



Open Access

ORIGINAL ARTICLE

Erectile Function

Chronic administration of sildenafil improves erectile function in a rat model of chronic renal failure

Nilgun Gurbuz¹, Arif Kol², Tumay Ipekci², Erhan Ates², Asli Baykal¹, Mustafa F Usta²

The relationship between erectile dysfunction (ED) and chronic renal failure (CRF) has been reported in several studies. This study aimed to investigate whether the chronic use of sildenafil could enhance the erectile capacity in CRF-induced rats. In addition, we assessed the effect of that treatment on certain molecules, which have been suggested to play crucial roles in erectile physiology and CRF-related ED as well. Three groups of animals were utilized: (1) age-matched control rats, (2) CRF-induced rats, (3) CRF-induced rats treated with chronic administration of sildenafil (5 mg kg⁻¹ p.o. for 6 weeks [treatment started after 6 weeks of CRF induction]). At 3 months, all animals underwent cavernosal nerve stimulation (CNS) to assess erectile function. Penile tissue advanced glycation end products (AGE's)/5-hydroxymethyl-2-furaldehyde, malondialdehyde (MDA), cGMP (ELISA), inducible nitric oxide synthase (iNOS) and neuronal NOS (nNOS) (Western blot) analyses were performed in all rat groups. CRF-induced rats had a significant decrease in erectile function when compared to control rats ($P < 0.05$). The increase in both intracavernosal pressure (ICP) and area under the curve of CRF-induced rats treated with sildenafil (Group 3) was greater than CRF-induced rats (Group 2). Additionally, sildenafil treatment decreased AGE, MDA and iNOS levels, while it preserved nNOS and cGMP contents in CRF-induced penile tissue. Decreased AGE, MDA, iNOS and increased nNOS, cGMP levels at the sildenafil-treated group increased both ICP and Total ICP to CNS, which led to improve erectile function in CRF-induced rats. The results of the present study revealed the therapeutic effect of chronic sildenafil administration on erectile function in CRF-induced rats.

Asian Journal of Andrology (2015) 17, 797–801; doi: 10.4103/1008-682X.146973; published online: 30 January 2015

Keywords: chronic renal failure; erectile function; sildenafil

INTRODUCTION

Erectile dysfunction (ED), the inability to attain or maintain penile erection for satisfactory sexual intercourse, is highly prevalent among men with chronic renal failure (CRF).^{1,2} Recently, it has been shown that the prevalence of ED is between 70% and 86% within this population.² Further studies have shown the effect of both renal transplantation and hemodialysis on erectile function in men with CRF-related ED.³ Moreover, the efficacy of on-demand use of sildenafil on erectile function in men with CRF has also been studied in several clinical trials.^{4,5} However, the effect of chronic administration of sildenafil on erectile physiology has not been investigated either in men with CRF or in an experimental CRF model yet.

Previously, the exact pathological effect of CRF on ED was not completely determined, as studies have focused on neuropathy, hyperparathyroidism, hyperprolactinemia, hypogonadism and anemia due to erythropoietin deficiency.⁶ Yet in recent reports, CRF-induced disturbances of erectile physiology has been investigated in detail, and the detrimental effect of CRF on the vascular component is well documented.⁷ Several factors, including increased penile tissue advanced glycation end products (AGEs) levels, decreased connexin 43 protein content has been reported to cause CRF associated ED in animal models.^{7,8} Furthermore, *in vitro* models revealed the effect of CRF on purinergic responses and nonadrenergic,

noncholinergic-mediated relaxation of cavernosal smooth muscle cells.^{9,10}

In previous studies, we investigated the connection between increased penile tissue AGE levels and CRF using an experimental model. In CRF model, we showed that penile tissue inducible nitric oxide synthase (iNOS) was significantly increased by which AGEs may produce their detrimental vascular effect.⁷ In further investigations, it was shown that, within the vascular wall, AGEs trap plasma proteins, quench NO and interact with specific AGE receptors to induce the release of cytokines and growth factors.¹¹

The aim of the present study was to investigate the possible relationship between AGE levels in penile tissue and CRF using an experimental model. Additionally, the efficacy of chronic use of sildenafil on erectile function in rats with CRF was assessed. To the best of our knowledge, the present animal model is the first model to investigate the effect of chronic sildenafil administration both on erectile physiology and on certain important molecules, which play crucial roles in normal erectile function.

