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Sakai T[1], Aikawa K[1], Ishibashi K[1], Kawashima Y[1], Kushida N[1], Shishido K[1], Yanagida T[1], Nomiya M[1], Yamaguchi O[1]

1. *Department of Urology, Fukushima Medical University*

THE INVOLVEMENT OF ANGIOTENSIN II TYPE 1 RECEPTOR ON BLADDER HYPERTROPHY AND DYSFUNCTION IN OBSTRUCTED BLADDER OF RAT

Hypothesis / aims of study

Bladder hypertrophy is not only a compensatory response to bladder outlet obstruction (BOO), but also a major risk factor of bladder dysfunction. It is thus very important to understand the mechanism that underlies the development of bladder hypertrophy. It is clear from clinical pressure-flow studies and ultrasound measurements that high-pressure voiding and increased bladder thickness are consistently observed in patients with BOO. Similar results were observed in rats with BOO, showing that micturition pressure and bladder weight in obstructed bladders were significantly increased compared with controls. An increase in bladder wall tension (mechanical stretch stress), therefore, is considered to be the trigger that induces both hypertrophic structural response and functional alterations observed after bladder obstruction. Repetitive stretch and relaxation applied to bladder smooth muscle cells in vitro has been used to model the urodynamically overloaded detrusor muscle under conditions of BOO. Recent evidence indicates that the peptide hormone angiotensin II (ANGII) is released from bladder smooth muscle cells in response to such repetitive stretch stimulus, which then activates angiotensin II type 1 receptors (AT1) in an autocrine fashion. This AT1 activation was demonstrated to mediate the heparin-binding epidermal growth factor-like growth factor (HB-EGF) gene expression. These basic studies have suggested that the local renin–angiotensin system is activated by urodynamic overload and that the AT1 has a crucial role in the development of load-induced bladder hypertrophy.
Thus, the present study attempted to determine whether long-term administration of AT1 antagonists improves morphology and function in the obstructed bladder of rat.

Study design, materials and methods

Forty male SD rats (12 weeks old) were used. In thirty rats BOO was created and ten rats underwent sham operation. Two weeks after the operation the BOO rats were separated at random into 3 groups of 10 each. Using osmotic pump, long-term (4-week) administration of AT1 antagonists or vehicle was performed. Candesartan (0.2mg/kg/day) was administered to candesartan group (obstructed candesartan-treated rats) and losartan (0.3mg/kg/day) was administered to losartan group (obstructed losartan-treated rats). Similarly, the vehicle (saline) was administered to BOO group (obstructed control rats). Six weeks after the operation rats were sacrificed, and the bladders were removed and weighed. Two longitudinal (7 x 2mm) strips were cut from each bladder for muscle contractile studies. The remainder of the bladder was used for Elastica-Masson stain.
The strips were mounted in 25-ml organ baths containing oxygenated Krebs-buffer solution at 37˚C. The strips were suspended between two metal hooks by 6-0 silk ligatures, and their tensions were recorded with an isometric transducer (Nihonkohden, TB-612T). To confirm that the administered AT1 antagonists could block AT1 on the bladder, the contractile response to ANGII (10^{-7} M) was measured. Electrical field stimulation (EFS) was performed at a frequency of 2 to 40 Hz by a SEN3201stimulator (Nihonkohden) connected to two platinum electrodes lying parallel to the strips in the organ baths. To evaluate the muscarinic receptor function, the responses to carbachol (2×10^{-5} M) was recorded. Furthermore, the contractile responses to membrane depolarization and purinergic receptor stimulation were evaluated by determining the responses to KCl (8×10^{-2}M) or ATP (10^{-3}M). The remainder of the bladder was fixed with 4% paraformaldehyde overnight at 4˚C, processed and embedded in Tissue-Tek O.C.T. compound blocks. Cryostat sections (10 μm) were cut and mounted on Silane-coated slides. Sections were stained with Elastica-Masson method for bladder smooth muscle and collagen fiber. To quantify the percent volume of smooth muscle or collagen fiber in the bladder morphometric analysis of full-thickness sections were analyzed using Image Pro Plus (Media Cybernetics, Silver Springs, Maryland) software. Data are shown as mean ± SE. A two-tailed Student's t-test was used to compare sham and BOO, or drug-treated and vehicle-treated specimens under BOO. Values of $p < 0.05$ were considered statistically significant.

