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Effect of tadalafil in chronic renal failure rabbits: relevance to erectile dysfunction

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Abstract: It is of great importance to investigate an effective and reliable medication against chronic renal failure (CRF)-related erectile dysfunction (ED), which aims to improve patients' life qualities. The concentrations of cyclic guanosine monophosphate (cGMP) in the corpus cavernosal smooth muscle of both CRF and control rabbits were measured. The effects of various concentrations of tadalafil, papaverine, and sodium nitroprusside on the relaxation responses of corpus cavernosal smooth muscle pre-contracted with phenylephrine in CRF rabbits were observed. There was significant difference in the concentration of cGMP between CRF and control rabbits (P<0.01). Tadalafil had the greatest impacts on CRF rabbits when given the same concentration of papaverine or sodium nitroprusside and particularly significant differences were identified under the concentration levels of 10^{-5} and 10^{-4} mol/L (P<0.01). The results suggest that the cGMP concentrations of the corpus cavernosum had been greatly reduced in CRF rabbits compared with control rabbits and that tadalafil may be an ideal medication for use in the treatment of CRF-related ED.

Key words: Chronic renal failure, Erectile dysfunction, Phosphodiesterase inhibitor, Tadalafil
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1 Introduction

For patients at the terminal phase of nephropathy, erectile dysfunction (ED) incurrence rate reaches as high as 85.4%, including 25.4% of complete ED, 35.4% of medium ED, and 24.6% of light ED. Incurrence rate of complete ED is 52.6% among the patients at the terminal phase of nephropathy who are under 50 years old while 70.5% among those above 50 years old (Neto *et al*., 2002). Similarly to age, including sex and many others influential factors, ED has become an independent factor that bears close influence to chronic renal failure (CRF) patients' life qualities (Nassir, 2009). Thus, it is of great significance to search for effective and reliable clinical medications for use in treating CRF-related ED. Among previously reported medications, sildenafil, a type of phosphodiesterase type 5 (PDE5) inhibitor which functions to relax corpus cavernosal smooth muscle by increasing the cyclic guanosine monophosphate (cGMP) level in cells, has been extensively applied in clinical treatment against ED (McCullough *et al*., 2008). The effective rate of sildenafil in the treatment of CRF-related ED is as high as 80% and the treatment has been proven safe and effective for most patients (Chen *et al*., 2001). Sildenafil can help 66% patients of the renal transplantation-related ED to improve penile erection and sexual performance (Barrou *et al*., 2003).

However, there is no report so far on the effectiveness of tadalafil (Yip *et al*., 2006; Mirone *et al*., 2007), another new type of PDE5 inhibitor, in treating CRF-related ED. By observing the effects of CRF on cGMP concentration in the corpus cavernosum of rabbits and the effects of tadalafil on the relaxation of corpus cavernosal smooth muscle in CRF rabbits, the present research is in an attempt to provide theoretical

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basis for the use of tadalafil in clinical treatment of CRF-related ED.

2 Materials and methods

2.1 Establishment of animal model

The study comprised 20 male New Zealand white rabbits (body weight 2.2–2.8 kg); the local ethical committee approved the study and use of the animals. The rabbits have been randomly divided into two groups: CRF group (10 rabbits) and control group (10 rabbits). All rabbits were anesthetized by intravenous injection with 0.03 g/ml sodium pentobarbital (1 ml/kg body weight). Operations were performed through back incision. Within the CRF group, the rabbits underwent a surgical procedure for the induction of uremia (2/3 partial excision of the left kidney and followed by right nephrectomy one week later). For the control group, similar anatomical procedures were undertaken except that no part of the kidney was resected but only the surrounding fat was removed (Kilicarslan *et al*., 2002). Increased levels of both blood urea nitrogen (BUN) and serum creatinine (Scr) were accepted as diagnostic of renal failure. Four weeks after the establishment of the animal model, the entire penes were taken out of both CRF and control rabbits for further examination.

2.2 Measurement of cGMP concentrations

A total of 25 mg of the corpus cavernosal smooth muscle tissue was put into a test tube which contained 1 ml of cold 50 mmol/L pH 4.75 acetic acid buffer solution. After homogenization, 1 ml of absolute ethyl alcohol was added and well mixed. Following a 5-min stagnation, 3500 r/min centrifugation was performed for 15 min. Supernatant fluid was collected into the small breaker. The sediment was mixed with 75% (v/v) ethyl alcohol, followed by a 15-min 3500 r/min centrifugation. The supernatant fluid was also collected. The two parts of supernatant fluid were then combined and dried by baking in the oven at 60 °C. The residue was stored at 4 °C. A 100-μl sample was collected to be added in 5 μl acetylation reagents, 100 μl ^{125}I, and 100 μl anti-serum. The mixture was thoroughly shaken and stored overnight at 4 °C. Then it was added with 100 μl rabbit serum and 100 μl goat anti-rabbit IgG, and incubated overnight at 4 °C, and then centrifuged at 3000 r/min for 15 min. The supernatant was collected to measure the magnitude of γ-ray. We obtained standard curve and determined cGMP assay. The ^{125}I radioimmunoassay determination of cGMP kit was from the Department of Isotope (Shanghai University of Traditional Chinese Medicine, China).

