

·Original Article·

Effects of chronic renal failure on the expression of connexin 43 in the rat's corpus cavernosum

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Abstract

Aim: To explore the mechanism of chronic renal failure (CRF)-related erectile dysfunction (ED). **Methods:** CRF experimental models were established by 5/6 nephrectomy from male Sprague-Dawley rats. Both the rats from the control group (NCRF group, $n = 6$) and the experimental group (CRF group, $n = 30$) were injected with a low dose (80 $\mu\text{g}/\text{kg}$) of apomorphine in the 12th week after resection surgery to measure corresponding penile erections. Western blot method was thereafter conducted to measure the expression of connexin 43 (CX43) in the rat corpus cavernosum in the 12th week after the resection surgery. **Results:** There was one death in the NCRF group and five in the CRF group. The penile erection ratio of the CRF group was 28% (7/25), whereas that of the NCRF group was 100% (5/5), which presents a significant difference between the two groups ($P < 0.05$). In terms of penile erection frequency, the average of the CRF group was 1.0 ± 0.0 , which was significantly different from that of the NCRF group (2.2 ± 0.8) ($P < 0.05$). As for the expression of CX43 in the rat corpus cavernosum, a notable difference existed between the CRF group (0.21 ± 0.07) and the NCRF group (0.53 ± 0.27) ($P < 0.01$). **Conclusion:** CRF significantly reduces the erectile function of rats. A close correlation exists between the expression of CX43 in rats' corpus cavernosum and CRF-related ED. (*Asian J Androl* 2008 Mar; 10: 286–289)

Keywords: erectile dysfunction; chronic renal failure; connexin; corpus cavernosum

1 Introduction

Chronic renal failure (CRF) occurs as a clinical syndrome. Of CRF cases, 57.9% are associated with erection dysfunction (ED) [1]. ED occurs in 85.4% of patients at the terminal phase of nephropathy, including

25.4% with complete ED, 35.4% with medium ED and 24.6% with slight ED. Of patients at the terminal phase of nephropathy, 52.6% of those below the age of 50 are subject to medium and complete ED while the figure amounts up to 70.5% when it is regarded with those above 50 years old [2]. The rate of ED incidence after renal transplantation is approximately 48.9% [3]. ED negatively influences a patient's life, and can be an independent contributor to quality of life (QoL), like other variables such as age and sex [4]. A lack of studies on the mechanism of CRF-related ED has to some extent hindered clinical treatment. The present study, therefore, attempts to explore the pathophysiology of CRF-related

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ED by measuring the expression of connexin 43 (CX43) in CRF rats' corpus cavernosum. It is hoped that the pathophysiological study will provide a theoretical basis for clinical treatment of CRF-related ED.

2 Materials and methods

2.1 Establishment and grouping of a CRF rat model

Selected for the present experiment were 36 2-month-old male Sprague-Dawley (SD) rats weighing approximately 150–200 g, provided by the Center for Laboratory Animals, Shandong University (Jinan, China). During the entire experimental process, free eating and drinking was guaranteed, lab temperature was set at 18–22°C, and relative humidity was kept at approximately 60%–70%. The 36 were divided into two groups: the control group (NCRF group, $n = 6$) and the experimental group (CRF group, $n = 30$). Within the experimental group, the treatment rendered was 5/6 nephrectomy (i.e. 2/3-off resection on the left kidney and total removal of the right one within a single backside incision). For the control group, no resection was made on either kidney, except for the removal of surrounding fat. Referring to Abdel-Gadad's approach [5], the 12 week time point was adopted as a standard. After 12 weeks, blood was drawn from the portal vein for measurement of serum creatinine (Scr) and blood urea nitrogen (BUN) in the serum using with fully automatic biochemical instruments (7170A; Hiachi, Tokyo, Japan). The normal range for Scr value is $40.67 \pm 11.48 \mu\text{mol/L}$. The normal range for BUN value is $5.53 \pm 1.58 \text{ mmol/L}$ [6]. The rat corpus cavernosum was anatomized away from penile skin, glans and urethra sponge for further testing in the 12th week after resection.