Results

Bladder weight of BOO rats increased 3.7-fold compared with sham rats (161 ± 56mg). The increase in bladder weight was significantly inhibited by candesartan (360 ± 142mg) and losartan (331 ± 125mg) administration in BOO rats. Figure shows the maximal contractile responses of bladder strips to ANGII, EFS, carbachol, ATP and KCl. There was no response to ANGII in AT1 antagonist-treated rats. The response for all modes of stimulation except ANGII was significantly lower for BOO group than for sham group. The responses to EFS, carbachol and KCl for AT1 antagonist-treated groups were significantly higher than in the BOO group. The responses to ATP of AT1 antagonist-treated groups were higher than the response of BOO group, although the difference did not achieve statistical significance in candesartan group. Microscopic evaluation of tissue specimens using Elastica-Masson stain revealed obvious differences between BOO and sham rat bladders. Morphometric analysis of the collagen fiber-to-smooth muscle ratio in the detrusor showed that there was a difference between the BOO and sham groups (0.81 and 0.55, respectively). Furthermore, the increase in the collagen fiber-to-smooth muscle ratio was significantly inhibited by AT1 antagonists administration in BOO rats.

Interpretation of results

Contractile responses of bladder strips to ANGII in AT1 antagonist-treated rats almost disappeared. This indicates that the AT1 of bladder is almost completely blocked by doses and route of 2 drugs used in this study. Contractile responses to KCl and morphological findings show that the decreased contractile function induced by BOO is due to increased fibrosis, and AT1 antagonists inhibit fibrosis and improve contractility. It is conceivable that AT1 antagonists inhibit bladder hypertrophy caused by BOO, and prevent fibrosis associated with relative ischemia.

Concluding message

The clinically used AT1 antagonists prevented bladder hypertrophy, fibrosis and contractile dysfunction related to obstructed bladders. These findings suggest that AT1 of rat bladders exposed to outlet obstruction was activated, which might be associated with the pathophysiology of bladder hypertrophy, fibrosis and dysfunction.

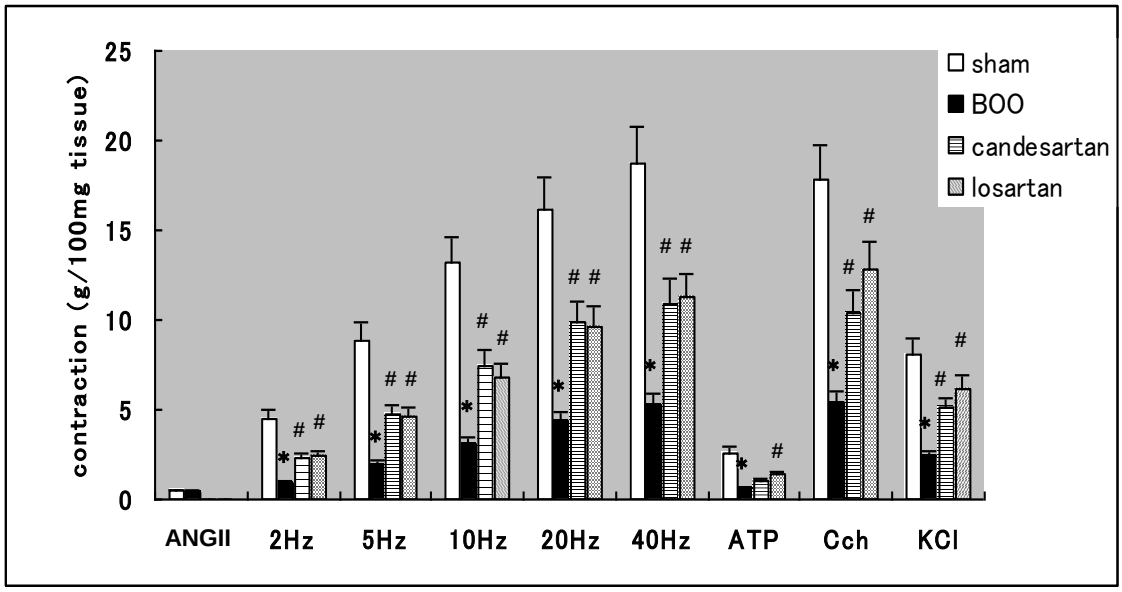

Figure. Maximum contractile responses to each stimulation.
* p<0.05 between sham and BOO, # p<0.05 vs BOO

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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