2.3 Measurement of the relaxation in corpus cavernosal smooth muscle of rabbit

As reported previously (Kilicarslan *et al*., 2002), corpus cavernosum of CRF rabbits was put into a bath of Krebs solution (NaCl 118 mmol/L, KCl 4.7 mmol/L, $CaCl_2$ 2.5 mmol/L, $NaHCO_3$ 25 mmol/L, $MgSO_4$ 1.2 mmol/L, and KH_2PO_4 1.2 mmol/L). Attached fascia and corpus cavernosum urethrae were removed and corpus cavernosal smooth muscle was cut into two muscle strips of 1.5 cm×0.2 cm×0.2 cm. The muscle strips were then put into another Krebs solution bath which contains a tension detector. The bath was set at 37 °C and inlet with 95% of O_2 and 5% of CO_2. One end of the muscle strip was fixed while the other was connected to a transducer (BL-420E Biotechnical Experiment System, Chengdu Taimeng Technology Ltd., China). Signals of the muscular tension were shown on the computer screen of the transducer. The bath was washed three times using Krebs solution and the muscular tension signals were recorded once they were stabilized. Phenylephrine of 10 μmol/L was added to measure the maximum contraction which is marked as F_1. Papaverine (Jiangsu Hengrui Pharmacy Ltd., Lianyungang, China), sodium nitroprusside, and tadalafil (Eli Lilly and Company Limited, Basingstoke, UK) of concentrations from 10^{-8} to 10^{-4} mol/L were added. The tissues were washed for 15 min before adding vehicle. Maximum relaxation forces (F_2) were measured under various concentrations. The relaxation ratio of each medicine under different concentrations, calculated as F_1/F_2 (%), was calculated afterwards.

2.4 Statistical analysis

Experimental values were expressed as mean± standard error of the mean (SEM). Comparisons between groups were assessed using the unpaired *t*-test with SSPS 10.0 statistical software (SPSS Inc., Chicago, USA). P values <0.05 were considered to indicate significant differences.

3 Results

3.1 Animal model

Four weeks after the rabbit model was established, the Scr levels of CRF rabbits were recorded to be (126.62±10.05) μmol/L, significantly higher than those in control group which were only (51.40±4.01) μmol/L (P<0.05). The BUN levels of CRF rabbits were recorded to be (27.35±2.51) mmol/L, significantly higher than those in control group which were only (5.40±0.41) mmol/L (P<0.05).

3.2 Effect of CRF on cGMP in corpus cavernosum

The cGMP concentrations in the corpus cavernosum of CRF rabbits were recorded to be (1.29±0.55) pmol/mg, significantly lower than those of control group which were (2.37±0.53) pmol/mg (P<0.05).

3.3 Effect of medications on the relaxation in corpus cavernosal smooth muscle of CRF rabbits

As shown in Fig. 1, at various concentration levels of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} mol/L, the relaxation ratios of corpus cavernosal smooth muscle in CRF rabbits induced by papaverine were recorded to be (27±10)%, (38±14)%, (48±16)%, (60±17)%, and (75±13)%, respectively. With regard to sodium nitroprusside, the ratios were (28±15)%, (47±20)%, (56±20)%, (62±22)%, and (70±24)%, respectively. As for tadalafil, the ratios were (40±26)%, (64±41)%, (81±49)%, (133±29)%, and (138±29)%, respectively. At the same concentration level, no significant difference has been identified between papaverine and sodium nitroprusside in terms of their effects on the relaxation ratios of corpus cavernosal smooth muscle in CRF rabbits (P>0.05). However, at the same concentration level, the effect of tadalafil was significantly elevated compared with papaverine and sodium nitroprusside. The relaxation ratios induced by tadalafil were significantly higher than those induced by papaverine and sodium nitroprusside, especially under the concentrations of 10^{-5} and 10^{-4} mol/L (P<0.01) (Fig. 1).