2.2 Penile erection experiment

Referring to Heaton's approach [7], in the 12th week after resection, the rats were firstly set in a transparent observation kit in a tranquil lab for 10 min to adapt to the new surroundings before the light was turned down and each of them was injected with 80 $\mu\text{g/kg}$ of apomorphine (APO; Sigma, St. Louis, MO, USA). Close observation to record the status and frequency of penile erection took place after the injection. Each glans engorgement and the appearance of penile shaft indicated one erection. The observation time was 30 min. Erection rate refers to the quotient between positive erection rats and total rats and erectile frequency is erection times in 30 min.

2.3 Western blot analysis

Tissue from penile corpus cavernosum was homogenized with the dounce homogenizer and also re-suspended in a preparation of modified radio immunoprecipitation buffer (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 1 mmol/L PMSF, 1 mmol/L EDTA, 1% Triton \times 100, 1% sodium deoxycholate and 0.1% SDS). A bicinchoninic acid protein assay kit (Bio-Rad, Hercules, CA, USA) was used to determine total protein concentration. 50 μg of total protein was drawn from each group to be electrophoresed by SDS-PAGE, leaving the produced gel to be equilibrated later in transfer buffer. Tissues were immersed in twice-distilled water for 10 min and transferred to transfer buffer for 5 min. Filter and nitrocellulose (NC) membrane (Hybond Company, Louisville, KY, USA) were then processed together. Filter, gel, NC membrane and a second filter were placed on a mat which was then put into the transfer tank at 100 mA for 2–4 h with the membrane placed towards the positive pole and gel towards the negative pole. Transferred NC membrane was then incubated for 1–2 h in 5% degreased milk powder reagent, before it was taken out to be washed with PBS three times, for 5–10 min each time. NC membrane was then immersed into a plate or a small bag with appropriately diluted CX43 rabbit polyclonal anti-rat antibodies (Santa Cruz Biotech, Santa Cruz, CA, USA) at room temperature for 1.0–1.5 h, before it was washed with PBS three times, for 5–10 min each time. NC membrane was immersed in the appropriately diluted peroxidase-conjugated secondary antibodies goat anti-rabbit IgG (Zhongshan, Beijing, China) at room temperature for 0.5–1.0 h, and was then washed with PBS three times, each time for 5–10 min. The membrane was then put into diaminobenzidine color development liquid until the effect was satisfactory. Once the color had been developed, the membrane was put into twice-distilled water to stop color development. Using an image analysis system (Pharmacia Biotech, San Francisco, CA, USA), absorbency scanning was performed to calculate the comparative expression of protein on the basis of absorbency ratio between target and β -actin (Santa Cruz Biotech, Santa Cruz, CA, USA) bandings. The dilution of the β -actin was 1:5 000.

2.4 Statistical analysis

The results were expressed as mean \pm SD. The statistical significance of difference in measured quantities was determined using unpaired *t*-test or χ^2 -test with

Table 1. Comparison of serum creatinine (Scr) and blood urea nitrogen (BUN) in blood serum. CRF, chronic renal failure; NCRF, control. ^b $P < 0.05$, compared with NCRF group.

Group	<i>n</i>	Scr ($\mu\text{mol/L}$)	BUN (mmol/L)
CRF	25	126.62 \pm 10.05 ^b	27.35 \pm 2.51 ^b
NCRF	5	51.40 \pm 4.01	5.40 \pm 0.42

Table 2. Comparison of penile erection and connexin 43 (CX43) expression. Erection rate refers to the quotient between positive erection rats and total rats and erectile frequency is erection times in 30 min. CRF, chronic renal failure; NCRF, control. ^b $P < 0.05$, ^c $P < 0.01$, compared with NCRF group.

Group	<i>n</i>	Erection rate (%)	Erection frequency	CX43
CRF	25	28 ^b	1.0 \pm 0.0 ^b	0.18 \pm 0.08 ^c
NCRF	5	100	2.2 \pm 0.8	0.53 \pm 0.27

SPSS 10.0 statistical software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3 Results

3.1 Animal model

There was one death in the NCRF group and five in the CRF group in the week following the operation. Scr and BUN in the blood serum of CRF rats exceeded that of the NCRF counterparts ($P < 0.05$) (Table 1).

3.2 Impact of CRF on rats' penile erection

After the injection of APO, the CRF group reported less penile erection than the NCRF group ($P < 0.05$) (Table 2).