MATERIALS AND METHODS

Study design

Male Harlan Sprague-Dawley rats weighing 340–490 g, 18–20 weeks old, were used for all experimental analyses. Akdeniz University

¹Department of Biochemistry, Akdeniz University School of Medicine, Antalya, Turkey; ²Department of Urology, Section of Andrology, Akdeniz University School of Medicine, Antalya, Turkey.

Correspondence: Dr. MF Usta (musta@akdeniz.edu.tr)

Received: 06 May 2014; Revised: 26 June 2014; Accepted: 26 July 2014

Animal Ethics Committee approved the research protocol. Twenty-four male rats were randomly divided into three groups: (I) age-matched control group ($n = 8$) without any operation, (II) CRF group ($n = 8$), which underwent 5/6 nephrectomy of the right kidney and total nephrectomy of the left kidney, followed by anesthesia with pentobarbital (50 mg kg^{-1} i.p.) and (III) CRF-treatment group ($n = 8$) receiving 5 mg kg^{-1} per day (p.o) of sildenafil citrate (Pfizer, New York, NY, USA) for 6 weeks after 6 weeks of the nephrectomy procedure. All rats were fed the same diet. CRF development was evaluated by measuring serum blood urea nitrogen (BUN) and creatinine levels in all rats before and after the operation, once a month. Development of renal failure was considered if serum BUN and creatinine levels were two-fold higher than the control serum level ($>20 \text{ mg dl}^{-1}$; $>1.2 \text{ mg dl}^{-1}$).

Advanced glycation end product (5-hydroxymethyl-2-furaldehyde) levels in cavernosal tissue

Advanced glycation end product content was evaluated through 5-hydroxymethyl-2-furaldehyde (5-HMF) levels in penile samples of control, CRF and sildenafil treatment groups by the colorimetric method of Kasai *et al.*¹² Briefly, 20 mg of the penile tissue hydrolyzed in 0.33 mol l^{-1} oxalic acid by boiling for 5 h was added to ice-cold 40% trichloroacetic acid to precipitate protein. After centrifugation at $1500 \times g$ for 10 min, the supernatant was incubated with 0.05 mol l^{-1} thiobarbituric acid (TBA) at 37°C for 30 min. 5-HMF levels were measured at 443 nm absorbance and expressed as nmol mg^{-1} tissue.

Malondialdehyde levels in cavernosal tissue

As a marker of lipid peroxidation generation, malondialdehyde (MDA) were measured in penile tissue samples of all groups by the fluorometric method of Wasowicz *et al.*¹³ Briefly, 20 mg tissue homogenized in 0.15 mol l^{-1} sodium phosphate buffer, pH 7.4, was reacted first with TBA by boiling for 1 h. and then with butanol for phase extraction. After centrifugation at $2500 \times g$ for 10 min, the fluorescence of the supernatant was measured at $\lambda_{\text{ex}} = 525 \text{ nm}$ and $\lambda_{\text{em}} = 547 \text{ nm}$. MDA levels were calculated based on the calibration curve using malonaldehyde bis dimethyl acetal in concentrations of 0, 0.5, 1, 2.5, 5, 7.5, 10 nmol ml^{-1} , and expressed as nmol mg^{-1} protein. Protein amounts were quantified by Lowry methods based on a complex formation of divalent copper ion with peptide bonds in which it was reduced to a monovalent ion under alkaline conditions.¹³

cGMP levels in cavernosal tissue

Cavernosal cGMP contents were measured in control and CRF-induced penile tissue and sildenafil-treated groups ($n = 3$ samples from each group). For the determination of cGMP levels, penile tissue samples were assayed by using an enzyme-linked immunoassay kit (Cayman Chemical Company, Ann Arbor, MI, USA) based on the competition between free-cGMP and a cGMP-acetylcholinesterase conjugate for a limited amount of cGMP-specific rabbit antibody sites, as previously described.¹⁴

Protein expression of neuronal nitric oxide synthase in cavernosal tissue

Cytosolic fractions of cavernosal tissues from control, CRF and therapeutic sildenafil rats were used for Western blot analyses to demonstrate neuronal NOS (nNOS) protein expression. Equal amounts of protein, 40 μg , were first run on 7.5% sodium dodecyl sulfate/polyacrylamide gel electrophoresis gels and transferred electrophoretically to $0.2 \mu\text{m}$ nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were first treated with 5% milk in TBST (120 mmol l^{-1} Tris-HCl, pH 7.4,

