

**THE EFFICACY OF HYPERBARIC OXYGEN TREATMENT FOR RODENT INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME MODEL**

Hypothesis / aims of study

The etiology of IC/PBS includes a diversity of factors and remains poorly understood. Therefore, appropriate therapeutic options have not been established from both the clinical and basic experimental evidences. We previously reported the clinical effect of hyperbaric oxygen (HBO) therapy in the IC/PBS patients.<sup>(1-2)</sup> A brand-new IC/PBS-like rodent model, which has the chronic cystitis caused by intravesical infusion of hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>), was recently reported.<sup>(3)</sup> This study's aim is to investigate whether HBO treatment is effective for the pathological condition using this IC/PBS-like rodent model. The results derived from this study will be the first experimental data of HBO treatment that can recover the condition of IC/PBS.

Study design, materials and methods

We used 6 weeks old ICR female mice, and examined separately in each four experimental group mentioned as below.

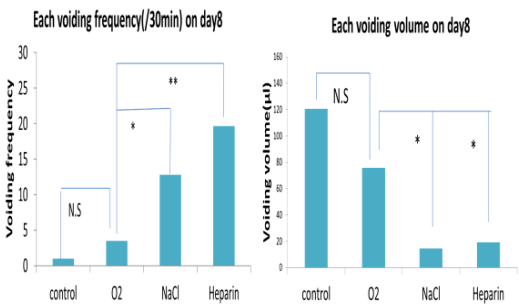
- Group 1 (Control): Control without both the instillation of H<sub>2</sub>O<sub>2</sub> and followed treatments.
- Group 2(HBO): After the intravesical instillation of H<sub>2</sub>O<sub>2</sub> for 20 minutes (min) on the day 1 and 3, followed by the treatment with HBO (100 % O<sub>2</sub>, 2 ATA, 30 min/day) through the day 4 to 7.
- Group 3(NaCl): After the intravesical instillation of H<sub>2</sub>O<sub>2</sub> for 20 min on the day 1 and 3, followed by the instillation of vehicle for 10 min on the day 4 and 7.
- Group 4(Heparin): After the intravesical instillation of H<sub>2</sub>O<sub>2</sub> for 20 min on the day 1 and 3, followed by the intravesical instillation of heparin for 10 minutes on the day 4 and 7.

On the day1, 3, 4 and 6, we measured body weight, voiding frequency (free void in each gauge for 30 minutes), voiding volume and the individual bladder pain threshold (on the suprapubic region) using von-Frey test. On the day 7, we injected intravenously the fluorescent agent taken specifically in inflammatory region and took the whole body images using IVIS(Lumina SeriesIII) on the day 8. Finally, we sacrificed these mice and resected each bladder for histopathological examination and RT-PCR. Subsequently, we performed immunohistochemical stain and measured the mRNA expression of several biomarkers (e.g., IL-6, eNos) associated with IC/PBS using the some specific TaqMan probes. One-way ANOVA was used for the statistical analysis.

Results

On the day 4, the voiding frequency significantly increased, and tidal voiding volume and bladder pain thresholds significantly decreased in mice groups with the intravesical instillation of H<sub>2</sub>O<sub>2</sub> compared with the control. These changes in the vehicle-treated mice (group 3) were sustained until the day 8. HBO-treated mice (group 2) recovered bladder capacity and hyperalgesia at the same level as equal to the control (group 1) on the day 8. The mice treated with the intravesical instillation of heparin (group 4) had no significant change in these factors compared with the vehicle-treated mice (group 3). In the histopathological examination, inflammatory cell (neutrophil/mast cells) infiltration was remarkably suppressed in the bladder tissue of HBO-treated mice. The mRNA expression of some IC/PBS-associated biomarkers (IL-6, IL-1b, TNF, CCL2) was inhibited by HBO compared with the vehicle-treated control. On the other hand, the mRNA expression of eNos was significantly induced by HBO compared with any other groups. The uptake of inflammatory fluorescent agent in bladder was markedly inhibited by HBO treatment compared with either the vehicle- or heparin-instillation.

**Figure1: Voiding frequency and voiding volume on day8**



**Figure 2 : RT-PCR Log2 IL-6 Log2 eNos**

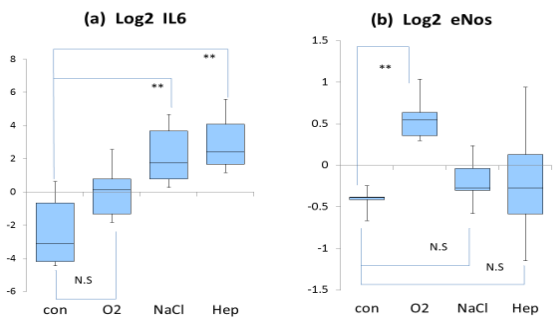
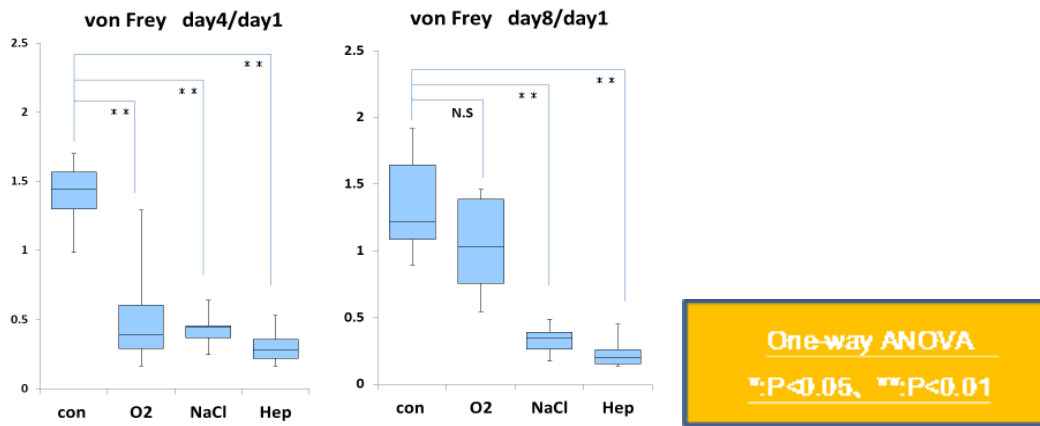


Figure 3: von Frey(g) ratio day4/day1 von Frey(g) day8/day1



#### Interpretation of results

The treatment with HBO can induce the early recovery of bladder capacity and pain, concomitant with the simultaneous biological changes in the IC/PBS-mimic mouse bladder. The experimental data from this study will secure the possibility of improvement of IC/PBS patients' symptoms on the basis of these evidences.

#### Concluding message

The HBO treatment may be a powerful option for IC/PBS through the enhanced repairment of bladder tissue with severe chronic inflammation.

#### References

1. Tanaka T et al. Hyperbaric oxygen therapy for painful bladder syndrome/interstitial cystitis resistant to conventional treatments: long-term results of a case series in Japan. BMC Urol. 2011;24;11:11-15.
2. Tanaka T et al. Hyperbaric oxygen therapy for interstitial cystitis resistant to conventional treatments. Int J Urol. 2007;14(6):563-5.
3. Homan T et al. Novel mouse model of chronic inflammatory and overactive bladder by a single intravesical injection of hydrogen peroxide. J Pharmacol Sci. 2013;121: 327-37

#### Disclosures

**Funding:** No conflict of interest **Clinical Trial:** No **Subjects:** ANIMAL **Species:** mouse **Ethics Committee:** The Institutional Animal Care and Use Committee of Osaka City University