

ENHANCED EXPRESSION OF HEME OXYGENASE ACCOMPANIED WITH INCREASE OF COLLAGEN TYPE III IN THE RAT BLADDER AFTER PARTIAL BLADDER OUTLET OBSTRUCTION

Aims of Study

Carbon monoxide (CO) is produced by the degradation of heme to biliverdin and CO, a reaction catalyzed by the enzyme heme oxygenase (HO). Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS). CO and NO are generally accepted as gaseous non-adrenergic, non-cholinergic mediators of urethral smooth muscle relaxation in urological organs (1,2). Recently, inducible NOS (iNOS) in the early phase after partial bladder outlet obstruction was suggested to contribute in a protective way to obstruction induced ischemia in the mouse model (2). Recently, we have reported that histological changes in the detrusor muscle correlated well with the degree of bladder hypertrophy due to bladder outlet obstruction, and demonstrated a significant increase of connective tissue, especially collagen type III (3). We hypothesized that the relationship between increase of collagen type III and gaseous non-adrenergic, non-cholinergic mediators (including CO as one candidate) could be involved in the process of bladder dysfunction after bladder outlet obstruction. The objective of the study was to evaluate the gene expression and localization of heme oxygenase (HO), nitric oxide synthase (NOS), and collagen type III in the rat bladder after partial bladder outlet obstruction.

Methods

A total of 39 Sprague-Dawley female rats with an approximate weight of 200 gm. initially were used. The experimental procedure was permitted by the Committee for Animal Research in our institution. Partial bladder outlet obstruction was induced in the manner previously described by Mattiasson and Uvelius. Sham-operated animals, which had undergone urethral manipulation without the urethra being ligated, served as control. On days 4, 7, 10, 14, 28 and 42 following initial obstruction, whole bladders were removed, snap frozen and stored at -80 °C for real-time quantitative reverse transcription-polymerase chain reaction (real-time RT-PCR) assay. To assess the gene transcription changes, the target transcripts were measured using the ABI Prism 7700 Sequence Detection System (PE-Applied Biosystems, Inc). To compare the obstructed bladders with control bladder in terms of the expression of the genes of interest, real-time RT-PCR using TaqMan probe was carried out with TaqMan Universal PCR Master Mix (The Perkin-Elmer Corp.). Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same specimens was also measured and the value was used to normalize the target gene results. The relative concentration of GAPDH and the target molecule of tissue samples obtained on post-operative days 4, 7, 10, 14, 28 and 42 were calculated from the standard curve, and the ratio of the relative concentration of the target molecule to GAPDH (target molecule/GAPDH) of the samples was calculated. The expression of target molecules (including collagen type III, HO, subtype HO-1 and 2, NOS, subtype iNOS, nNOS, eNOS) and relative expression of HO, the sum of HO-1 and 2, and NOS, the sum of iNOS, nNOS and eNOS, to 5×10^5 copies of GAPDH mRNA were obtained.

For histological analysis, bladders of controls as well as of post-operative day 14 rats were fixed, and paraffin-embedded for immunohistochemistry for HO-1, HO-2, iNOS, protein gene product 9.5 (PGP-9.5). In immunohistochemistry for PGP-9.5, the sections were viewed under a microscope with a high Nikon-3CCD color video camera, and captured video images were displayed on a color monitor and simultaneously digitized using a personal computer. Using a computer-assisted image analysis system (Mac Scope, Mitani Corporation, Fukui, Japan), the ratio of PGP-9.5 to muscular layers was analyzed.

Results

Bladder weight increased to five- and seven-fold compared with controls on post-operative days 14 and 42, respectively. Real-time RT-PCR assay demonstrated significantly enhanced expression of the HO gene from the post-operative 4 (p 0.001) to 14 day (p 0.01) (early to middle phase after obstruction). Similarly, expression of collagen type III increased from post-operative day 4 to the post-operative day 14 (p 0.001) (early to middle phase after obstruction), and then decreased. Enhanced expression of the NOS gene was seen only on post-operative day 4 (p 0.01) (early phase after obstruction).

The ratio of PGP-9.5 immunoreactive area to muscular layer area were significantly (p 0.0001) increased from the control (0.990±0.161) to the rats on post-operative day 14 (2.019±0.283). Immunohistochemistry

revealed that immunoreactivity for HO-1 had a lot in common with that for PGP-9.5, although immunoreactivity for HO-2 and iNOS were relatively weak in the cell which was positive for PGP-9.5. There was no difference in localization of the immunoreactive cells for HO-1, HO-2 as well as iNOS between the controls and the rats on post-operative day 14.

Conclusions

This study suggested that gene expression of HO as well as collagen type III were up-regulated simultaneously accompanied by relative increase of nerve fibers in the hypertrophied muscular layer after partial bladder outlet obstruction. CO/HO systems may play an important role in the etiology of bladder dysfunction after bladder outlet obstruction.

References

- 1) Br J Pharmacol 120: 312-8, 1997
- 2) J Urol 161: 1015-22, 1999
- 3) Acta Histochem. Cytochem. 33: 131-9, 2000