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THE EFFECTS OF CHRONIC NITRIC OXIDE SYNTHESIS INHIBITION ON BLADDER FUNCTION IN PARTIAL OUTLET OBSTRUCTED MICE

Hypothesis / aims of study

Benign prostatic obstruction (BPO) represents a clinically significant cause of bladder outflow obstruction in men. Clinical manifestations are related to obstruction of the urethra and bladder dysfunction. Bladder outlet obstruction (BOO) results in an impaired ability of the urinary bladder to store and empty urine. In animal models, BOO results in bladder dysfunction and alterations of the contractile machinery in detrusor smooth muscle (DSM), similar to those found in men with BPO [1]. Nitric oxide (NO) is a neurotransmitter formed from L-arginine by a family of enzymes known as nitric oxide synthase (NOS) [2]. Neuronal and endothelial NOS (nNOS and eNOS, respectively) are constitutive isoforms whereas inducible NOS (iNOS) is produced by inflammatory stimulus [3]. Various studies have been made in order to establish the role of these enzymes in physiopathology of BOO. This study aimed to investigate the bladder function in mice treated with L-NAME (non-selective NOS inhibitor) and aminoguanidine (selective iNOS inhibitor) after 5 weeks of partial BOO in mice.

Study design, materials and methods

All animal procedures were approved by the Ethical Principles in Animal Research (CEUA 2030-1). C57BL/6 male mice (28-32 g) were anaesthetized with xilazine (30 mg/kg i.p.) and ketamine (2 mg/kg i.p.) for surgical procedure. BOO was performed using nylon ligature in the bladder neck. Six groups with 3 to 10 animals per group were obtained: Sham, Sham + L-NAME, Sham + aminoguanidine, BOO, BOO + L-NAME and BOO + aminoguanidine. L-NAME and aminoguanidine were administered in drinking water at a dose of approximately 150 mg/Kg and 20 mg/Kg, respectively. After 5 weeks, *in vivo* and *in vitro* studies were performed. Continuous cystometry was carried out by infusing saline into the bladder at a rate of 0.6 mL/h for 30 min after the first micturition cycle in anaesthetized mice. In separate groups, bladder and body weights were obtained and bladder weight to body weight ratio was calculated. Bladder contractility was also evaluated in vitro by performing concentration-response curves to carbachol (CCh; 1 nM to 30 µM) and electrical-field stimulation (EFS; 2-16 Hz, 50 V, 0.2 ms, 10 s interval).

Results

BOO animals presented heavier bladders compared to Sham animals (bladder weight to body weight ratio of 2.45 ± 0.16 and 1.24 ± 0.05, respectively). L-NAME treatment prevented the increased bladder weight in BOO group (bladder weight to body weight ratio of 1.02 ± 0.05). Cystometric evaluation showed that L-NAME worsened the non-voiding contractions (NVC), diminished bladder capacity and compliance, while aminoguanidine decreased NVC and bladder capacity and compliance. Tissue bath protocols revealed that BOO mice had lower contractile response to CCh (E_{max}: 0.49 ± 0.04 and 1.34 ± 0.13 mN, respectively) and EFS when compared to Sham group. Besides, BOO mice treated with L-NAME had higher contractile responses to CCh (?) (E_{max}: 1.13 ± 0.06 mN), whereas aminoguanidine failed to modify CCh-induced contractions in BOO mice (E_{max}: 0.54 ± 0.04 mN).

Interpretation of results

L-NAME diminished bladder gain mass and improved contractile responses in BOO animals, suggesting that non-selective inhibition of NOS isoforms could have a protective effect. However, cystometric results showed that increased NVC seen in these animals would be related to poor bladder function. Aminoguanidine enhanced *in vivo* bladder response, but did not prevent increase in bladder weight or restored *in vitro* contractility.

Concluding message

Selective inhibition of iNOS in BOO mice showed good cystometric responses, which could be related to less oxidative stress and bladder fibrosis. Non-selective inhibition of NOS isoforms worsens bladder function, suggesting that absence of the endothelial and neuronal NOS are important for maintenance of bladder integrity.

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Disclosures

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