

## COLLAGEN AND ELASTIC SYSTEM CHANGES INDUCED BY DETRUSOR OVERACTIVITY IN RABBITS. THE ACTION OF OXYBUTYNYN

### Hypothesis / aims of study

Overactive bladder (OAB) is clinically characterized by symptoms such as frequency, nocturia and urgency, with or without urinary incontinence (1). Morphological alterations in the vesical wall occur frequently in partial bladder outlet obstruction (PBOO) and include progressive hypertrophy of smooth muscle and changes in collagen and in fibers of the elastic system (2). Additionally, we have shown recently that PBOO is associated with ultrastructural cellular damage in the bladder, which can be prevented by oxybutynin treatment<sup>3</sup>. However, little is known about the changes in the composition of vesical connective tissue and smooth muscle that underly the beneficial effects of oxybutynin on detrusor hyperactivity (DH).

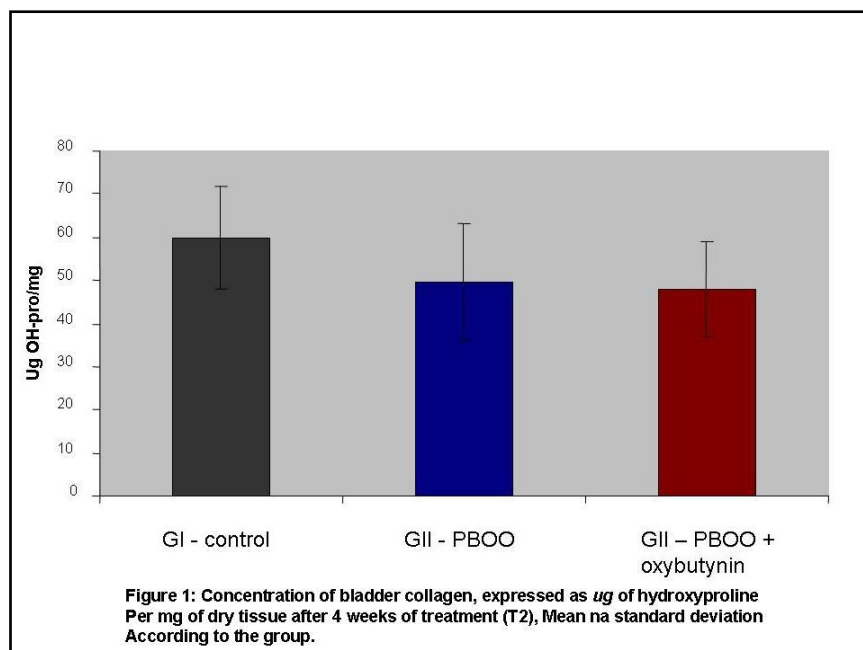
This study aims to evaluate the effects of intravesical oxybutynin on major components of bladder wall, such as smooth muscle, collagen, and elastic fibers, using morphological and biochemical methods and an experimental rabbit model of DH with PBOO.

### Study design, materials and methods

Forty-five North Folk male rabbits, weighting on average 2,207.4 g, were randomly divided into three groups: Group I (GI, n=15) was not submitted to surgical interventions or treatments and were used as controls; The experiments started at T0 with the remaining animals undergoing PBOO as previously described (3). Twenty nine animals developed DH, as verified by a cystometric study conducted one week after surgery (T1), and were randomly divided into groups 2 (GII, n=14) and 3 (GIII, n=15). In GIII, the animals were treated with an intravesical instillation of liquid oxybutynin (0.5 mg/kg body weight, daily for 4 weeks. Serum creatinine, urine culture, and cystometric study were performed on all animals one (T1) and five (T2) weeks after surgery, when the animals were sacrificed. Diameter of smooth muscle fibers was measured on HE-stained bladder sections and is given in  $\mu\text{m}$ . Sections were also stained by Picro-Sirius Red technique for evidencing collagen and by Weigert's Fuchsin-Resorcin for characterizing the elastic system fibers. Morphometric analyses were carried out using the programs Pro- Image Plus and Image J. The concentration of total collagen in the bladder wall was determined by a hydroxyproline assay and was expressed as  $\mu\text{g}$  hydroxyproline per mg dry, defatted tissue.

