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Testosterone Deficiency Causes Endothelial Dysfunction via Elevation of Asymmetric Dimethylarginine and Oxidative Stress in Castrated Rats

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ABSTRACT

Background: Testosterone is believed to mediate the penile erectile response by producing adequate nitric oxide; therefore, testosterone deficiency results in erectile dysfunction through decreased nitric oxide bioavailability. However, the mechanisms underlying endothelial dysfunction in testosterone deficiency remain unclear.

Aim: To investigate the mechanism of endothelial dysfunction in a rat model of testosterone deficiency.

Methods: Rats were distributed into 3 groups: castrated (Cast), castrated and supplemented with testosterone (Cast + T), and sham (Sham). In the Cast + T group, castrated rats were treated daily with subcutaneous testosterone (3 mg/kg daily) for 4 weeks; Sham and Cast rats received only the vehicle.

Outcomes: Erectile function using intracavernosal pressure and mean arterial pressure measurements after electrical stimulation of the cavernous nerve, endothelial function using isometric tension, asymmetric dimethylarginine (ADMA) levels using ultra-performance liquid chromatography and tandem mass spectrometry, and inflammatory biomarker expression were performed 4 weeks after the operation.

Results: In the Cast group, the ratio of intracavernosal pressure to mean arterial pressure significantly decreased, acetylcholine-induced relaxation was lower, and serum ADMA, oxidative stress, and inflammation biomarker levels were significantly increased ($P < .01$). Testosterone injection significantly improved each of these parameters ($P < .01$).

Clinical Translation: The present results provide scientific evidence of the effect of testosterone deficiency on erectile function and the effect of testosterone replacement therapy.

Strengths and Limitations: This study provides evidence of the influence of testosterone deficiency on endothelial function by investigating ADMA and oxidative stress. A major limitation of this study is the lack of a direct link of increased ADMA by oxidative stress to inflammation.

Conclusion: Testosterone deficiency increased not only ADMA levels but also oxidative stress and inflammation in castrated rats, which can cause damage to the corpus cavernosum, resulting in erectile dysfunction. **Kataoka T, Hotta Y, Maeda Y, Kimura K. Testosterone Deficiency Causes Endothelial Dysfunction via Elevation of Asymmetric Dimethylarginine and Oxidative Stress in Castrated Rats. J Sex Med 2017;14:1540–1548.**

Key Words: Testosterone; Erectile Dysfunction; Asymmetric Dimethylarginine; Endothelial Dysfunction; Inflammation

INTRODUCTION

Androgens are essential for male physical activity and normal erectile function.[1–5] Age-related androgen deficiency, known as late-onset hypogonadism, is considered a risk factor for erectile dysfunction (ED).[1,2] Several studies have reported that androgen replacement therapy mitigates the symptoms of late-onset hypogonadism and ED. In this context, bioidentical or synthetic testosterone facilitates erectile function by maintaining an adequate supply of nitric oxide (NO), penile structure, and the endothelial function of the corpus cavernosum (CC).[6–9] Thus, decreased NO bioavailability is believed to be the main cause of ED in individuals with testosterone deficiency[10]; however, the pathophysiologic mechanisms underlying this process remain unclear and require further study.

Several reports have identified a relation between ED and levels of asymmetric dimethylarginine (ADMA),[11,12] an endogenous NO synthase (NOS) inhibitor that increases in response

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to inflammation.[13] Interestingly, castrated mice exhibit symptoms of Parkinson disease because of increased inducible NOS (iNOS) levels in cerebral blood vessels.[14] Moreover, an in vitro study found that testosterone ameliorates H_2O_2-induced inflammation in blood vessel cells.[15,16] We previously reported that androgen replacement therapy improved erectile function in rats with type 2 diabetes through suppression of CC inflammation[17]; therefore, inflammation and ADMA seem to have specific effects on endothelial function in rat models, which could indicate a connection between these factors in ED.

In this study, we investigated the effects of testosterone deficiency on erectile function, ADMA levels, and inflammatory parameters in rats to better define the mechanisms of endothelial dysfunction and pathologic damage in testosterone deficiency.

METHODS

Animals

Male Wistar/ST rats were purchased from Japan SLC, Inc (Hamamatsu, Japan). All experimental protocols were approved by the ethics review board of Nagoya City University (Nagoya, Japan) and conducted in accordance with institutional standards for the care and use of animals.

