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EFFECT OF PROLYL 4-HYDROXYLASE INHIBITOR ON THE BLADDER FIBROSIS IN THE RAT MODEL WITH PARTIAL BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study
The Prolyl 4-hydroxylase (P4H) has been reported a critical enzyme in the process of the tissue fibrosis. This study was performed to investigate P4H expression changes and its inhibitor (2,4-Diethylpyridine dicarboxylate)-mediated effect on the bladder fibrosis in the rat with partial bladder outlet obstruction (pBOO).

Study design, materials and methods
Twenty female Sprague-Dawley rats (200-250g) were divided into four groups; pBOO with P4H inhibitor 2mg/kg (group A, n=5), pBOO with P4H inhibitor 20mg/kg (group B, n=5), pBOO with normal saline (group C, n=5), and normal control (group D, n=5). After pBOO for 2 weeks in A, B, and C groups, each amount of inhibitor was administered orally once a day for 2 weeks. After total 4 weeks, the bladders in all group were removed. To evaluate the changes due to pBOO and P4H inhibitor, the Masson's trichrome stain for muscle change and the immunohistochemical stain for P4H, collagen I and III protein expression were performed in all group.

Results
The muscle thickness calculated from Masson's trichrome stain was 0.85±0.22, 1.06±0.15, 1.19±0.3, and 0.49±0.1mm in A, B, C, and D group, respectively. These results showed 1) that the muscle thickness of pBOO groups A, B, and C increased significantly than normal group D (p=0.002), and 2) that, compared with that of the group C with normal saline (p=0.07), the groups A and B treated with P4H inhibitor had substantially reduced muscle thickness. The overall P4H expression percentage was 65.7±15.2, 13.4±8.4, 73.8±15.5, and 10±10% in A, B, C, and D group, respectively. As a result of pBOO, P4H expression in groups A, B, and C was augmented compared with D group (p=0.002), and furthermore, it was down-regulated in P4H inhibitor groups, more deferentially in group B than in group C (p=0.003). The overall collagen I and III protein expression percentage were 16.9±18.0, 17.0±24.1, 30.5±13.4, 8.8±8.7% and 9.6±4.2, and 8.8±2.9, 12.5±10.6, and 7.5±3.5% in A, B, C, and D group, respectively. pBOO led to the increasing collagen I and III protein expression compared with D group (p=0.05 and p=0.003), and those in P4H inhibitor-treated groups marginally decreased compared with that in C group (p=0.135 and p=0.811). The ratio of collagen I/III was 1.8, 1.9, 2.4 and 1.2 in A, B, C, and D group, respectively.

Table 1. Data summary of muscle thickness, P4H, collagen I and III

	Group A	Group B	Group C	Group D
Muscle thickness (mm)	0.85±0.22	1.06±0.15	1.19±0.30*	0.49±0.10†
P4H expression (%)	65.7±15.2	13.4±8.4‡‡	73.8±15.5‡	10.0±10.0§
Collagen I expression (%)	16.9±18.0	17.0±24.1	30.5±13.4//	8.8±8.7¶
Collagen III expression (%)	9.6±4.2	8.8±2.9	12.5±10.6**	7.5±3.5††
Collagen I/III ratio	1.8	1.9	2.4	1.2

P4H: prolyl 4-hydroxylase, Group A: partial bladder outlet obstruction (pBOO) with P4H inhibitor 2mg/kg, Group B: pBOO with P4H inhibitor 20mg/kg, Group C: pBOO with normal saline, Group D: normal control.
*p=0.07, ‡ p=0.003, // p=0.135, **p=0.811 as compared to the group A and B.
† p=0.002, §p=0.002, ¶p=0.05, ††p=0.815 as compared to the group A, B and C.
‡ ‡ p=0.003 as compared to the group A and B.

Interpretation of results
Our data suggest that P4H inhibitor decrease the muscle thickness and the expression of P4H and collagen I and III protein in the rat model with pBOO.

Concluding message
The P4H inhibitor may be potentially utilized to reduce the bladder fibrosis caused by pBOO.

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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**
Name of ethics committee	**PNUH IRB (Pusan National University Hospital Institutional Review Board)**