Temporal Changes in Erectile Function and Endothelium-Dependent Relaxing Response of Corpus Cavernosal Smooth Muscle After Ischemia by Ligation of Bilateral Internal Iliac Arteries in the Rabbit

Yuko Abe¹, Yuji Hotta¹, Kana Okumura¹, Tomoya Kataoka¹, Yasuhiro Maeda¹, and Kazunori Kimura¹,²,*

¹Department of Hospital Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe do-ri, Mizuho-ku, Nagoya 467-8603, Japan
²Department of Clinical Pharmacy, Graduate School of Medical Sciences, Nagoya City University, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

Received May 10, 2012; Accepted September 3, 2012

Abstract. We aimed to elucidate the changes in the relaxation function of corpus cavernosal smooth muscle (CCSM) and erectile function using acute penile ischemic rabbits. Relaxation response to acetylcholine (Ach) was unchanged at 3 days after ischemia. The response to ACh had significantly decreased at 1 week, but had recovered completely at 4 weeks. The reaction to sodium nitroprusside and electrical field stimulation was unchanged by ischemia at all time points. Erectile function was changed in the same manner as the response of ACh. The endothelium-dependent relaxing responses of CCSM and erectile function were found to be initially decreased, but subsequently improved completely.

Keywords: arteriogenic erectile dysfunction, corpus cavernosal smooth muscle, endothelium

Arteriogenic erectile dysfunction (ED) is caused by insufficiency of arterial inflow to sinusoidal spaces as a result of traumatic or atherosclerotic arterial occlusive disease of the pudendal, cavernous, or helicine arteries (1–3). There have been several basic studies on arteriogenic ED caused by atherosclerosis (4–6). These reports suggest that corpus cavernosal smooth muscle (CCSM) relaxation in response to electrical field stimulation (EFS) is diminished (4, 5).

On the other hand, there have been few reports on arteriogenic ED in acute ischemic conditions, such as trauma or pelvic surgery. It is uncertain how the relaxation response of CCSM changes temporally after acute ischemia. Therefore, the aim of this study was to investigate temporal changes in the relaxation response of CCSM induced by acetylcholine (ACh), sodium nitroprusside (SNP), and EFS after acute ischemia induced by ligation of the bilateral internal iliac arteries in rabbits.

This experiment was performed using male New Zealand White Rabbits (SLC Inc., Shizuoka) weighing 2.0–3.0 kg. Animals were housed in an environmentally (temperature and humidity) controlled room with a 12-h light/dark cycle and had free access to laboratory chow and water. All experiments were carried out in accordance with the guiding principles for the care and use of laboratory animals of The Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science and Technology and were approved by the Institutional Animal Care and Use Committee at Nagoya City University.

Each animal was anesthetized with an injection of pentobarbital sodium (30 mg/kg) into the auricular vein. A midline longitudinal low abdominal incision was made, and the internal iliac arteries were identified, isolated, and triply ligated. After bilateral ligation, the incision was sutured.

After the observation period (3 days, 1 week, or 4 weeks), rabbits were euthanized and penises were rapidly extirpated and placed in chilled Krebs solution with the following composition: 119 mM NaCl, 4.6 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgCl₂, 15 mM NaHCO₃, 1.2 mM Na₂HPO₄, and 11 mM glucose. The glans, urethra, and blood vessels were carefully removed from the penis. In addition, the tunica albuginea was removed and a strip of
CC (length: 10–15 mm) was prepared. Strips were vertically mounted in 10-ml organ chambers containing Krebs solution at 37°C and continuously bubbled with 95% O₂ and 5% CO₂ (pH 7.4). One side of the strip was connected to a force transducer (UL-10GR; Minebea Co., Ltd., Nagano) using thread. Tissues were equilibrated for 90–120 min under a resting tension of 10 mN. After contraction with 30 μM norepinephrine (NE) and washout, relaxation was measured and recorded with PowerLab 2/25 (AdInstruments Pty Ltd., Bella Vista, Australia).

The cumulative concentration–responses induced by the muscarinic agonist ACh (10⁻⁸ – 10⁻⁴ M) and the NO-donor compound SNP (10⁻⁸ – 10⁻⁴ M) were measured in cavernosal strips precontracted with NE (30 μM).