150 mmol l^{-1} NaCl and 0.05% Tween 20) for 1 h at room temperature to block nonspecific binding sites, and then incubated with polyclonal rabbit anti-nNOS (1:500 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) overnight and with horseradish peroxidase-conjugated secondary antibody (1:1000 dilution; Invitrogen-Biosource Inc., Carlsbad, CA, USA) for 1 h at room temperature. The bands were visualized by using enhanced chemiluminescence detection reagent kit (Amersham Biosciences, Arlington, IL, USA). Equal loading and transfer were confirmed by blotting the same membrane with anti-GAPDH (1:5000 dilution; Chemicon International, Temecula, CA, USA). Quantification of band intensity was performed by Image J version 1.24 software (National Institutes of Health, Bethesda, Maryland, USA). Data are representative of 5 independent experiments with nearly identical results.^{14,15}

Measurement of erectile responses

The erectile capacity was evaluated by intracavernous pressure measurements in control, CRF and sildenafil therapeutic rats after 12 weeks of nephrectomy procedure, as previously described in several studies.¹⁴⁻¹⁸ Of note, sildenafil treatment was given up 3 days before the "erectile response measurement" due to the wash-out issue of the drug.

Statistical analysis

All results expressed as mean \pm SEM were statistically analyzed using a software program for statistics (SPSS 13.0 Version, SPSS Inc., Chicago, IL, USA). The differences among groups were evaluated by Kruskal-Wallis one-way ANOVA. Since our data were nonparametric and independent, example, the comparison of *in vivo* data between control and CRF groups at only specific 7.5 V value, Mann-Whitney U-test was used to analyze the differences between two groups. *P* values were adjusted with a Bonferroni correction. Level of significance < 0.05 was considered as significant.

RESULTS

In our study, we were able to induce CRF in 16 animals (Group II and Group III). However, due to the technical difficulty of the method, an additional 5 and 4 animals died during the study period in Group II and Group III, respectively. Autopsies in those animals showed intraperitoneal bleeding and necrosis of the remaining kidney. Additionally, we failed to induce CRF in 3 and 2 animals from Group II and Group III, respectively, which were excluded from the study. At the end of the study, 16 CRF-induced rats were able to be included (Group II and III). As shown in **Figure 1a** and **1b** there was a significant rise in serum BUN and creatinine levels in Group II and III when compared to the control group ($P < 0.05$). While the mean BUN and creatinine levels were $11.2 \pm 0.6 \text{ mg dl}^{-1}$ and $0.59 \pm 0.01 \text{ mg dl}^{-1}$ for the control group; the mean BUN and creatinine values for Group II and III was found $22.75 \pm 1.97 \text{ mg dl}^{-1}$; 1.41 mg dl^{-1} and $32.12 \pm 4.75 \text{ mg dl}^{-1}$; $1.61 \pm 0.13 \text{ mg dl}^{-1}$, respectively.

Cavernosal advanced glycation end product (5-hydroxymethyl-2-furaldehyde) and malondialdehyde levels

5-hydroxymethyl-2-furaldehyde served as a marker of penile AGE content, while MDA was considered as a marker for reactive oxygen species. As shown in **Figures 2** and **3**, both 5-HMF and MDA levels were significantly increased in the penile tissue of CRF-induced rats (Group II and Group III) when compared to the control group. However, chronic use of sildenafil caused a significant decrease in 5-HMF and MDA levels in the penile tissue of CRF-induced rats (Group-III) when compared to CRF-induced rats without treatment (Group II).

Cavernosal c-GMP levels

Cavernosal tissue concentrations of cGMP were measured in the control rats, CRF-induced rats and the therapeutic group, and these data are shown in **Figure 4**. Cavernosal cGMP levels were significantly lower ($P < 0.05$) in the CRF-induced rats (Group II) when compared to control rats. Chronic sildenafil treatment significantly increased penile tissue c-GMP levels when compared to CRF-induced rats (Group II vs Group III; $P < 0.05$).