3.3 Impact of chronic renal failure on CX43 expression

CX43 expression in the corpus cavernosum of the CRF group show apparent scarcity, compared with the NCRF group ($P < 0.01$) (Table 2, Figure 1).

4 Discussion

Using 5/6 nephrectomy, the most widely-applied modeling approach, injury to other organs and systems is avoided. Possible changes in experimental variables caused by dead renal tissue is also less likely when compared to any form of chemical or bio-modeling approach. Apparently, this approach is most suitable for the present pathological study [8]. Unlike conventional 5/6 nephre-

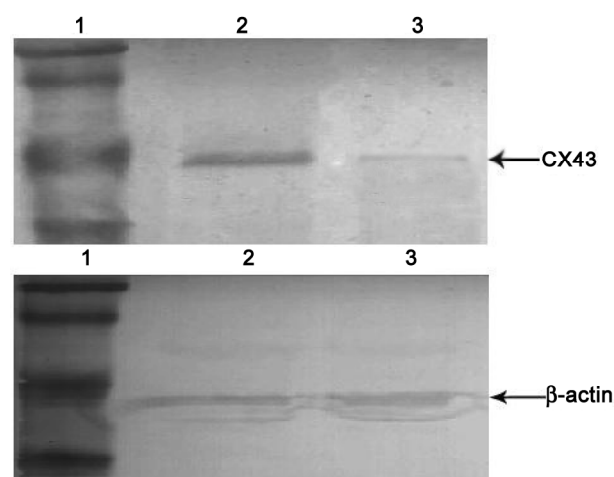


Figure 1. Connexin 43 (CX43) expression in rats' corpus cavernosum. Line 1: marker; Line 2: control group; Line 3: chronic renal failure (CRF) group.

ctomy for CRF rat modeling, which is usually performed via either single ventro-incision [9] or two backside incisions [10], we made it through a single backside incision without increasing the death rate and only shortening the experimental duration. The key to the success of such resection lies in the familiarity with animal anatomization and sophisticated operations with little room for error.

Apomorphine contributes to penile erection by acting on both the centre nerve [11] and dopamine receptors (D_1 , D_2) [12] in the smooth muscle of the corpus cavernosum. The present study determined 100% erection within the NCRF group and reduced erection rate and frequency within the CRF group after APO injection, a clear indication of significant correlation between CRF and rat erectile function.

The gap junction is a special membrane structure linking two neighboring cells [13]. Gap junction intercellular communication (GJIC) facilitates the exchange of information, energy and substances, participates in the metabolic coupling in intercellular substantial exchange and electronic coupling in electronic signal transferring, and plays a regulating role in the whole physiological process of cellular metabolism, homeostasis and cell differentiation [14]. The gap junctions, with each of two neighboring cells providing a single corpuscle, is a channel bestriding the cell gaps. The junction corpuscle is a hexagon with six subunit connexins forming a ring around a hydrophilic channel in the middle. Connexin usually appears on the plasma membrane in clusters to form gap

junction speckles, the amount of which directly influences the functions of GJIC after connexin is processed, decorated and phosphorylated. Connexin, as a big protein family, has many subsets, like CX26, CX32, CX36, CX43, CX45 and CX50 [15, 16]. Gap junctions in the corpus cavernosum smooth muscle cell are mainly composed of CX43 [17], which through electronic couple and metabolic couple connects the numerous cells in the corpus cavernosum smooth muscle as a functional entity to regulate the synchronic diastole among smooth muscle cells, induce the relaxation of smooth muscle of corpus cavernosum and maintain penile erection. The expression of CX43 in the corpus cavernosum of the hypertensive ED rat exhibited an apparent decrease [18].

The reduction of dense tissue and basal lamina, and the increase of interstitial collagen fiber in the cytoplasm of male CRF patients' corpus cavernosum leads to weakness of the cell junction [19]. The fact that CX43 expression of CRF rats is obviously lower than that of their NCRF counterparts further proves that CRF leads to lesions in synchronic diastole mechanism in corpus cavernosum and, accordingly, to ED.

To sum up, decreased expression of CX43 in corpus cavernosum, which is caused by chronic renal failure, might be one of the causes of CRF-related ED, and possibly provides a novel theoretical basis for the clinical treatment of CRF-related ED.

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