell phenotype in smooth muscle cells or the onset of a fibroblast–myofibroblast transition, and in the case of the corporal or penile arterial tissue, the reduction of the smooth muscle cellular compartment underlies vasculogenic ED. This histopathology leads either to localized plaques or nodules in penile tissue, or to the diffuse fibrosis causing impairment of tissue compliance that underlies corporal veno-occlusive dysfunction and arteriogenic ED. The antifibrotic role of the sustained stimulation of the nitric oxide/cyclic guanosine monophosphate pathway in the penis and its possible relevance to exogenous and endogenous stem cell differentiation may explain the collagen biosynthesis. The relationship between collagen and prolidase activity was observed during fibrotic processes, where an increase in prolidase activity was accompanied by increase in tissue collagen deposition.[27] The negative effect of free radicals is mediated by degradative agents such as proteolytic enzymes and the last step of collagen degradation is mediated by prolidase.[12] Surazynski et al. pointed out that prolidase may also have a role in angiogenesis as prolidase deficiency is associated with angiopathy.[13] Oxidative stress resulted in collagen degradation and this process is mediated by prolidase.[12] Moreover the degree of severity of oxidative stress is directly related to the inhibition of collagen production, and prolidase is thought to be the target enzyme of this process.[28] Yildiz et al. showed that increased serum prolidase activity was significantly associated with the presence and severity of coronary artery disease.[29] In accordance with their study we have shown that serum prolidase activity is significantly associated with the presence and severity of vasculogenic ED, and elevated serum prolidase activity may be an independent predictor of ED.[10] In addition, it was suggested that hypertension and its duration is associated with increased serum prolidase activity and might be useful as a marker for the follow-up of hypertensive patients.[11]

Several potential limitations of this study should be considered. First, is the cross-sectional design of this study. Usually, to assess the effect of any agent on a molecule the investigation should be based on a chronic treatment set-up but in this study the acute effect of the PDE5 inhibitor was investigated. To extend the findings of our study, assessment of serum prolidase activity in endothelial cells, and evaluation of the association of serum prolidase activity with the presence and extent of atherosclerosis in other regions of the arterial system would better clarify the pathophysiological role of prolidase activity in endothelial dysfunction. Evaluation of the association of serum prolidase activity with the extent of endothelial ischemia would identify the role of endothelial ischemia in increased collagen turnover in patients with vasculogenic ED. Finally, measurement of serum oxidative status and prolidase enzyme activities after daily treatment with PDE5 inhibitor would have added to the value of this study, but this was beyond the scope of this present work.

In conclusion, the results of this preliminary study confirm the beneficial acute effects of PD5 inhibitor in patients with ED by evaluating serum oxidative status and prolidase activity. However, further clinical long-term studies of this issue, particularly including groups at high risk, should be carried out.

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