Western blot analysis of inducible nitric oxide synthase and neuronal nitric oxide synthase

Levels of iNOS, nNOS and GADPH protein were measured in rat cavernous tissue from control, CRF rats and CRF rats treated with sildenafil (**Figure 5a** and **5b**). Penile tissue iNOS (130 kDa) protein levels were significantly increased in cavernous tissue of CRF rats and CRF rats treated with sildenafil compared to the control group ($P < 0.05$). However, in the CRF rats treated with sildenafil the

expression of iNOS was significantly lower than the CRF rats without treatment. Furthermore, while penile tissue nNOS (155 kDa) protein levels were significantly decreased in cavernous tissue in CRF rats compared with control group ($P < 0.05$), nNOS levels were preserved in penile tissues from CRF rats treated with sildenafil.

In vivo erectile function

Erectile responses to cavernosal nerve stimulation (CNS) are given in **Figure 6**. The magnitude of the increase in intracavernosal pressure/mean arterial pressure (ICP/MAP) in response to CNS in the CRF rats was significantly lower ($P < 0.05$) than the control rats, whereas rats treated with sildenafil had greater responses to CNS (at 5 V and 7.5 V settings) that was similar to the response obtained in the control rats (**Figure 6a**). Furthermore, the total ICP (total duration of erection) in the CRF rats was significantly lower than the control rats. However, in the CRF rats treated with sildenafil, the total ICP level was significantly increased both at the 5 V and 7.5 V settings which was similar to the control group.

DISCUSSION

The present study demonstrated a significant increase in AGE, MDA and iNOS protein levels in CRF-induced rats' penises. Furthermore, chronic sildenafil treatment in these animals caused a significant decrease in AGE, MDA and iNOS levels. Additionally, we have shown that cavernosal cGMP and nNOS protein contents were preserved in the CRF-induced rats treated with sildenafil. Concurrent *in vivo* erectile function studies revealed that CRF-induced rats, which underwent chronic sildenafil treatment had significant improvements in peak and total ICP.

Currently, it is believed that CRF causes neurological and vascular changes in the penis. However, despite extensive research into the

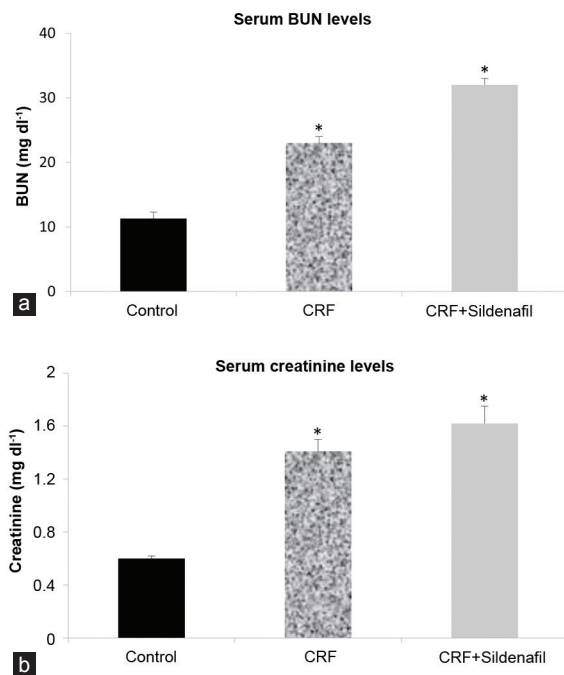


Figure 1: (a) Serum blood urea nitrogen levels. (b) Serum creatinine levels. * $P < 0.05$, compared with control.

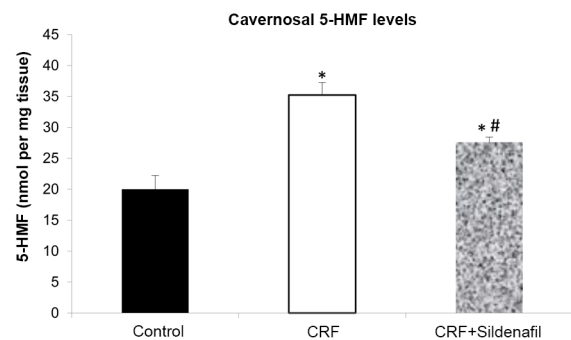


Figure 2: Cavernosal advanced glycation end product levels. * $P < 0.05$, compared with control. # $P < 0.05$, compared with chronic renal failure.

