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BLADDER DYSFUNCTION AND INCREASED BLADDER EXPRESSION RATIO OF ANGIOTENSIN II TYPE I RECEPTORS AGAINST ANGIOTENSIN II TYPE II RECEPTORS IN AGED RATS

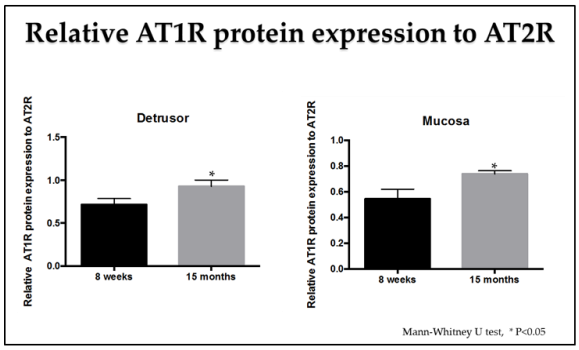
Hypothesis / aims of study
Aged patients with bladder dysfunction have been increasing. Angiotensin II acts through two types of receptors termed angiotensin II type I (AT1) receptors and angiotensin II type II (AT2) receptors. It has recently been reported that telmisartan, an AT1 receptor antagonist, suppresses detrusor overactivity in association with reduced expression of AT1 receptors in the bladder from rats with partial bladder outlet obstruction (P-BOO) [1]. We have also reported that AT1 receptors are highly expressed in the bladder mucosa of P-BOO rats [2], and that the expression of AT1 receptors and gap junctional protein connexin43 (Cx43) were increased in the bladder submucosa of rats with HCl-induced bladder inflammation. The AT1 receptor is known to induce interstitial fibrosis and activation of MAP kinase (MAPK) leading to an increase in Cx43 expression, which is considered as a cause of OAB [3]. On the other hand, the AT2 receptor is known to suppress AT1 receptor induced remodelling, which is lead to organ homeostasis. Furthermore, AT1 receptor antagonists such as telmisartan can activate directly to PPARγ to secrete adiponectin, which leads to anti-remodelling and organ homeostasis. However, it is not known whether changes in the expression of AT1 receptor, AT2 receptors and PPARγ are involved in the development of bladder dysfunction in the aging process. Therefore, we investigated the alternation of the expression of AT1 receptors, AT2 receptors, PPARγ, adiponectin, adiponectin receptor I, Cx43, MAPK, and also collagen I in the bladder in aged rats with bladder dysfunction.

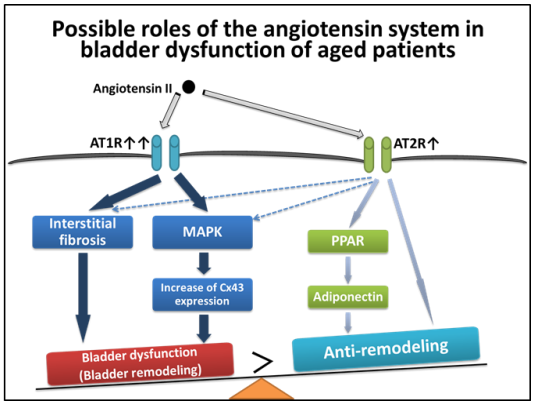
Study design, materials and methods
Female Sprague-Dawley rats of three different ages at 8 weeks old (8W) (n=5), 9 months old (9M) (n=5) and 15 months old (15M) (n=5) were used. Cystometrograms (CMG) were obtained under urethane anaesthesia (1.2mg/kg, S.C) in all three groups, and urodynamic parameters were evaluated, including non-voiding contraction (NVC), pressure threshold during the storage phase(PT), maximum voiding pressure (MVP), voided volume (VV) and post-void residual volume (RV). After the CMG investigation, the bladder was excised and separated into mucosa and detrusor muscle layers under a microscope. The protein and mRNA expression levels of AT1 receptor, AT2 receptor, PPARγ, Cx43 and MAPK1 were investigated by Western blot analysis and RT-qPCR, respectively. The mRNA levels of adiponectin, adiponectin receptor 1 and collagen I were investigated by RT-qPCR. The localization of AT1 and AT2 receptors were investigated by immunohistochemistry. Statistical analysis was performed using Mann-Whitney U test or Dunn's multiple comparison test, with $P<0.05$ being considered significant. Data are presented as means ± SEM.

Results
In CMG investigation, the mean number of NVCs was significantly greater in the 15M group than in 8W ($P<0.01$). PT was also significantly increased in the 15M group than in the 8W group ($P<0.01$). MVP and VV were significantly decreased in the 15M group, compared with those in the 8W group ($P<0.05$) and RV was significantly increased in the 15M group, compared with the 8W group ($P<0.01$).
Because there was no significant difference in any parameters between 8W and 9M groups, the protein and mRNA expression levels were compared between 8W and 15M groups. In RT-qPCR analyses, the mRNA expression of AT1 receptors, AT2 receptors, Cx43, MAPK1, PPARγ, adiponectin, adiponectin receptor 1 and collagen I in the mucosa and detrusor were significantly increased in the 15M group than in the 8W group ($P<0.05$). In Western blot analysis, the protein expression of angiotensin II, AT1 receptors, AT2 receptors, Cx43, MAPK1 and PPARγ in the mucosa and detrusor were significantly increased in the 15M group than in the 8W group ($P<0.05$).
Furthermore, the relative expression ratio of AT1 receptor protein against AT2 receptor protein in the mucosa and detrusor was significantly increased in 15M group compared with 8W group (Figure 1). In immunohistochemistry, there was no difference of the localization of AT1 receptor and AT2 receptor in 8W and 15M groups.

Interpretation of results
These results indicate that aged rats exhibit not only bladder overactivity during storage phase evidenced by increased NVCs, but also impaired voiding evidenced by decreased MVP and increased RV, which are associated with upregulation of AT1 receptors, AT1/AT2 ratio, Cx43, and MAPK1/2 in the bladder. Thus, it is suggested that, during the aging process, bladder dysfunction including both bladder overactivity and impaired voiding, which are often seen in aged lower urinary tract symptoms (LUTS) patients is developed. Our results also suggest that the upregulation of AT1 receptors, which can increase Cx43 expression via activation of MAPK signalling pathways, may play a significant role in the emergence of bladder overactivity during aging, and that increased collagen I expression may contribute to impaired voiding during aging. On the other hand, the upregulation of AT2 receptors may play a significant role in the suppressing of AT1 receptors induced remodelling in the bladder with aged rats. Furthermore, the upregulation of PPARγ leads to secretion of adiponectin in the bladder, which also may play a significant role in suppressing of AT1 receptor-induced remodelling and keeping the bladder homeostasis. However, because AT1 receptor upregulation is more dominant than AT2 receptor increases as evidenced by the higher AT1/AT2 ratio in aged rats, AT2 receptor activation may not be sufficient to suppress AT1 receptor stimulation in aged rats (Figure 2).

Figure 1 Figure 2





Concluding message

AT1 receptor upregulation could be an important pathophysiological process to induce both storage and voiding LUTS in elderly people. Therefore, the third-generation AT1 receptor antagonists such as telmisartan, which can suppress AT1 receptors and also directly stimulate PPARγ to secret adiponectin, might be effective for the treatment of aged patients with LUTS.

References

1. Urology. 2012; 80:1163.e1-7
2. Urol Int. 2012; 89:241-5
3. Urology. 2005; 65:1254-8

Disclosures

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