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THE IMMUNOHISTOCHEMICAL STUDY FOR THE EXPRESSION OF ICAM-1, NEUTROPHIL, MACROPHAGE AND INOS IN RATS WITH PARTIALLY OBSTRUCTED BLADDER OUTLET.

Hypothesis / aims of study

Bladder outlet obstruction causes structural and functional changes of bladder including detrusor hypertrophy, increased intravesical pressure during voiding, detrusor overactivity and decrease of detrusor contractility. These changes are related with neuronal damage of bladder wall, and according to many reports neuronal damage is related with the excessive manifestation of nitric oxide (NO). Intercellular adhesion molecule-1 (ICAM-1) is up-regulated in the inflammatory condition, and increased ICAM-1 causes migration of neutrophil and macrophage through the wall of blood vessel. Inducible nitric oxide synthase (iNOS) exists in the cytoplasm of inflammatory cells like neutrophil and macrophage, and if stimulated by cytokine, it creates excessive nitric oxide. This study was designed for evaluating the manifestation of ICAM-1, neutrophil, macrophage and iNOS in rats with partially obstructed bladder outlet. And we evaluated the relationship between each manifestations.

Study design, materials and methods

Thirty-two female Sprague-Dawley rats weighting 250-300 gm were used. Partial bladder outlet obstruction was performed by modified Levin and Wein's method. We injected chloral hydrate (400mg/Kg) into rat's abdominal cavity, incised at lower abdomen and dissected bladder neck and urethra from adjacent fat tissue. After dissection, placed needle (1mm external diameter) just beside urethra and ligated uretha with needle by 4-0 silk. Control group was operated by same method except urethral ligation. Each bladders were resected at postoperative 6 hours, 12 hours, 1 day, 2 days, 3 days, 5 days and 7 days from rats with partially obstructed bladder outlet and from sham-operated rats. Resected bladders were put into liquid nitrogen and kept in refrigerator (-70^{o}C). Immunohistochemical stain was done with 4 tissues per each bladder, and the manifestation of ICAM-1, neutrophil, macrophage and iNOS was analized by Image Pro Plus. Student's t-test and ANOVA test were used for statistical analysis.

Results

In the partially obstructed group, the manifestation of ICAM-1 was started at 12 hours (412.9±55.0 μm^2) in the endothelial cells of blood vessel, and most increased at postoperative 1 day (481.7±55.7 μm^2, $p<0.05$ vs. control) and decreased rapidly after 2 days. It was not present after 3 days. In the control group, ICAM-1 was not expressed. In the partially obstructed group, the manifestation of neutrophil was started at postoperative 12 hours (54.6±14.2/mm^2) and most increased from postoperative 1 day (59.4±23.0/mm^2, $p<0.05$ vs. control) to 2 days (47.9±15.2/mm^2, $p<0.05$ vs. control). In the control group, neutrophil was not expressed. In the partially obstructed group, the manifestation of macrophage was started at postoperative 6 hours (6.0±3.7/mm^2), and most increased from 2 days to 5 days (74.9±18.7/mm^2, $p<0.05$ vs. control). In the control group, it was not expressed. In the partially obstructed group, the manifestation of iNOS was started at postoperative 6 hours (0.05±0.03 mm^2), and most increased from 2 days to 5 days (0.22±0.03 mm^2, $p<0.05$ vs. control). In the control group, it was not expressed. There was high correlation between manifestation of neutrophil, macrophage and iNOS ($p<0.05$).

Interpretation of results

Intercellular adhesion molecule-1 (ICAM-1) is up-regulated in the inflammatory condition, and increased ICAM-1 causes migration of neutrophil and macrophage through the wall of blood vessel. Inducible nitric oxide synthase (iNOS) exists in the cytoplasm of inflammatory cells like neutrophil and macrophage, and if stimulated by cytokine, it creates excessive nitric oxide. In this animal experiment, ICAM-1, neutrophil, macrophage and iNOS was expressed in bladder after partial obstruction of bladder outlet. And the time sequence of each menifestation corresponded to above-mentioned theory. And there was high correlation between expression of neutrophil, macrophage and iNOS. So authors think these results suggest that the sequential inflammatory reaction after partial obstruction of bladder outlet causes high expression of iNOS and over-expression of NO, and it may cause structural and functional changes of bladder after partial obstruction of bladder outlet.

Concluding message

According to this results of animal experiment, authors suggest that the sequential inflammatory reaction after partial obstruction of bladder outlet causes high expression of iNOS and over-expression of NO, and it may cause structural and functional changes of bladder.

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