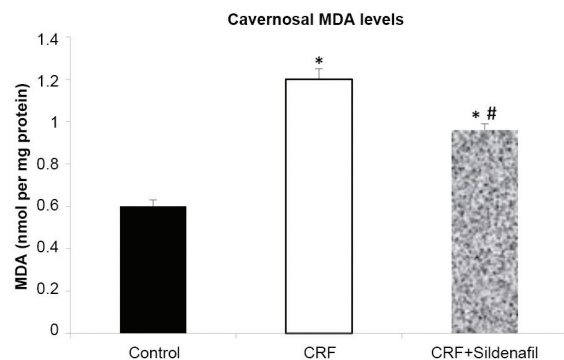


Figure 3: Cavernosal malondialdehyde levels. * $P < 0.05$, compared with control. # $P < 0.05$, compared with chronic renal failure.

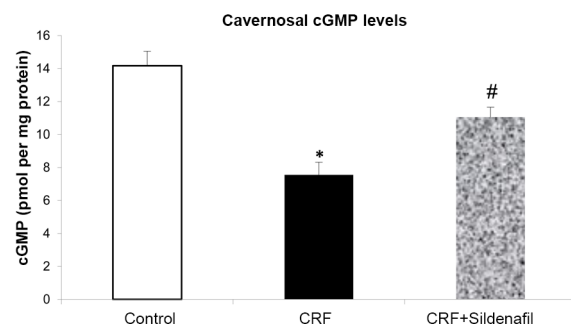


Figure 4: Cavernosal cGMP levels. * $P < 0.05$, compared with control. # $P < 0.05$, compared with chronic renal failure.

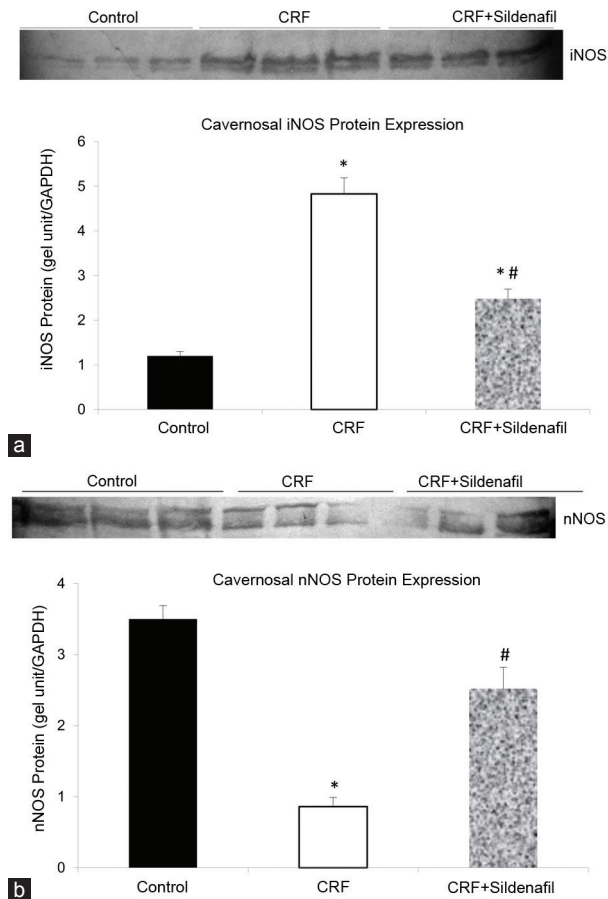


Figure 5: (a) Cavernosal inducible nitric oxide synthase (NOS) protein expression. * $P < 0.05$, compared with control. # $P < 0.05$, compared with chronic renal failure (CRF). (b) Cavernosal neuronal NOS protein expression. * $P < 0.05$, compared with control. # $P < 0.05$, compared with CRF.

etiology of CRF-related ED, the exact mechanism by which erectile failure occurs remains unknown. It has been reported that uremia produces an alteration in the metabolic environment with its local and systemic pathologic effects. Furthermore, researchers have focused on uremic neuropathy, hyperparathyroidism, hyperprolactinemia, medications, disturbance of the autonomic nervous system, low testosterone levels, and anemia due to erythropoietin deficiency.^{6,7}

