

ผลของแคปโตพริลต่อภาวะเสื่อมสมรรถภาพทางเพศและคุณภาพของอสุจิ ในหนูทดลองความดันเลือดสูง

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Effect of Captopril on Erectile Dysfunction and Sperm Quality in Hypertensive Rats

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หลักการและวัตถุประสงค์: ภาวะความดันเลือดสูงถูกพบว่ามีสัมพันธ์กับภาวะเสื่อมสมรรถภาพทางเพศและการทำงานที่ผิดปกติของอัณฑะ การศึกษาครั้งนี้เพื่อศึกษาผลของยาแคปโตพริลต่อภาวะเสื่อมสมรรถภาพทางเพศและการทำงานของอัณฑะในหนูความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

วิธีการศึกษา: หนูทดลอง Sprague-Dawley เพศผู้ ถูกแบ่งเป็น 3 กลุ่ม กลุ่มควบคุมได้รับน้ำเปล่า กลุ่มแอลเนมได้รับแอลเนม 40 มก/กก/วัน นาน 5 สัปดาห์และกลุ่มแคปโตพริลได้รับแอลเนมและแคปโตพริล 5 มก/กก/วัน ช่วง 2 สัปดาห์สุดท้ายเมื่อจบการทดลอง ความดันเลือด การแข็งตัวขององคชาติตอบสนองต่อการกระตุ้นเส้นประสาท ปริมาณและการเคลื่อนที่ของอสุจิ และตัวชี้วัดภาวะเครียดออกซิเดชันถูกวัด

ผลการศึกษา: แอลเนมมีผลเพิ่มความดันเลือด ลดการแข็งตัวขององคชาติ ลดปริมาณและการเคลื่อนที่ของอสุจิในหนู และพบการสร้าง superoxide สาร malondialdehyde ในเลือดและเนื้อเยื่อเพิ่มขึ้น ขณะที่ plasma nitric oxide metabolite ลดลงในหนูความดันเลือดสูงเมื่อเทียบกับกับหนูกลุ่มควบคุม ($p < 0.05$) การให้แคปโตพริลมีผลลดความผิดปกติข้างต้นที่เกิดจากแอลเนม

สรุป: แคปโตพริลสามารถฟื้นฟูภาวะเสื่อมสมรรถภาพทางเพศและปรับปรุงคุณภาพของอสุจิในหนูทดลองความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม กลไกที่เกี่ยวข้องอาจเป็นผลจากฤทธิ์ต้านอนุมูลอิสระ

คำสำคัญ: แคปโตพริล; ภาวะเสื่อมสมรรถภาพทางเพศ; หนูทดลองความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม; อสุจิ; ภาวะเครียดออกซิเดชัน

Background and Objectives: Hypertension has been reported to be associated with erectile and testicular dysfunction. This study aimed to evaluate the role of the anti-hypertensive drug, captopril, on erectile and testicular function in L-NAME-induced hypertensive rats.

Methods: Male Sprague-Dawley rats were divided into 3 groups including control group received tap water, L-NAME group received L-NAME at dose 40 mg/kg/day for five weeks and captopril group received L-NAME and captopril at dose 5 mg/kg/day for the last two weeks. At the end of the study, systolic blood pressure, erectile response to nerve stimulation, sperm concentration, sperm motility, and oxidative stress markers were determined.

Results: L-NAME administration caused high blood pressure and decreased erectile response to nerve stimulation, reduced sperm concentrations, and sperm motility in rats. Moreover, the high level of vascular superoxide production, plasma, and tissue malondialdehyde, and low level of plasma nitric oxide metabolites were also observed in hypertensive rats compared to control rats ($p < 0.05$). Treatment with captopril alleviated these reproductive alterations caused by L-NAME.

Conclusion: Captopril improved erectile dysfunction and sperm quality in L-NAME-induced hypertensive rats. The possible mechanism may associate with its antihypertensive and antioxidant properties.

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Keywords: Captopril; erectile dysfunction; L-NAME hypertensive rats; sperm; oxidative stress