### Results

At T2, bladder weight was significantly higher in animals from GII and GIII as compared to GI ( $9.61 \pm 5.36$  and  $6.00 \pm 2.18$  vs  $2.35 \pm 0.55$ , respectively,  $p < 0.05$ ). In the obstructed animals which received intravesical oxybutynin, a significantly lower bladder weight was noted when compared to those that did not receive the drug ( $p < 0.05$ ). Creatinine levels remained normal throughout. Cystometric study revealed that vesical capacity was significantly higher in GII at T2 compared to T1 ( $35.13 \pm 13.60$  vs  $54.53 \pm 22.77$ ,  $p < 0.05$ ), but no statistical difference was observed between GI and GIII groups. Maximum detrusor pressure showed no statistical difference between T1 and T2 for all groups. Bladder compliance was significantly higher in animals using intravesical oxybutynin at T2 as compared to T1 ( $p < 0.05$ ). There was no statistical difference in groups GI and GII in different moments. At T2, detrusor involuntary contraction (DIC) was detected in all animals of GII, in 47% of GIII, and in none of GI. GII animals presented significantly more DIC as compared to GI and GIII ( $p < 0.05$ ). Smooth muscle fibers were significantly thicker in the obstructed group (GII) with regards to GI and GIII ( $180.2 \pm 33.2 \mu\text{m}$  vs.  $126.8 \mu\text{m} \pm 27.3$  and  $133.1 \mu\text{m} \pm 41.2$ ,  $p < 0.05$ ). There was no statistically significant difference between the GI and GIII groups. Collagen volumetric density was significantly higher in GI animals with relation to groups GII and GIII ( $461,807.1 \pm 118,695.3 \mu\text{m}^2$  vs.  $269,637.9 \mu\text{m}^2 \pm 147,046.2$  and  $292,879.7 \mu\text{m}^2 \pm 106,548.9$ ,  $p < 0.05$ ). There was no statistically significant difference between the GII and GIII groups.



The volumetric density of elastic fibers was significantly higher in GII and GIII as compared to control group. Collagen concentration in GIII was not different from that of GII ( $48.00 \pm 11.16$  vs  $49.56 \pm 13.63$ ). However, comparing these two groups against G1 ( $59.93 \pm 11.84$ ) showed that PBOO caused a ~18% decrease in vesical collagen content ( $p < 0.05$ ) (Fig. 1).

#### Interpretation of results

Bladder weight was significantly higher in the animals of groups GII and GIII with regards to G1. However, we observed a significantly lower weight in the group treated with intravesical oxybutynin (GIII) compared to the untreated group (GII). This implies that intravesical oxybutynin has a possible protective effect, in view of the fact that IDC also disappeared in 53% of the animals in GIII. After 4 weeks of treatment with intravesical oxybutynin (T2) involuntary detrusor contractions disappeared in 53% of the animals in GIII, demonstrating the effectiveness of intravesical oxybutynin. At T2, all the animals retained IDC in GII, thus suggesting that this is a good experimental model of DH.

In the histomorphometric assessment, it was observed that thickness of smooth muscle cells increased by approximately 42%, comparing the animals of GII to G1. In GIII, the thickness was not different from the control group, suggesting a good response to the treatment with intravesical oxybutynin. In our experimental setting, using rabbits with partial bladder outlet obstruction and DH without bladder compliance change, a collagen concentration decrease and an increase of the elastic system fibers was observed after 4 weeks in the obstructed groups. In the linear association between variables, we observed a negative correlation between the collagen and elastic fibers, demonstrating that the increase of elastic fibers was related to the reduction of collagen. These changes are probably due to adaptation of the bladder to the obstruction process, in which elastic fibers play a role by imparting resistance to distension forces.

There was a functional bladder improvement in the GIII group after using intravesical oxybutynin. Accordingly, smooth muscle cell thickness decreased when compared to that of the group not treated with the drug, which may explain the disappearance of involuntary detrusor contractions.

Further studies are needed for a better understanding of the quantitative changes in the extracellular matrix during voiding dysfunction due to obstructive bladder dysfunction, as well as their regulatory mechanisms. The use of drugs that block this progressive process may enable reversal or even interruption of the deterioration of the structural and functional properties of the bladder wall.

#### Concluding message

These results show that intravesical oxybutynin protects the bladder against functional and structural changes of OAB associated to PBOO, and also prevents alterations in collagen and elastic fibers. Future studies should assess the effect of intravesical oxybutynin in bladders with low compliance and longer obstruction time.

#### References

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