The role of AGEs on erectile pathophysiology was first reported by Seftel *et al.*, who hypothesized that AGEs may affect erectile function by interacting with NO.¹⁹ AGEs are formed through a series of irreversible oxidative and nonoxidative reactions in tissues over time, in particular, endothelial and vascular smooth muscle cells.^{20,21} A common consequence of AGE formation is the pathologic cross-linking of collagen, which leads to vascular thickening, endothelial dysfunction, and ultimately atherosclerosis of the vascular branches. Moreover, studies have shown that AGEs quench NO *in vitro*, and AGE formation causes accelerated superoxide anion formation. AGEs accumulate in endothelial and smooth muscle cells and lead sustained cellular activation of numerous proteins and generation of oxygen-derived free radicals.^{21,22} Previously, it has been reported that a possible explanation accounting for CRF-related ED resides in AGE formation. The increased penile AGE levels in CRF-induced rats have been shown.^{7,19} In this study, cavernous tissue from CRF-induced rats showed an increase in AGE's, MDA and iNOS protein expression. These findings suggest that AGEs formation

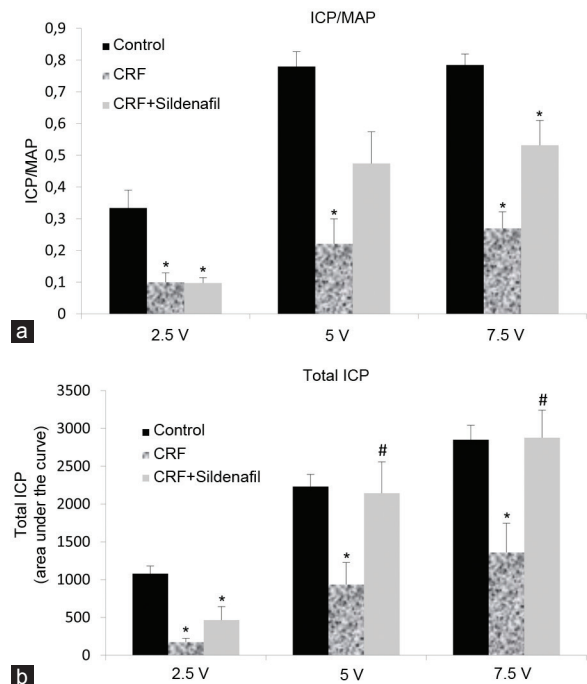


Figure 6: The voltage depending erectile response. (a) Intracavernosal pressure (ICP)/mean arterial pressure and (b) total ICP (area under the curve; mmHg s) after cavernosal nerve stimulation for 1 min in all groups of animals. * $P < 0.05$, compared with control. # $P < 0.05$, compared with chronic renal failure.

and related increase in iNOS may contribute to ED in CRF-induced rats. Recently, we have shown that while AGEs, MDA, iNOS, NF- κ B, MAP kinase and apoptosis levels were significantly increased in diabetic penises, the chronic use of sildenafil was able to decrease the content of all of those parameters, which led to improved erectile capacity.¹⁵ On the other hand, in recent literature there are several papers suggesting the contributing effect of iNOS on erectile function in the presence of an inflammatory environment.²³ To our knowledge, the study revealed for the first time the protective effect of chronic sildenafil use on erectile function in CRF-induced rats. According to our results, the mentioned effect probably occurred due to the protection of penile cGMP and nNOS protein contents in CRF-induced rats treated with sildenafil.

During the last 2 decades, phosphodiesterase-5 (PDE-5) inhibitors have been used as the first-line therapeutic option for the treatment of ED world-wide. In several studies, it has been shown that sildenafil is an effective and well-tolerated treatment for ED in men with CRF who are on chronic dialysis.^{5,24} On the other hand, there is only limited experimental information explaining the impact of sildenafil in CRF-induced ED, and the possible effect of sildenafil in both erectile physiology and pathophysiology has not been determined in detail.

Kilcarslan *et al.* have investigated the effect of CRF on PDE-5 inhibitor-induced relaxation response in rabbit cavernosal strips. The results of this *in vitro* model showed that the decreased production or availability of endogenous NO in CRF animals led to decreased efficacy of PDE-5 inhibitors to induce relaxation.¹⁰ However, the mentioned study has not assessed the effect of CRF on some important mediators which are involved in either normal erectile physiology or pathophysiology. Additionally, because the design of that study is an *in vitro* one, the assessment related to erectile function seems somewhat debatable.

