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Oxidative Stress in the Liver of Diabetic Rats Treated with a Combination of Sildenafil Citrate and a Free Radical Scavenger*

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Abstract: Erectile Dysfunction (ED) is a common problem within diabetic patients. The liver is one of the most affected vital organs by diabetic consequences. Oxidative stress is the most known intermediary pathway initiating liver diseases among diabetics. The present study was designed to investigate the protective effect of alpha toccopherol (α-TP) against possible oxidative stress, that may be elicited by administration of Sildenafil Citrate (SC) and to assess whether SC may negatively affect the liver in an experimental diabetic model. SC was given to groups of normo-glycemic and diabetic rats, either alone or in combination with α-TP, by oral route for two weeks. Hepatic tissue content of malondialdehyde-a thiobarbituric acid reactive oxygen species (TBARS) and reduced glutathione (GSH) were determined as biomarkers for oxidative stress in liver tissue. TBARS was significantly up-regulated in diabetic than normo-glycemic rats. SC significantly down-regulated TBARS content, an effect which was synergized by α-TP co-administration. SC treatment depleted GSH in both normo-glycemic and hyperglycemic rats, this effect was completely reversed by α-TP co-administration. α-TP could not correct the effect of diabetes on liver GSH and TBARS contents and it couldn't restore theses parameters in diabetic to non-diabetic values. In conclusion, our study explored the usefulness of α-TP co-administration in protecting the liver against GSH depletion, induced by SC administration. We also elucidated that SC down-regulated TBARS in liver tissue, an effect which was potentiated by α-TP co-administration. We recommend the use of α-TP as an adjuvant therapy to SC, specially for diabetic patients who are considered to be the most extensive users of the drug.

Key words: Liver, lipid peroxidation, glutathione, sildenafil, diabetes, rats

INTRODUCTION

Oxidative stress is believed to mediate the development of diabetes-associated vasculopathy, endothelial dysfunction and neuropathy within erectile tissue.

It was proposed that adequate levels of a free radical scavenger, as vitamin E, improves Erectile Dysfunction (ED), with a synergistic potential to phosphodi-esterase type 5 (PDE-5) inhibitors (De Young *et al*., 2004).

In addition, diabetes is often associated with hypogonadism, both conditions represent major risk factors for ED. Testosterone normalization in diabetic models maintains neural nitric oxide, PDE-5 and reinstates sensitivity to relaxant stimuli and responsiveness to Sildenafil Citrate (SC) (Zhang *et al*., 2006).

However, SC enhanced liver injury caused by ethanol (Li *et al*., 2005). It is metabolized by cytochrome p-450, 3A4 and 2 C and any inhibitor of these enzymes, may result in delayed metabolism and require dose adjustment (McCullough, 2002). Diabetes represents a major risk factor for ED as

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reported in people with long term Insulin Dependent Diabetes Mellitus (IDDM) (Klein *et al*., 1996). This is why special attention should be paid for patients with ED-risk factors as hypertension, diabetes and coronary heart diseases (Kalsi and Kell, 2004). In experimental models of diabetic ED, it was found that increased levels of free radicals in diabetes can divert nitric oxide away from erectogenic pathway, through its conversion to peroxynitrite (Khan *et al*., 2001). Pharmacokinetic studies of SC demonstrated similarities between the rat and human in metabolite formation *in vivo* (Walker *et al*., 1999). This drug was widely used for male ED, showing a selective PDE 5 inhibitory potential, preventing cycloguanosine monophosphate (cGMP) degradation. These actions enhance the effect of nitric oxide at the target tissue (Leung and Yip, 1999). In addition, high levels of malondialdehyde-MDA-(lipoperoxide product) and low levels of nitric oxide in peripheral blood of diabetic men, having ED correlates strongly with the severity of ED (El-Latif *et al*., 2006). Malondialdehyde is always up-regulated in liver cells in response to hepatocellular injury (El Sisi *et al*., 1993). Biomarkers of oxidative stress such as MDA and reduced glutathione (GSH) have been considered as specific indicators to oxidative status (Mayne, 2003). Data provided evidence that nitric oxide deficiency, possibly due to the membrane lipid peroxidation and defective glutathione levels, may contribute to the development of diabetic ED and thus is involved in the pathogenesis of ED in diabetic patients (Alper *et al*., 2003).

The present study was designed to investigate the possible effect of SC on liver tissue oxidative status in diabetic rats, trying α-TP as a free radical scavenger in both normo-glycemic and hyperglycemic subjects.