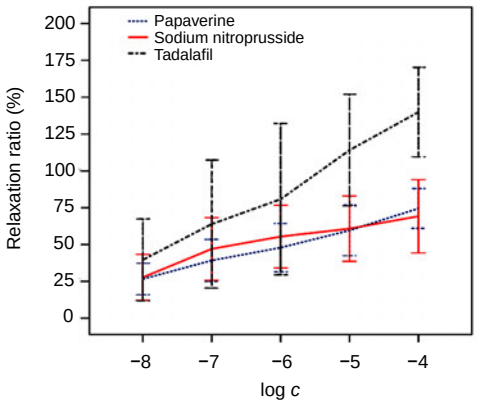

Fig. 1 Relaxation ratios of corpus cavernosal smooth muscle in CRF rabbits under various concentrations (c) of papaverine, sodium nitroprusside, and tadalafil
The relaxation ratios induced by tadalafil were significantly higher, especially under 10^{-5} and 10^{-4} mol/L (P<0.01), compared with papaverine and sodium nitroprusside between which there was no significant difference has been identified (P>0.05)

4 Discussion

Penile erection is a biological process regulated by neuroendocrine and induced by changes in penile hemodynamics. Nitric oxide (NO) is the most important medium in this process (Kilicarslan *et al*., 2003). NO stimulates the soluble guanylyl cyclase to convert guanosine triphosphate (GTP) to the active second messenger cGMP. cGMP in turn activates a specific protein kinase, which phosphorylates certain proteins and ion channels, resulting in the opening of potassium channels, hyperpolarization of the muscle-cell membrane, sequestration of intracellular calcium by the endoplasmic reticulum, and blocking of calcium influx by the inhibition of calcium channels. The consequence is a drop in cytosolic calcium concentrations and relaxation of the smooth muscle. As a result, blood flow around the penile artery and cavernous sinus exceeds the outflow through the vein, and penile erection occurs. As the second important messenger, cGMP becomes nonvital as it is hydrolyzed by PDE5 into 5′GMP (Montani *et al*., 2009) and thus the penile weakens. The present study discovers that cGMP concentrations in the corpus cavernosal smooth muscle of CRF rabbits were notably lower than those of non-CRF rabbits. It denotes that CRF affects the capacity of cGMP in corpus cavernosum and medicines that increase the cGMP capacity would be potentially applied into the treatment against ED.

Sodium nitroprusside is a medicine that carries and releases NO. It breaks down spontaneously in

aqueous solution to generate NO (Moncada *et al*., 1991). Papaverine, as nonspecific phosphodiesterase inhibitor, relaxes corpus cavernosal smooth muscle by interdicting the degradations of cGMP and cyclic adenosine monophosphate (cAMP) and reducing intracellular Ca^{2+} concentrations (Fu *et al*., 2000; Shamloul *et al*., 2005). Tadalafil is a selective PDE5 inhibitor, which competes to depress the combination of cGMP and PDE5. As a result, PDE5 will be devitalized and the hydrolyzation of cGMP will be depressed. Consequently, cGMP concentration increases and then erectile performance will be improved (Frajese *et al*., 2006). There are existing 11 families of PDEs scattering all over the body as suggested. Expressed and functioning in corpus cavernosum and surrounding blood vessels is PDE5 (Burnett, 2008). Three PDE5 inhibitors have been applied in clinic treatment, i.e., sildenafil, vardenafil, and tadalafil. All three medicines work under the same mechanism but with manifestations of different biological effects and clinical features due to their distinct molecular constitutions (Ahn *et al*., 2007; Gratzke *et al*., 2007). Compared to sildenafil and vardenafil, tadalafil can be peroral and hardly influenced by hyper-fat diet. It has a longer half-life of 17.5 h and performs well from the 16th minute to the 36th hour after per os administration (Eardley *et al*., 2004). Within the 30th minute and the 36th hour after a per os administration of 20 mg of tadalafil, the sexual success rate is recorded as high as 73% to 80% (Brock *et al*., 2002).

Diabetes can reduce the effect of sodium nitroprusside on the relaxation response in rabbit's corpus cavernosal smooth muscle (Khan *et al*., 2001). The relaxation response in CRF rabbit's corpus cavernosal smooth muscle is dependant on the concentrations of papaverine and sodium nitroprusside but with no clear statistical difference between the control group and the experimental group (Bagcivan *et al*., 2003).

The present study shows that the relaxation of corpus cavernosal smooth muscle in CRF rabbits is highly dependent on the concentrations of papaverine, sodium nitroprusside, and tadalafil. At the same concentration level, no significant difference has been identified between papaverine and sodium nitroprusside in terms of their effects on the relaxation ratio of corpus cavernosal smooth muscle in CRF rabbits. Compared with papaverine and sodium nitroprusside, tadalafil indicated better effect on the relaxation ratio of corpus cavernosal smooth muscle in CRF rabbits, with a particular distinctive effect at the relaxation in 10^{-5} and 10^{-4} mol/L. It denotes that tadalafil, as a specific phosphodiesterase inhibitor, has a better effect in the regard than papaverine, which is a non-specific phosphodiesterase inhibitor, and sodium nitroprusside, which is an NO provider.

The distinctive effect of tadalafil on improving relaxation response in the corpus cavernosal smooth muscle of CRF rabbits provides a solid theoretical basis for its clinical application as the ideal medication against CRF-related ED.

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