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WATER AVOIDANCE STRESS UP-REGULATES CYCLOOXYGENASE-2 ON THE RAT BLADDER

Hypothesis / aims of study

Water avoidance stress (WAS) represents a potent psychological stressor(1) associated with visceral hyperalgesia, such as irritable bowel syndrome (IBS). In this WAS rat model of IBS, there exists some erosive changes at the bladder mucosa(2) as well as the bowel mucosa. We certified the voiding pattern of the WAS rats which had been previously reported as urinary frequency(3), and we investigated the histological and genetic changes in the WAS rat's bladder to find out the process between psychological stress and urinary frequency.

Study design, materials and methods

Twenty four adult male Sprague-Dawley rats (200-250g) were divided into 2 groups. In WAS group, rats were subjected to water avoidance stress 2 hours a day for 10 consecutive days. Rats were placed on a glass platform in the center of a plastic container filled with 25°C water up to 1 cm below the top of the glass platform. In the control group, the plastic container was empty.

After day 10 of WAS or control, rats were placed in a metabolic cage for a 24-hour voiding assessment. Then, cystometrography (CMG) was performed under the anesthesia of urethane. We investigated the basal, threshold and maximum voiding pressure. We also counted voiding interval time and residual urine volume.

All animals were sacrificed after CMG. The bladders were removed and each was divided into two pieces; one was homogenized and used for extraction of mRNA, the other was fixed and sectioned to perform histological examination.

Agilent double-labeled cDNA microarray analysis was used to identify the gene expression profiles in the bladder to investigate the genes up-regulated after the exposure to WAS. Then, we quantified the gene expression by performing RT-PCR.

We investigated the voiding pattern of WAS rats under the inhibition of a candidate among up-regulated genes. We administered the inhibitory drug to the eight adult male rats with WAS for consecutive 10 days, as a group of treated WAS. And we compared CMG parameters (described as above) of the treated WAS rats with that of WAS only rats (n=8).

The data were presented as the mean ± standard error of the mean. Differences between groups were analyzed by Mann-Whitney's U-test. P values < 0.05 were considered statistically significant.

Results

In the metabolic cage, voiding interval time was significantly shorter in WAS rats (1.69 ± 0.19 hr) than in normal controls (2.40 ± 0.34 hr). Voided volume was significantly less in WAS rats (0.58 ± 0.09 ml) than in normal controls (0.93 ± 0.19 ml). Total urine volume revealed no significant differences between the two groups. In CMG, voiding interval time was significantly shorter in WAS rats (7.58 ± 2.07 min) than in normal controls (10.0 ± 2.85 min). Intravesical pressure and residual urine volume revealed no significant differences between the two groups.

The microarray analysis showed higher expression of cyclooxygenase-2 (COX-2) and interleukin-1 (IL-1) beta in the WAS rat bladder, respectively 5.9 fold and 12.3 fold versus normal control. Using RT-PCR, significant up-regulation of COX-2 (6.28 ± 2.45 times as much as normal control) was detected in the bladder of WAS rats. Although IL-1 beta was up-regulated in the bladder of WAS rats (2.39 ± 1.33 times as much as normal control), the increase of gene expression was not significant. This discrepancy of the results of microarray analysis and RT-PCR in IL-1 beta was considered to be caused by individual deviation.

The immunohistochemistry of COX-2 in the WAS rats bladders showed intensive expression in the smooth muscle layer compared with normal controls.

As a COX-2 inhibitor, Etodolac was used. It was suspended in 0.5% methylcellulose solution, and administered orally at a dose of 10 mg/kg. In CMG, voiding interval time was significantly longer in treated WAS rats than in WAS only rats. Intravesical pressure and residual urine volume revealed no significant differences between the two groups.

Interpretation of results

The WAS rats showed voiding frequency in both the metabolic cage and CMG. High expression of COX-2 on the bladder of WAS rats were seen. It was found that voiding frequency of the WAS rats was improved with the treatment of COX-2 inhibitor.

Concluding message

COX-2 may have some important role to mediate voiding frequency due to psychological stress. COX-2 inhibitor could be a useful treatment of the stress-induced voiding frequency.

Comparison of CMG parameters between WAS and normal control rats

	normal control	WAS	
Voiding interval time (min)	10.0 ± 2.85	7.58 ± 2.07	<0.05
Residual urine volume (ml)	0.08 ± 0.10	0.06 ± 0.08	NS
Intravesical pressure (cmH_2O)			
basal	6.18 ± 1.89	7.51 ± 2.69	NS
threshold	14.3 ± 3.46	16.5 ± 4.59	NS
maximum	36.0 ± 6.15	38.8 ± 6.86	NS

(mean ± SD)

References
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Is this a clinical trial?	**No**
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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