Preventive Effect of Hydrogen Water on the Development of Detrusor Overactivity in a Rat Model of Bladder Outlet Obstruction

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Abbreviations and Acronyms

8-OHdG = 8-hydroxy-2′-deoxyguanosine
BOO = bladder outlet obstruction
Cch = carbachol
EFS = electrical field stimulation
HW = H_2 water
LUTS = lower urinary tract symptoms
MDA = malondialdehyde
NGF = nerve growth factor
OW = ordinary drinking water
PVR = post-void residual urine volume

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Purpose: Bladder ischemia and oxidative stress contribute to the pathogenesis of bladder dysfunction caused by bladder outlet obstruction. H_2 reportedly acts as an effective antioxidant. We investigated whether oral ingestion of H_2 water would have a beneficial effect on bladder function in a rat model of bladder outlet obstruction.

Materials and Methods: H_2 water was made by dissolving H_2 gas in ordinary drinking water using a hydrogen water producing apparatus. The bladder outlet obstruction model was surgically induced in male rats. Rats with obstruction were fed H_2 water or ordinary drinking water. On week 4 postoperatively cystometry was performed. Oxidative stress markers and the bladder nerve growth factor level were determined. Bladder tissues were processed for pharmacological studies and histological analysis.

Results: The micturition interval and micturition volume significantly decreased in obstructed rats given ordinary drinking water. These decreases were significantly suppressed by oral ingestion of H_2 water. Increased post-void residual volume in obstructed rats was significantly reduced by H_2 water. Obstruction led to a significant increase in bladder weight, oxidative stress markers and nerve growth factor. H_2 water significantly suppressed these increases without affecting bladder weight. There was no significant difference in histological findings between rats with bladder obstruction given H_2 water and ordinary drinking water. Decreased responses of detrusor muscle strips from obstructed bladders to KCl, carbachol and electrical field stimulation were reversed by H_2 water ingestion.

Conclusions: Results suggest that H_2 water could ameliorate bladder dysfunction secondary to bladder outlet obstruction by attenuating oxidative stress.

Key Words: urethra, bladder neck obstruction, drinking water, hydrogen, oxidative stress

Bladder dysfunction and LUTS are highly prevalent in older men and women.[1,2] In men as a result of benign prostatic enlargement BOO is one of the most common causes of detrusor overactivity and OAB symptoms. In addition, BOO may impair detrusor contractility, progressing to inability to empty the bladder.

In the obstructed bladder there is a reduction in bladder blood flow. This hemodynamic change has been

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demonstrated in the animal model of BOO.[3] Koritsiadis et al noted a significant increase in the expression of hypoxia-inducible factor-1α, a cellular marker of hypoxia, in the obstructed human bladder.[4] These studies suggest that BOO induces a decrease in bladder blood flow and chronic ischemia of the bladder. A decrease in blood flow (ischemia phase) resulting in a decrease in oxygen tension (hypoxia) in the bladder is followed by an increase in blood flow and oxygen tension after micturition (reperfusion phase). Thus, chronic bladder ischemia and repeat ischemia-reperfusion during a micturition cycle may produce oxidative stress, leading to bladder denervation and the expression of tissue damaging molecules in the bladder such as NGF and prostaglandins.[5–7] This may be responsible for the development of bladder dysfunction in the obstructed bladder. In this context antioxidants would be beneficial to prevent and treat bladder dysfunction and LUTS in male patients with benign prostatic obstruction.

Recently attention has focused on the novel role of H_2 as an antioxidant. Ohsawa et al reported that H_2 selectively reduced strong oxidants such as hydroxyl radical and peroxynitrite in cells but did not react with other reactive oxygen species that have physiological roles, thereby protecting cells against oxidative stress.[8] The preventive and therapeutic applications of H_2 have been reported in a variety of oxidative stress associated diseases and in their animal models. Administration of H_2, inhalation of H_2 gas or oral ingestion of water in which H_2 was dissolved water (HW) has shown beneficial effect on ischemia-reperfusion injuries in cerebral and myocardial infarction,[8,9] metabolic syndrome,[10] organ transplantation,[11] cognitive impairment[12] and neurodegeneration in an animal model of Parkinson disease[13,14] and in patients with Parkinson disease.[15] However, despite a strong link between oxidative stress and obstructive bladder dysfunction to our knowledge the effect of H_2 on this condition has not been elucidated to date.

Recently attention has focused on bladder ischemia and oxidative stress as a common pathophysiological mechanism for bladder dysfunction.[16] Particularly NGF expression associated with oxidative stress may have a major role in the pathogenesis of bladder dysfunction, including BOO induced bladder hyperactivity.[17–19] Thus, we investigated whether oral ingestion of HW would prevent bladder dysfunction by alleviating oxidative stress in a rat model of BOO.

MATERIALS AND METHODS

H_2 Water

HW was made by dissolving H_2 gas in drinking water under high pressure (0.4 MPa) using a hydrogen water generating apparatus (Bio Coke Laboratory, Tokyo, Japan). Rats were provided with HW (0.7 mM) through a closed glass vessel (300 ml) equipped with an outlet line containing 2 ball bearings to prevent water degassing. The H_2 concentration of HW was measured by a hydrogen sensor (Unisense, Aarhus, Denmark).

Animals and Experimental Design

The experimental protocol was reviewed and approved by the Nihon University animal ethic committee. Seven-week-old male Sprague Dawley® rats weighing 280 to 320 gm were used. The rats received OW or HW for 7 days preoperatively.[13,14] They then underwent BOO or sham surgery at age 8 weeks. From the day of operation BOO rats continued to receive OW (BOO plus OW group) or HW (BOO plus HW group) for 4 weeks after surgery. Sham operated rats also continued to receive OW (sham operated group) for 4 weeks.

On week 4 postoperatively urine was collected from the rats in a metabolic cage in a 24-hour period to determine urinary concentrations of creatinine and oxidative stress markers. Continuous cystometry was done in conscious rats from each group. After cystometrogram recording the rats were sacrificed and the bladder was removed and weighed. Removed bladders were used for organ bath study, histological analysis and measurement of the levels of oxidative stress markers and NGF in bladder tissue.

BOO Operation

Eight-week-old rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). BOO was created by incomplete ligation of the urethra using a standardized method.[20] Sham operated rats underwent similar surgery but no obstruction was created.

Continuous Cystometry

Three days before continuous cystometry each rat was anesthetized with the mentioned anesthetics. A PE-50 polyethylene tube was inserted in the bladder dome. The other end of the tube was brought out through the subcutaneous tunnel in the back. Three days later, continuous cystometry was performed in conscious rats without restraint in a metabolic cage.[21] The bladder tube was connected through a T-tube to a DPT-200 pressure transducer (Utah Medical Products, Salt Lake City, Utah) for bladder pressure recording and to a SP-80s infusion pump (Techtron, Osaka, Japan) for continuous saline infusion into the bladder. An analytical balance was placed under the metabolic cage. The transducer and balance were connected to a PowerLab® system to record bladder pressure and micturition volume while saline was infused into the bladder at a rate of 10 ml per hour.

To measure PVR saline infusion was stopped immediately after voiding and saline remaining in the bladder was aspirated through a cystostomy tube. The cystometric parameters evaluated were micturition pressure, micturition volume, micturition interval, PVR, bladder capacity (micturition volume plus PVR) and bladder voiding efficiency (micturition volume divided by bladder capacity).

Measurement

Oxidative Stress Markers. We determined the levels of 8-OHdG and MDA as a marker of DNA damage and lipid