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Introduction

Hypertension is a chronic medical condition that is the major cause of cardiovascular disease and increases risks of other organ damage. There is evidence to show that 30 percent of hypertensive patients had erectile dysfunction¹. Additionally, poor sperm quality is present in most of hypertensive patients². The exact underlying mechanism of which is unclear, however, depletion of nitric oxide (NO) and oxidative stress have been proposed to participate in these reproductive alterations in hypertension³. It has been reported that patients with hypertension had high level of reactive oxygen species and low level of plasma nitric oxide⁴. N^ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase inhibitor, is widely used to induce hypertension in rats⁵⁻⁷. L-NAME causes NO deficiency and systemic vasoconstriction and hypertension^{8,9}. Furthermore, systemic oxidative stress markers were elevated in L-NAME hypertensive rats^{10,11}. Previous study, rats treated with L-NAME exhibited hypertension, erectile dysfunction and testicular damage that was associated with reducing NO and increasing oxidative stress markers¹². Captopril is a common anti-hypertensive drug that inhibits angiotensin-converting enzyme (ACE) activity¹³. Several other actions of captopril have been reported such as reducing oxidative stress, improving vascular function and preventing cardiovascular remodeling¹⁴⁻¹⁶. For instance, treatment with captopril at dose 30 mg/kg/day has been found to reduce blood pressure as well as angiotensin II and ET-1 levels, improve left ventricular remodeling and have anti oxidative properties¹⁷. Additionally, previous study reported that supplementation with captopril at dose 5 mg/kg/day significantly prevented the increase in blood pressure and cardiovascular remodeling in NO-deficient rats⁵. Nevertheless, the effect of captopril on male reproductive alterations in hypertensive rats has little information. This study aimed to investigate whether captopril could alleviate L-NAME induced erectile and testicular dysfunction in rats.

Materials and methods

Chemicals

L-NAME and captopril were purchased from Sigma Aldrich Corp (St Louis, MO, USA).

Animals

Adult male Sprague-Dawley rats (220-240 g) were purchased from the Nomura Siam International Co., Ltd., Bangkok, Thailand. Five rats/cage were housed in an animal room with controlled the temperature (25±2°C) and a light/dark cycle (12:12 h) at the Northeast Laboratory Animal Center, Khon Kaen university. A standard chow diet and drinking water were available ad libitum. All animal procedures were performed in compliance with the guidelines for use and care of laboratory animals established by the Ethics of Animal Experimentation of the National Research Council of Thailand and approved by the Institutional Animal Care and Use Committee of Khon Kaen university, Thailand (IACUC-KKU-49/61).

Experimental protocols

Rats were assigned into 3 groups of 5 rats in each. Over the 5 weeks of the study, control rats were given tap water while hypertensive rats were given L-NAME (40 mg/kg/day) in their drinking water to induced hypertension. Rats were given water as vehicle or captopril (5 mg/kg/day) for the last 2 weeks.

Blood pressure measurement

Systolic blood pressure (SP) was measured before and after 5 weeks of the experiment using non-invasive tail cuff plethysmography (IITC/Life Science Instrument model 229 and model 179 amplifier; Woodland Hills, CA, USA).

Intracavernous pressure (ICP) and mean arterial blood pressure (MAP) measurements

The ratio of ICP and MAP, an index of erectile function, was determined at the end of the study. Briefly, rats were anesthetized by intraperitoneal administration of thiopental sodium (65 mg/kg/day)

and placed on a heating pad. A polyethylene tube was inserted into the carotid artery for MAP monitoring. Thereafter, ICP was measured during the MAP measurements. In brief, the right side of penis was dissected, and the largest branches of the cavernous nerve was identified. Subsequently, the cavernous nerve was stimulated at 16 Hz for 1 min in different voltages (1, 2, 3 and 4 volts, respectively) using an electronic stimulator (Grass SD9 B Square Pulse Stimulator, Rhode Island, USA). All parameters were recorded using a LabChart7 program (PowerLab System, ADInstruments, New South Wales, Australia). The data were expressed as max ICP/MAP ratio (max ICP/MAP)¹⁸⁻²⁰.

Sperm concentration and sperm motility assessment

After an ICP measurement, semen was collected from the caudal epididymis and diluted with 4 ml of phosphate-buffered saline (PBS), pH 7.4 solution (37 °C). Thereafter, 10 µl of the sperm suspension was placed on a Neubauer hemocytometer (BOECO, Hamburg, Germany) and then sperm concentrations and motility were counted and assessed under light microscopy (OLYMPUS BH Series, Pennsylvania, USA) using a phase-contrast microscope. The numbers and motility of sperm were counted from 5 randomly selected rows. The total number and the percent motility of sperm were calculated using the formulas as follows:

The caudal epididymal sperm concentration (1 ml) = (sperm count × 4 × 10³)/ 0.02

Percent motility of sperm = (total number of motile sperm/ total number of sperm) × 100

Oxidative stress markers measurements

Assay of O₂^{•-} production in thoracic aorta

The production of vascular O₂^{•-} production was examined in the thoracic aorta using lucigenin-enhanced chemiluminescence as previously described by Lu et al. in 1996²¹. The aortic segments were incubated in the tube filled with oxygenated Krebs-KCL solution (37°C) for 30 minutes. Afterward, the sample tubes with lucigenin were placed in a luminometer (Turner Biosystems, CA, USA). The luminometer detections were counted every 30 seconds for 5 minutes. The production of O₂^{•-} was shown as relative light unit counts per minute per dried weight of the artery.