We believe that the impact of chronic sildenafil treatment on molecules including AGE, MDA, iNOS, nNOS and cGMP, which have been suggested to play crucial roles in CRF related to ED pathophysiology, has not been investigated yet. The main goal of this study was to determine the CRF-related ED pathophysiology in a molecular point of view. Furthermore, we aimed to show whether or not chronic sildenafil administration has a therapeutic effect on erectile function *in vivo* CRF model.

Our results clearly showed that penile tissue nNOS and cGMP contents could be preserved by chronic sildenafil administration in a CRF-induced rat model. Additionally, we revealed, for the first time, the therapeutic effect of chronic sildenafil therapy on erectile function in CRF-induced rats.

To produce renal failure we performed the left total nephrectomy and right 5/6 nephrectomy technique. This is a commonly used method including excision of upper and lower poles, which allow for the consistent removal of a nearly equal amount of tissue from each pole of the kidney and controlled bleeding.^{6,7} Previous studies have shown that this technique causes the development of ED by impairing NO/cGMP-signaling pathway and increasing RhoA/Rho-kinase pathway. Furthermore, it has been reported that there was a lower erectile response in 5/6 nephrectomized rats following injection of apomorphine, and postulated that the CRF caused a decrease in the expression of a certain gap junction protein (C × 43), which is responsible for relaxation of corporeal smooth muscle and penile erection.^{8,25} Although this technique is widely used in several studies, in our study 30% of animals died after the surgical procedure and in ~ 16% of rats, we were not able to induce CRF. We believe in a meticulous surgical approach in order to avoid high grade of animal loss and unsatisfactory CRF induction.

CONCLUSIONS

The present experimental model has confirmed the detrimental effect of CRF on erectile function. A possible explanation, which could account for CRF-associated ED, may occur through the formation of AGEs. Chronic use of sildenafil has a therapeutic effect on erectile function in CRF-induced rats by decreasing penile tissue AGE levels and protecting cGMP and nNOS protein contents. The results of the present study broaden our understanding about CRF-related ED and may suggest new therapeutic avenues such as chronic administration of PDE-5 inhibitors and the possible use of AGE inhibitors.

AUTHOR CONTRIBUTIONS

NG: carried out the molecular studies, participated in the drafting procedure of the paper and performed the statistical analysis. AK: carried out the *in vivo* studies. TI: participated in the design of the study. EA: participated in the *in vivo* studies. AB: participated in the design of the study and helped draft the manuscript. MU: has made substantial contributions to conception and design.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was supported by the Akdeniz University Research Foundation. Sildenafil was kindly provided by Pfizer, New York, NY, USA. Part of the present study was presented during the AUA Annual Meeting, San Diego, USA, 2013.

