Diploma Thesis The “point of no return” in bladder dysfunction: When macrophages infiltrate

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1. Abstract

Bladder outlet obstruction (BOO) is a highly prevalent disease and occurs particularly often in elderly men due to benign prostatic hyperplasia (BPH), when the growing prostate progressively occludes the urethra and interferes with urine outflow. When left untreated, the obstruction progress from bladder inflammation to hypertrophy to fibrosis and ultimately leads to bladder failure. In addition to examining bladder biopsies of the BOO patients, bladder tissue from two different mouse studies is assessed: One mouse model is used to study bladder function after surgical induction of partial BOO (pBOO) and the other to measure the activity of the external urethral sphincter (EUS) by electromyography (EMG) during micturition in awake and anaesthetized mice. Collected bladder tissue is cryosectioned, stained with Masson’s Trichrome for a collagen quantification and with different immunohistochemical markers to examine the presence and morphology of specific cell types. The bladder-to body- weight ratio was significantly increased in the group of 7 weeks post pBOO, the collagen quantification showed no significance between the test groups in both mouse models. This suggests that the duration of the obstruction was not long enough to induce the significant fibrotic changes in the bladder wall. The catheter and EUS-EMG electrode implantation seem not to induce morphological changes of the bladder tissue. Due to the SARS-CoV2 outbreak no IHC staining for the macrophage detection on mouse bladders could be done. Another mouse experiment will be started where the changes in the bladder following spinal cord injury will be investigated and compared to the findings in this thesis.

2. Introduction

The bladder is a hollow organ, which can histologically be seperated in 3 layers: The innermost layer, called Mucosa is lined by a multi-layer urothelium. The Tunica muscularis is built from smooth muscle cells, and the mucle they form is called Musculus detrusor vesicae (Detrusor). The outermost layer called Tunica adventitia, consists of loose connective tissue and a layer of fat. [1]

Bladder outlet obstruciton (BOO) is a narrowing of the urethra directly underneath the bladder. This slows down or even prevents the urinary flow. The most common reason is a benign prostate hyperplasie (BPH) in elderly men, which may lead to a benign prostatic obstruction (BPO), a form of BOO. [2] The patients often suffer from lower urinary tract symptoms (LUTS). Therfore the term BOO needs a confirmation by urodynymic investigation. [3] Typical urodynamic findings in BOO patients are decreased bladder compliance, detrusor overactivity (DO) or underactivity (UA) and changes in sensitivity. The chronical increased pressure in the bladder due to the obstruction leads to functional as well as structural changes in the bladder wall, which results in bladder hypertrophy and leads to fibrosis. The thickening of the bladder wall correlates with the severity of the obstruction. [2] an often used therapy method is a surgical relief of the obstruction through porstatectomy. The surgery often brings symptomatic and fuctional impariment or even bladder failure after surgery. As BOO progresses fast from inflammation to hypertrophy and to fibrosis, it is very important to identify structural changes as early as possible, in order no surpass the “point of no return”. [4]