Assays of plasma and tissue malondialdehyde (MDA) levels

The level of MDA in plasma, penis and testes were determined using thiobarbituric acid reactive substances (TBARS) following a previous method of Lungaram et al.²² The absorbance of the supernatant was measured at the wavelength of 532 nm by a spectrophotometer. A standard curve was generated using appropriate concentrations of standard TEP (0.3-10 mmol/l).

Assay of plasma nitric oxide metabolites (NOx) level

Plasma NOx level were determined using an enzymatic conversion method as previously described by Verdon et al. in 1995²³ with minor modifications²². Briefly, the plasma was deproteinized and the supernatant was mixed with NADPH, G-6-P, 1 G-6-PD and nitrate reductase before incubated at 30 °C for 30 minutes. Thereafter, the mixer was reacted with a Griess solution for 15 minutes. The absorbance of the samples was detected at wavelength 540 nm using a microplate reader (Tecan GmbH., Groding Australia). A standard curve was established with a set of serial dilution of NaNO₂²⁴.

Statistical analysis

Data were expressed as the mean ± SEM. Statistical analyses were tested by one-way ANOVA followed by a post-hoc test, Tukey's Honest Significant Difference (HSD) test. A p-value of less than 0.05 was considered statistically significant.

Results

Effect of captopril on systolic blood pressure

There was no significant difference in SP among the group at the beginning of the experiment. After 5 weeks, it was found that L-NAME increased SP in rats compared to the control group (SP at week 5, 192.33±0.32 vs. 120.60 ± 3.02 mmHg, p<0.05) (Figure 1). Captopril had an antihypertensive effect as it decreased SP in the L-NAME-induced hypertensive rats (SP at week 5, 122.93 ± 1.80 mmHg, p< 0.05).

Effect of captopril on intracavernosal pressure response to cavernous nerve stimulation

L-NAME hypertensive rats showed a low value of the max ICP/MAP ratio compared to the control group (at 4 V, 6.70±1.59 vs. 46.73±2.65 %, p< 0.05). Treated

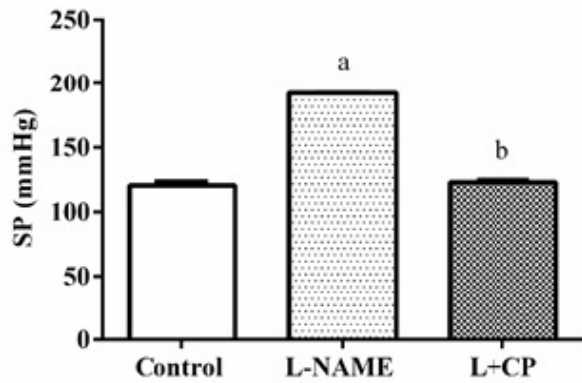


Figure 1 Effects of captopril on systolic blood pressure in L-NAME-induced hypertensive rats. Data are mean \pm SEM, (n= 5/group). ^ap<0.05 significant difference with control, ^bp<0.05 significant difference with L-NAME, L = L-NAME, CP = Captopril (5 mg/kg/day).

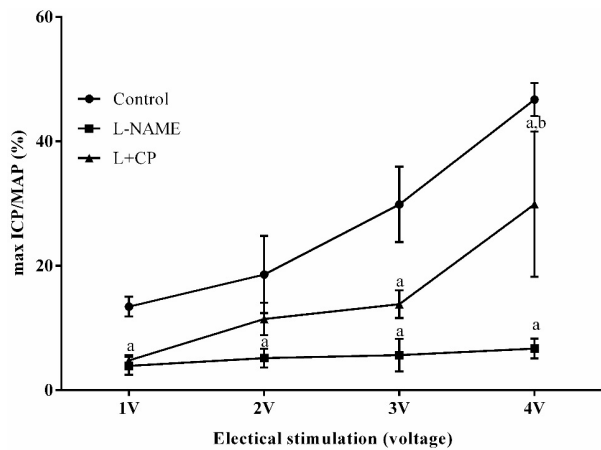


Figure 2 Max ICP/MAP ratios in all groups of rats. Data are expressed as mean \pm SEM, (n= 5/group). ^ap<0.05 significant difference with control, ^bp<0.05 significant difference with L-NAME. L = L-NAME, CP = Captopril (5 mg/kg/day).

with captopril significantly increased the max ICP/MAP ratio (at 4V, 29.91 \pm 11.67%) in L-NAME rats compared to the untreated rats (p < 0.05) (Figure 2).