MATERIALS AND METHODS

Animals and Experimental Design

Sixty four male Wistar rats weighing 100-120 g of ages 6-8 weeks were purchased from the animal house of the college of medicine, Assuit University, Egypt, around December 2004. The animals were kept in polyethylene cages of 60×40×30 cm dimensions at temperature range of 15-20°C, in fairly humid room at 12 h light/12 h dark adjusted cycles. Rats were fed standard rat chow and allowed to drink normal tap water ad libitum and left for 10 days to acclimatize before dosing started. They were classified into 8 equal groups and assigned from 1 to 8.

Group 1 was left as control and did not given any medication. Group 2 was given SC (formal chemical name (IUPAC) : 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d] pyrimidin-5-yl) phenylsulfonyl]-4-methylpiperazine, Pfizer, USA), as single dose of 3 mg kg^{-1} body weight by oral gavage (Baratti and Boccia, 1999). Group 3 was given α-TP (Farco, Egypt), dissolved in olive oil, as 300 mg kg^{-1} daily and orally (Fu and Liu, 1992). Group 4 was given both SC, then α-TP, 30 minutes apart, in the same doses on a daily basis. The first four groups were considered as normo-glycemic. The animals of group 5 were given 150 mg kg^{-1} alloxan, intra-peritoneally, blood glucose was measured daily, by blood puncture, the dose could be repeated till blood glucose level reached about 270 mg dL^{-1}. This level was considered to be the diabetic level (Kisel *et al*., 2001). This group served as diabetic control. Groups 6-8' animals were diabetized by the same way, then group 6 animals were given SC doses as group 2. Group 7 animals were given α-TP alone, those of group 8 were given SC plus α-TP as group 4. Dosing for both normo-glycemic and diabetic animals continued for two consecutive weeks. Groups 5-8 were considered as diabetic subjects. All animals were killed in the next morning of the last dose. Fasting blood samples were withdrawn, centrifuged, sera were divided into aliquots, livers were excised, blotted in filter papers, frozen in liquid nitrogen and all samples were kept frozen at -80°C right analysis.

Methods

Tissue content of GSH was colorimetrically determined, utilizing a reaction with 5, 5-dithiobis (2-nitrobenzoic acid, Illman's reagent) (Moron *et al*., 1979). Hepatic tissue lipid peroxidation product was estimated by thiobarbituric acid reaction (Ohkawa *et al*., 1979).

Statistical Analysis

Results were expressed as mean±SEM. The inter group variations was measured by one way analysis of variance (ANOVA). Statistical significance was considered at $p<0.05$. Analysis was by one way ANOVA and differences were calculated using Duncan's new multiple range test (Duncan's, 1955).

RESULTS AND DISCUSSION

Thiobarbituric acid reactive oxygen species, TBARS (a lipid peroxidation product) was significantly reduced by SC and more significantly by α-TP when given alone or as an adjuvant to SC ($p<0.01$). GSH was significantly down-regulated by SC, but administration of α-TP alone significantly up-regulated GSH, which was non-significantly up-regulated by the combination of SC with α-TP (Table 1). Effect of diabetes on liver contents of both TBARS and GSH is shown in Table 2.

Malondialdehyde is a known by-product of lipid peroxidation usually considered as a biomarker for oxidative stress. Peroxidation and reduced antioxidant reserve play an important role in the pathogenesis of diabetic vascular complications (Piconi *et al*., 2003). In our study, TBARS is significantly up-regulated in diabetic, compared to control normo-glycemic animals (Table 2). This observation is in agreement with that reported by Griesmacher *et al*. (1995). In addition, El-Latif *et al*.

Table 1: Effect of Sildenafil citrate (SC, 3 mg kg^{-1}, body weight) combination with α-TP (300 mg kg^{-1}, body weight) on liver lipid peroxide and glutathione contents in normo-glycemic and diabetic rats after two weeks of daily oral administration

	Groups							
	Normo-glycemic				Diabetic			
Parameters	Control	SC	α-TP	SC+α-TP	Control	SC	α-TP	SC+α-TP
TBARS (mmol mg^{-1} protein)	0.4±0.01	0.35±0.01*	0.33±0.01*	0.3±0.01*	1.4±0.01	0.5±0.01*	0.6±0.13*	0.41±0.01*
GSH (μg mg^{-1} protein)	26.2±0.47	19.60±0.07*	31.20±1.2*	27.7±0.62	16.9±0.32	13.2±0.73*	20.5±0.45*	17.60±0.32