EFS was performed in strips placed between two platinum plates (distance: 5 mm, length: 15 mm) using an Electronic Stimulator (Nihon Kohden, Tokyo). Stimulus conditions were voltage, 10 V; duration, 800 μs; frequency, 1, 2, 4, 8, 16, and 32 Hz; and time, 5 s. To assess the relaxation induced by the nitrergic nerve, strips were precontracted with NE and underwent EFS after preincubation with the beta-receptor blocker propranolol (1 μM), the muscarinic receptor blocker atropine (1 μM), and the ATP receptor blocker suramin (10 μM).

Penile tests were performed for rabbits under arousal before surgery, and at 3 days, 1 week, and 4 weeks after ligation, as described by Bischoff et al. (7). Briefly, rabbits were orally administered vardenafil (3 mg/kg), and SNP (0.2 mg/kg) was injected into the auricular vein at 1 h after treatment in order to induce erection. The maximum length of the penis after treatment was measured and the rate of penile extension (maximum penile length / penile length before treatment) was calculated.

Results are expressed as means ± standard error of the mean. Differences in the data were evaluated by Welch’s t-test for isometric tension study and paired t-test for the penile extension study. Differences of *P < 0.05 were considered to be significant.

In order to examine the changes in endothelium-dependent relaxation function of CCSM, the relaxation response induced by ACh was measured. In CC precontracted with NE, cumulative addition of ACh produced concentration-dependent relaxation. At 3 days after ligation, there were no differences between the Ligation and Control groups in the relaxation induced by ACh. However, at 1 week, the relaxation response in the Ligation group was significantly lower than that in the Control group (*P < 0.05, **P < 0.01). At 4 weeks, the response in the Ligation group had completely recovered (Fig. 1).

Next, to examine the reaction of CCSM to NO, we measured the reaction of relaxation induced by SNP.

![Figure 1](image-url)
rate before surgery. However, the extension rate was fully restored at 4 weeks after surgery (Fig. 3).

Arterial occlusive disease is recognized as one of the causes of organic ED (1–3, 8), and there have been several reports of arteriogenic ED caused by atherosclerosis (4–6). However, there have been few reports of arteriogenic ED caused by acute ischemia due to trauma or pelvic surgery. In this study, we therefore focused on arteriogenic ED caused by acute ischemia using an acute arteriogenic ED model rabbit induced by ligation of bilateral internal iliac arteries and evaluated the temporal changes in CCSM function. There have been no reports on this acute arteriogenic ED rabbit model, and the degree to which intracavernosal blood flow and pO2 changed remain unknown; thus, further study is necessary.

Interestingly, our data showed that the relaxation response of CCSM to ACh initially decreased, but later recovered fully. The reasons for the decrease in response remain unknown. However, possible reasons include 1) decrease in endothelial cells in CC, 2) changes in expression levels and/or sensitivity of muscarine receptors, and 3) changes in activity and expression of eNOS in endothelial cells. The recovery may be due to a compensatory change, such as the development of collateral circulation. In mice with hindlimb ischemia induced by resecting the right femoral and saphenous arteries, it was reported that blood flow of the ischemic leg recovered gradually after surgery (9). In addition, penile extension rate decreased at 1 week and had completely recovered at 4 weeks after surgery, and these results are similar to those for the relaxing response of CCSM to ACh. To improve erectile function, improvement in penile blood flow is necessary. Thus, collateral circulation is thought to have developed at 4 weeks after surgery.

The response of CCSM to SNP was slightly increased at 4 weeks after ligation, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week. The relaxation response of CCSM to SNP was slightly increased at 4 weeks after ligature, and this increase may be due to a compensatory mechanism, particularly considering that the response to ACh was lower at 1 week.
response to EFS did not change at all after ligature. Thus, the function of nitrergic nerves may not be damaged in this model.

In this study, we found that the endothelium-dependent responses of CCSM initially decreased significantly and then completely recovered after ligation surgery. In rats with ligated bilateral internal iliac arteries, the increase in intracavernous pressure by electrical stimulation was significantly lower soon after ligature (10 – 12). In this study, erectile function was initially decreased after ligation and later recovered completely. Thus, collateral circulation may have developed, although this requires further study. Therefore, we propose that patients should undergo therapy in the early stages after ischemic injury, as CCSM function may be restored.

References