

CHRONIC ISCHEMIA ALTERS THE BLADDER PROTEOMIC PROFILES AND ACTIVATES DEGENERATIVE PATHWAYS AND SURVIVAL SIGNALING

Hypothesis / aims of study

Growing clinical [1] and basic research [2] evidence suggests that peripheral arterial disease and pelvic ischemia contributes to aging-associated bladder dysfunction and lower urinary tract symptoms (LUTS). In animal models, aorto-iliac atherosclerotic occlusive disease induces pelvic ischemia, initiates bladder oxidative stress, mitochondrial injury, muscarinic receptor hypersensitivity, neurodegeneration, inflammation, and fibrosis leading to detrusor instability [3]. We hypothesized that differentially or newly expressed proteins under the ischemic conditions could activate downstream pathways of smooth muscle instability and fibrosis and lead to bladder dysfunction. Our goal was to perform a large-scale proteomic analysis to characterize differentially expressed proteins and explore consequent molecular pathways in the chronically ischemic bladder.

Study design, materials and methods

Adult male Sprague-Dawley rats were randomly divided into five treatment and five sham control groups. The treatment group was placed on a 2% cholesterol diet for two weeks then, under general anesthesia, iliac artery ballooning was performed using a 2F Fogarty catheter. After the ballooning procedure, the treated animals were placed on a 2% cholesterol diet for 8 weeks to create aorto-iliac atherosclerosis and bladder ischemia. The sham group underwent similar procedures without arterial ballooning. After eight weeks, changes in the treated animals were compared with sham controls. Voiding behavior including voiding frequency and voided volume were continuously recorded for 24 hours using the metabolic cage system. After this, the animals were anesthetized, iliac artery and bladder blood flow were recorded and cystometrograms were obtained. The bladder was isolated and proteomic profile of tissue lysates from ischemic and control bladders were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) followed by label-free quantification. Up- and down-regulated proteins were identified and classified with the DAVID 6.7 and Ingenuity Pathway Analysis (IPA) software. Gene Ontology (GO) bioinformatics tool was used for gene ontology analysis of differentially expressed proteins. Pathway and network analysis were performed with the Ingenuity Pathways Analysis software. Western blot was used to further confirm the up- and down-regulated proteins pertaining to bladder ischemia.

Results

Micturition frequency per 24 hours significantly increased while mean voided volume significantly decreased in bladder ischemia group compared with controls. Cystometry showed increased contractile activity in bladder ischemia group characterized by spontaneous fluctuations in intravesical pressure, suggesting detrusor instability. There were no significant differences between bladder ischemia and control groups in terms of body weight and bladder weight. LC-MS/MS followed by SEQUEST HT identified 4277 proteins in the ischemic and 4602 proteins in the control bladder tissues. Among the identified proteins, spectral count analysis revealed 172 up-regulated proteins and 527 down-regulated proteins in the ischemic bladders versus controls. The final panel of ischemically altered proteins was narrowed down by selecting their expression levels with at least 2-fold of increase or decrease, resulting in 97 up-regulated and 262 down-regulated proteins in comparison with controls. For references, 20 up-regulated and 46 down-regulated proteins were generated when over 5-fold of increase or decrease was considered. Gene ontology analysis revealed that the 66 ischemia-regulated proteins (20 up-regulated and 46 down-regulated at over 5.0 folds of changes) were classified in the categories involving proteolysis, peptidase activity, hydrolase activity, and protein metabolic processes, all of which were known for their roles in ischemia-related protein degeneration. Further analysis revealed that, based on their molecular functions, the 66 ischemia-regulated proteins were involved in peptidase and other enzymatic regulatory activities. Thus, both molecular function and biological properties of ischemically altered proteins in the bladder suggested their involvement in peptidase pathways and proteolysis mechanisms, implying a central role of protein degradation in the pathogenesis of bladder ischemia. Canonical pathway analysis linked the highest number of ischemia regulated proteins to ubiquitination pathway, nuclear factor erythroid-2 related factor (Nrf2)-mediated oxidative stress response and survival signaling, and extracellular signal-regulated kinase-1 (ERK1) / mitogen-activated protein kinase (MAPK) signaling pathway that are closely related to free radical incursion. Network of ischemia-regulated proteins revealed a close linked between altered proteins and disruption of glucose metabolism, alteration of cytoskeleton organization, and bladder degeneration. Western blotting confirmed differential expression of the representative proteins identified by LC-MS/MS.

Interpretation of results

Our study represents the first global comparison of bladder protein expression profiles in chronic ischemia versus sham control conditions. The profile of differentially expressed proteins of fold change cutoff of ≥ 2.0 along with modifications of relevant pathways in the ischemic bladder may potentially reflect the bladder's response to ischemic injury as well as the functional impairment and morphological aberrations of the ischemic bladder. The differentially expressed proteins identified in the present study provide new insight into molecular pathways underlying ischemia-associated bladder dysfunction and LUTS.

Concluding message

Our findings may provide a foundation for future research regarding validation and clinical translation of identified biomarkers to develop targeted diagnosis and therapies for bladder dysfunction and LUTS in elderly patients with pelvic arterial insufficiency.

References

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Disclosures

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Subjects: ANIMAL **Species:** Rat **Ethics Committee:** VA Boston Healthcare System Animal Care and Use Committee