REFERENCES

- Kaufman JM, Hatzichristou DG, Mulhall JP, Fitch WP, Goldstein I. Impotence and chronic renal failure: a study of the hemodynamic pathophysiology. *J Urol* 1994; 151: 612–8.
- Fernandes GV, dos Santos RR, Soares W, de Lima LG, de Macêdo BS, *et al*. The impact of erectile dysfunction on the quality of life of men undergoing hemodialysis and its association with depression. *J Sex Med* 2010; 7: 4003–10.
- Tavallaii SA, Mirzamani M, Heshmatzade Behzadi A, Assari S, Khoddami Vishteh HR, *et al*. Sexual function: a comparison between male renal transplant recipients and hemodialysis patients. *J Sex Med* 2009; 6: 142–8.
- Hyodo T, Ishida H, Masui N, Taira T, Yamamoto S, *et al*. Kidney disease quality of life of Japanese dialysis patients who desire administration of sildenafil and the treatment of erectile dysfunction using sildenafil. *Ther Apher Dial* 2004; 8: 340–6.
- Yeniçerioglu Y, Kefi A, Aslan G, Cavdar C, Esen AA, *et al*. Efficacy and safety of sildenafil for treating erectile dysfunction in patients on dialysis. *BJU Int* 2002; 90: 442–5.
- Abdel-Gawad M, Huynh H, Brock GB. The impact of chronic renal failure on nitric oxide synthase isoforms gene expression in the penis and pelvic ganglia of rats. *J Urol* 1999; 162: 1473–9.
- Usta MF, Tuncer M, Baykal A, Ciftçioğlu MA, Erdogru T, *et al*. Impact of chronic renal failure and peritoneal dialysis fluids on advanced glycation end product and iNOS levels in penile tissue: an experimental study. *Urology* 2002; 59: 953–7.
- Fu Q, Lv JJ, Zhang H. Effects of chronic renal failure on the expression of connexin 43 in the rat's corpus cavernosum. *Asian J Androl* 2008; 10: 286–9.
- Bagcivan I, Kilicarslan H, Sarac B, Gokce G, Yildirim S, *et al*. The evaluation of the effects of renal failure on erectile dysfunction in a rabbit model of chronic renal failure. *BJU Int* 2003; 91: 697–701.
- Kilicarslan H, Yildirim S, Bagcivan I, Gokce G, Sarac B, *et al*. The effect of chronic renal failure on phosphodiesterase inhibitor-induced relaxation responses in rabbit cavernosal strips. *Eur J Pharmacol* 2003; 462: 155–60.
- Usta MF, Bivalacqua TJ, Yang DY, Ramanitharan A, Sell DR, *et al*. The protective effect of aminoguanidine on erectile function in streptozotocin diabetic rats. *J Urol* 2003; 170: 1437–42.
- Kasai K, Nakamura T, Kase N, Hiraoka T, Suzuki R, *et al*. Increased glycosylation of proteins from cataractous lenses in diabetes. *Diabetologia* 1983; 25: 36–8.
- Wasowicz W, Nève J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 1993; 39: 2522–6.
- Bivalacqua TJ, Usta MF, Champion HC, Leungwattanakij S, Dabisch PA, *et al*. Effect of combination endothelial nitric oxide synthase gene therapy and sildenafil on erectile function in diabetic rats. *Int J Impot Res* 2004; 16: 21–9.
- Gurbuz N, Sagdic G, Sanli A, Ciftçioğlu A, Bassogru I, *et al*. Therapeutic effect of combination of alagebrium (ALT-711) and sildenafil on erectile function in diabetic rats. *Int J Impot Res* 2012; 24: 114–21.
- Bivalacqua TJ, Usta MF, Kendirci M, Pradhan L, Alvarez X, *et al*. Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of *in vivo* extracellular superoxide dismutase gene therapy. *J Sex Med* 2005; 2: 187–97.
- Usta MF, Bivalacqua TJ, Koksai IT, Toptas B, Surmen S, *et al*. The protective effect of aminoguanidine on erectile function in diabetic rats is not related to the timing of treatment. *BJU Int* 2004; 94: 429–32.
- Usta MF, Kendirci M, Gur S, Foxwell NA, Bivalacqua TJ, *et al*. The breakdown of preformed advanced glycation end products reverses erectile dysfunction in streptozotocin-induced diabetic rats: preventive versus curative treatment. *J Sex Med* 2006; 3: 242–50.
- Seftel AD, Vaziri ND, Ni Z, Razmjouei K, Fogarty J, *et al*. Advanced glycation end products in human penis: elevation in diabetic tissue, site of deposition, and possible effect through iNOS or eNOS. *Urology* 1997; 50: 1016–26.
- Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia* 2001; 44: 129–46.
- Bivalacqua TJ, Usta MF, Champion HC, Kadowitz PJ, Hellstrom WJ. Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. *J Androl* 2003; 24 6 Suppl: S17–37.
- Vlassara H. The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 2001; 17: 436–43.
- Kataoka T, Hotta Y, Maeda Y, Kimura K. Assessment of androgen replacement therapy for erectile function in rats with type 2 diabetes mellitus by examining nitric oxide-related and inflammatory factors. *J Sex Med* 2014; 11: 920–9.
- Chen J, Mabeesh NJ, Greenstein A, Nadu A, Matzkin H. Clinical efficacy of sildenafil in patients on chronic dialysis. *J Urol* 2001; 165: 819–21.
- Chung E, De Young L, Brock GB. Investigative models in erectile dysfunction: a state-of-the-art review of current animal models. *J Sex Med* 2011; 8: 3291–305.