Values are expressed in mean±SE (n = 8), *: Significantly different from control at $p<0.01$

Table 2: Role of diabetes on TBARS and GSH liver contents in rats treated by SC (3 mg kg^{-1}, body weight) and α-TP (300 mg kg^{-1}, body weight) combination after two weeks of daily oral administration

TBARS (mmol mg^{-1} protein)								
Control		SC		α-TP		SC+α-TP	
N	D	N	D	N	D	N	D
0.4±0.01	1.4±0.01**	0.35±0.01	0.5±0.01**	0.33±0.01	0.6±0.13**	0.3±0.01	0.41±0.01**
GSH (μg mg^{-1} protein)							
Control		SC		α-TP		SC+α-TP	
N	D	N	D	N	D	N	D
26.2±0.47	16.9±0.32**	19.6±0.07	13.2±0.73**	31.2±1.2	20.5±0.45**	27.7±0.62	17.6±0.32**

Values are expressed in mean±SE (n = 8), D = Diabetic, N = Normo-glycemic, **: Significantly different from normo-glycemic at $p<0.001$

(2006) registered that peripheral and cavernous TBARS levels were higher in diabetic, compared to normo-glycemic men. TBARS up-regulation was strongly correlated to ED in diabetic patients (Sozmen *et al*., 1999).

SC significantly down-regulated hepatic TBARS in normo-glycemic rats (compared to normo-glycemic control) and similarly in diabetic in comparison to control diabetic rats. This is might be due to maintaining nitric oxide production within a physiologic level acting as internal antioxidant (Tooby *et al*., 2004). It is also clear that diabetes played a significant role in TBARS regulation in SC users, which is mostly due to diabetes-induced vasculopathy, taking into account that SC mainly acts on vascular bed. On the contrary of that, a combination of SC and alcohol was reported to up-regulate TBARS in rat testicular tissues after the same period of administration. This is mostly due to increased tissue oxygenation and vascular congestion by both SC and alcohol (Sivasankaran *et al*., 2007). However, tissue response may differ in regard to SC vascular activity. In our findings, both SC and α-TP could nearly restore TBARS to normal non-diabetic control, specially when given together.

α-TP administration for both normo- and hyperglycemic rats down-regulated TBARS, whether given alone, or as an adjuvant to SC. Literatures correlating SC to hepatic TBARS content seem to be very sparse. This is why our observation may be reported for the first time. SC treatment exhibited a prominent antioxidant activity on hepatocellular level, comparable to α-TP and its combination to α-TP showed an additional synergistic effect as an antioxidant, taking into account that α-TP was reported to be hepatocellular antioxidant (Yakaryilmaz *et al*., 2007).

GSH content was significantly lower in diabetic than normo-glycemic subjects. In both cases, SC significantly depleted hepatic GSH, which was corrected by α-TP co-administration to control, non-treated levels, but it was up-regulated by α-TP when given alone. Our finding is comparable to that recently noticed by Sivasankaran *et al*. (2007), who found that SC and alcohol combination significantly depleted testicular GSH in treated rats. α-TP was reported to correct glucose-induced vascular dysfunction in diabetics (Kinlay *et al*., 1999). In addition, our results agree with that reported by De Young *et al*. (2003), who registered that α-TP combined with SC is better than either of each, when given solely in animal model of diabetes. Additionally, reactive oxygen species seems to be double-edged sword, serving as a key signal molecule in physiological processes, but also have a role in pathological pathways, which was greatly corrected by α-TP supplementation (Agarwal *et al*., 2005).

However, diabetes played a significant role in GSH down-regulation than normal rats. Existence of diabetes prohibited both SC (solely) or combined with α-TP from restoring GSH level to normal non-diabetic control, although α-TP co-administration significantly alleviated SC effect on GSH level (Table 2).

In conclusion, our study revealed that diabetes plays a key role in the effect of SC on oxidative stress and anti-oxidant therapy fails to combat this problem. SC can attenuate lipoperoxidation in hepatic tissue, which was significantly synergized by α-TP co-administration, in both diabetic and normo-glycemic rats. Meanwhile, it significantly depleted liver GSH, an effect could be combated by α-TP supplementation. It is suggested that, GSH depletion is mostly consumed in other alternative pathways than lipid peroxidation. By instance, SC administration by hepatic diseased and diabetic patients can be encouraged when used simultaneously with α-TP, although SC can't be considered as an adjuvant treatment for diabetic-induced GSH depletion, but greatly corrected reflected lipoperoxidation in diabetes.

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