SUPPLEMENTARY MATERIALS AND METHODS

WESTERN BLOT

Penile tissues were homogenized, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Chengdu Baihe Technology Co., Ltd., Chengdu, China), and transferred to polyvinylidene difluoride membrane (Merck Millipore, Billerica, MA, USA) by standard procedures. Primary antibodies to LOX (ab174316, 1:1000, Abcam, Cambridge, MA, USA) were used and the results were normalized to tubulin expression (Cat NO:200608, 1:2000, Zen BioScience Co., Ltd., Chengdu, China). Protein bands were collected using a Bio-Rad ChemiDoc MP (Bio-Rad, Berkeley, CA, USA) and band intensities were quantified by ImageJ software (National Institute of Health, Bethesda, MD, USA).

THE ACTIVITY OF LOX PROTEIN

LOX activity was determined using an Amplite™ Fluorimetric LOX assay kit (AAT Bioquest Inc., Sunnyvale, CA, USA). Briefly, the tissues were finely snipped and homogenized in PBS (self-configuration) at 4°C, then supernatants were collected after centrifugation (10 000 g, 10 min).[1,2] Fluorescence was measured using a BioTek Synergy Mx (BioTek Instruments Co., Ltd., Winooski, VT, USA) with excitation and emission wavelengths at 560 and 590 nm, respectively. LOX activity was normalized to total protein and expressed as relative fluorescent units per ug protein (RFUs ug^{-1} protein) (**Figure 1**).

TOTAL HYDROXYPROLINE (HYP) LEVEL

Hydroxyproline, a reliable collagen marker,[3,4] was quantified using the HYP detection kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China). Wet penile tissues (proximal-middle) were weighed, snipped, and hydrolyzed with 6 M HCl (Guangzhou Sagene Biotech Co., Ltd., Guangzhou, China) for 4 h at 100°C, until no visible lumps were observed. After centrifugation (16 000 g, 20 min), supernatant was collected and adjusted to a suitable pH value (6–8) with 10 nmol L^{-1} NaOH (Guangzhou Sagene Biotech Co., Ltd., Guangzhou, China). According to the manufacturer's instructions, HYP was measured at 560 nm by a Molecular Devices SpecbaMax 190 (Molecular Devices Corporation, San Jose, CA, USA). The results were normalized with respect to wet weight and expressed as μg mg^{-1} wet tissue.

TOTAL ELASTIN LEVEL

Total elastin level was measured by a Fastin Elastin assay (F2000; Biocolor Life Science Assays, Carrickfergus, UK). First, wet tissues (distal-middle) were weighed, finely minced, and solubilized three times in 0.25 M oxalic acid at 100°C for 1 h. Extracts were pooled, and equal amount of samples were added to elastin precipitating reagent and incubated for 15 min, then centrifuged. Dye reagent was added and incubated for 90 min to form an elastin-dye complex. After centrifugation, the complex was dissolved by incubation with dye dissociation reagent for 10 min. Absorbance was measured at 513 nm with a Molecular Devices SpecbaMax 190 (Molecular Devices Corporation, San Jose, CA, USA). The elastin content was expressed as μg mg^{-1} wet tissue.[5,6]

THE CROSSLINKING LEVEL OF COLLAGEN AND ELASTIN

Pyridinoline (PYD) and desmosines (DES) are mature crosslinking forms of collagen and elastin, respectively.[4,7] Penile tissues (as the same procedure of sample management, they share same proximal samples with LOX activity) were finely snipped, homogenized in PBS, and centrifuged (10 000 g, 10 min). The supernatant was obtained for PYD and DES quantification by the rat PYD enzyme-linked immunosorbent assay (ELISA) kit and the rat DES ELISA kit (Shanghai Jingkang Biological Engineering Co., Ltd., Shanghai, China), respectively. Absorbance was measured at 450 nm with the Molecular Devices SpecbaMax 190 (Molecular Devices Corporation, San Jose, CA, USA). PYD and DES concentrations were normalized to total protein and expressed as nmol L^{-1} and pg ml^{-1}, respectively.

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