Treatment Protocols

Rats were separated into the following groups: castrated (Cast; n = 14), castrated and supplemented with subcutaneous testosterone (3 mg/kg daily; Cast + T; n = 14), and sham (Sham, n = 14). Rats in the Cast and Cast + T groups were anesthetized using an intraperitoneal injection of pentobarbital sodium (40 mg/kg). After creating a 2-cm incision in the muscle and peritoneum, the testes were identified, extracted, and thus castrated. Then, the skin was sutured using 7-0 silk surgical sutures (Natsume Seisakusho Co, Ltd, Tokyo, Japan). In the Sham group, rats underwent sham laparotomies and the incision was sutured. In the Cast + T group, rats received daily subcutaneous doses of testosterone (3 mg/kg) for 4 weeks. The testosterone solution was composed of testosterone propionate (Wako Pure Chemical Industries, Osaka, Japan) dissolved in a vehicle composed of sesame oil (Nacalai Tesque, Kyoto, Japan) and 0.01% benzyl alcohol (Wako Pure Chemical Industries). The Sham and Cast groups received injections of the vehicle on the same schedule.

Blood and Tissue Sample Collection

Before endothelial function analysis, blood samples were obtained through the vena cava. After coagulation and centrifugal separation at 800*g* for 20 minutes at 4°C, serum samples were stored at −80°C until analysis. After the measurement of erectile response, the urethra and blood vessels were removed from the penis for real-time quantitative polymerase chain reaction (qRT-PCR) analysis and oxidative stress analysis. The CC was harvested and frozen in liquid nitrogen. In addition, penile shaft samples were embedded in optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan) and frozen in liquid nitrogen for Masson trichrome staining. All samples were stored at −80°C until analysis.

Examination of Erectile Function

Intracavernous pressure (ICP) was measured by electrical stimulation as previously reported.[17–21] Briefly, rats from each group (n = 7) were anaesthetized using isoflurane (Mylan, Canonsburg, PA, USA). The carotid artery was cannulated for continuous monitoring of the mean arterial pressure (MAP) and the left crus of the CC was cannulated using a 23-gauge needle for continuous ICP monitoring. The pressure transducer was connected through an amplifier to a data acquisition board (PowerLab 2/26; ADInstruments Pty Ltd, Dunedin, NSW, Australia). Stainless steel bipolar wire electrodes (Unique Medical, Osaka, Japan) and a pulse generator (Nihon Kohden, Tokyo, Japan) were used for the penile stimulations with the following parameters: 1 minute at 5 V, 1 to 16 Hz, and a square wave duration of 5 ms. Erectile function was evaluated using the maximum ICP/MAP ratio, because ICP is influenced by systemic arterial pressure.

Endothelial Function Analysis

Endothelial function was measured using isometric tension as previously reported.[17,18] Briefly, rat penises from each group (n = 7) were removed and CC strips were equilibrated for at least 60 minutes in an aerated organ bath containing Krebs solution (NaCl 119 mmol/L, KCl 4.6 mmol/L, $CaCl_2$ 1.5 mmol/L, $MgCl_2$ 1.2 mmol/L, $NaHCO_3$ 15 mmol/L, D-glucose 11 mmol/L, and NaH_2PO_4 1.2 mmol/L) at 37°C with 5% CO_2. The resting tissue force was set to 500 mg, and changes in isometric tension were recorded using a force transducer (Nihon Kohden) that was connected to a data acquisition board (PowerLab 2/26). Relaxant experiments were conducted using strips that were pretreated with noradrenaline 10 μmol/L (Sigma Aldrich, St Louis, MO, USA), and the relaxant effect was induced by acetylcholine (ACh; Wako Pure Chemical Industries). Cumulative dose response curves for ACh (10^{-10}–10^{-4} mol) were performed using separate tissue samples.

Biological Parameters

Bioavailable testosterone was measured as previously reported.[17,18,21] In brief, dexamethasone was used as the internal standard working solution, and concanavalin A was added to the serum samples to remove SHBG. To improve their detectability, testosterone and the internal standard were derivatized using picolinic acid. The prepared samples were subjected to ultra-performance liquid chromatography and tandem mass spectrometry (Aquity UPLC-MS/MS; Waters, Milford, MA, USA), using an Inertsil ODS-3 column (2.1 × 50 mm; GL Sciences, Tokyo, Japan) for separation in a mixture of water and acetonitrile (40:60, v/v) that contained 0.1% acetic acid as the mobile phase. NO secreted from the coronary artery is rapidly