Effect of captopril on sperm concentrations and sperm motility

The caudal epididymal sperm concentrations and percentages of sperm motility were significantly decreased in the L-NAME group (13.00 \pm 3.85 $\times 10^6$ /ml and 44.48 \pm 1.29%) compared to the control group (20.68 \pm 1.04 $\times 10^6$ /ml and 66.97 \pm 1.82%) (p<0.05). Treatment with captopril significantly improved caudal epididymal sperm concentrations and percentages of sperm motility (20.08 \pm 1.18 $\times 10^6$ /ml and 72.30 \pm 1.38%) compared to the untreated group (p<0.05) (Figure 3).

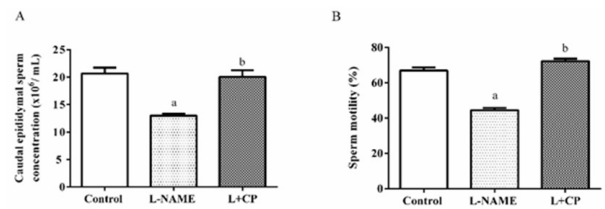


Figure 3 Effects of captopril on caudal epididymal sperm concentration (A) and sperm motility (B) in L-NAME-induced hypertensive rats. Data are mean \pm SEM, (n= 5/group). ^ap<0.05 significant difference with control, ^bp<0.05 significant difference with L-NAME, L = L-NAME, CP = Captopril (5 mg/kg/day).

Effect of captopril on oxidative stress markers

Table 1 shows significant increases in oxidative stress markers, vascular O₂^{•-} production, plasma, testicular and penile MDA levels in the L-NAME group compared with the control group (p<0.05). Treatment with captopril significantly decreased oxidative stress markers in the L-NAME rats compared with the untreated L-NAME group (p<0.05). In addition, a decrease in plasma NOx was found in hypertensive rats compared to control rats (p<0.05), however, captopril supplementation improved this alteration (p<0.05).

Discussion

This study found the beneficial effects of captopril on reproductive alterations induced by L-NAME in rats. The results showed that rats that received L-NAME had high blood pressure, decreased max ICP/MAP ratios and poor sperm number and motility. High levels of vascular O₂^{•-} production and MDA in plasma, testicular and penile tissue and low level of plasma NOx were observed in L-NAME hypertensive rats. Furthermore, captopril treatment in L-NAME rats reduced blood pressure and improved max ICP/MAP ratios and sperm number and motility. These results were associated with reducing systemic and local oxidative stress markers together with raising plasma NOx in captopril treated group.

It is well documented that L-NAME causes high blood pressure via reducing NO production and vascular diameter and hypertension²⁵. Subsequently, male reproductive dysfunction, low max ICP/MAP ratio and poor sperm quality has been reported in L-NAME treated rats²⁶. Under normal physiological conditions, NO plays an important role in regulation of erectile response and spermatogenesis^{27, 28}. This study, erectile dysfunction as indicated by low max ICP/MAP ratios was demonstrated that was a consequence of NO depletion in L-NAME hypertensive

rats. Interestingly, supplementation with captopril alleviated erectile dysfunction by increased NO bioavailability and subsequently improved max ICP/MAP ratios in L-NAME hypertensive rats. Impairment of sperm quality was also observed in L-NAME rats relevant to increased oxidative stress and reduced NO bioavailability. This information was supported by Adedara et al. in 2018²⁸ who found an impairment of testicular sperm number, epididymal sperm number and sperm progressive motility along with a low level of the antioxidant enzyme and high oxidative stress markers in L-NAME hypertensive rats. Antihypertensive effects of captopril have been well established to inhibit ACE activity¹³. Moreover, several lines of evidence reported that captopril also exhibited antioxidant properties since it contains thiol group²⁹. These data were consistent with the recent study that treatment with captopril alleviated the level of ROS production, decreased lipid peroxidation as well as suppressed the expression of NADPH oxidase subunit in NO-deficient rats¹⁵. In the current study, the antihypertensive effect of captopril was also related to antioxidant activity because captopril attenuated vascular $O_2^{\cdot-}$ production and lipid peroxidation in plasma, testicular and penile tissue relevant to raise NO bioavailability in hypertensive rats. Additionally, this study, captopril attenuated reproductive dysfunction in L-NAME hypertensive rats that might be link with at least two underlying mechanisms. Firstly, captopril reduced blood pressure and subsequently increased max ICP/MAP ratios. Secondly, captopril reduced oxidative stress since it contains free sulfhydryl groups to directly scavenge oxygen free radicals³⁰. Therefore, this anti-oxidative effect of captopril can enhance NO bioavailability in hypertensive rats and improve erectile dysfunction.

Conclusion

In conclusion, captopril reduced blood pressure and improved max ICP/MAP ratios as well as sperm quality in NO-deficient hypertensive rats. These effects might be mediated by its antihypertensive and anti-oxidative capacities.

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