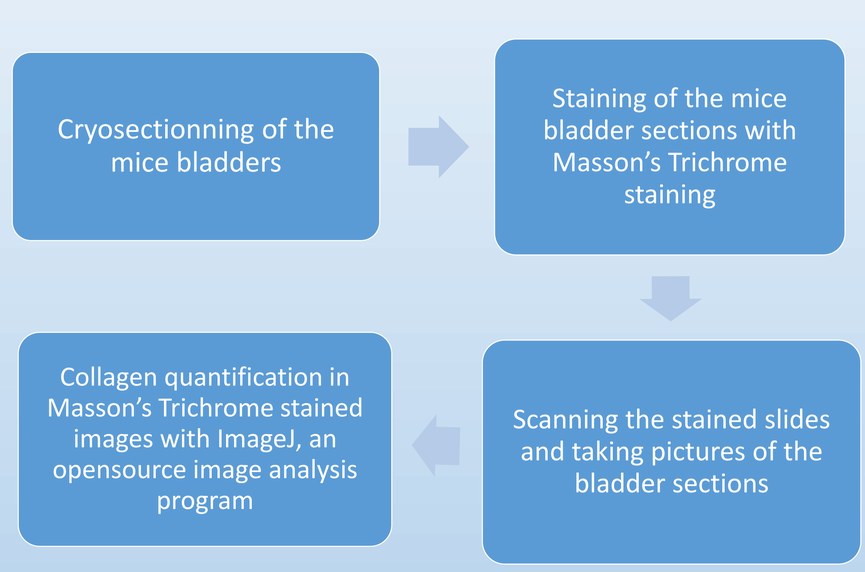
3. Aims/ Leading Question

Due to gene expression changes, a loss of contractility was suggested during PBO-induced bladder dysfunction. Therefore, we aim to find a link between bladder contractility, determined by urodynamic examination and the bladder fibrosis.

1. How much fibrosis occurs in the bladder of mice with experimentally induced pBOO within 1 week and 7 weeks?
2. Which morphological characteristics do pBOO mouse bladders and bladders of mice undergoing electrode implantation next to the external urethral sphincter (EUS) have? What are the differences?

4. Material and Methods

Visualized workflow of all steps and methods of the experiment:



5. Results

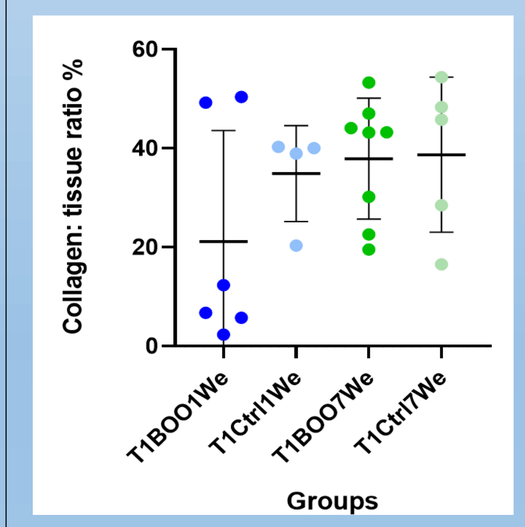


Fig. 5.1 Ratio of collagen to total bladder tissue. Displayed are group mean and standard deviation, including the value of each animal represented as dots.
P value < 0.05

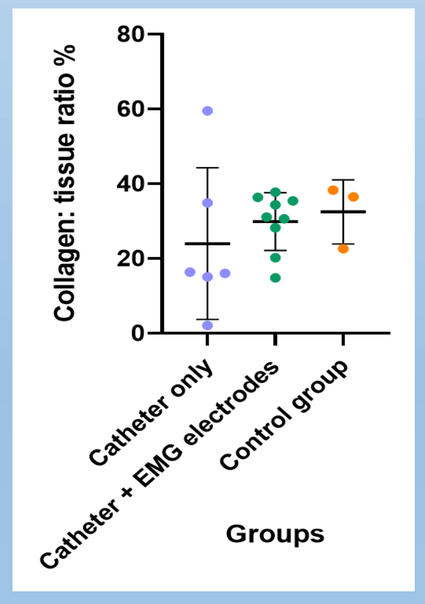


Fig. 5.2 Ratio of collagen to total stained section of each group. Displayed are group mean and standard deviation, including the value of each animal represented as dots. P value < 0.05

Figure 5.1 shows the ratio of collagen to the total stained bladder section of pBOO and control the group. Although no significant difference was detected, the mean of the mice with one week pBOO is lower than the means of every other group indicating relative increase of non-collagenous tissue, including smooth muscle, early during obstruction.

Figure 5.2 shows the ratio of collagen to the total stained section of catheter and/or EUS-EMG and the control group. All group mean values are close together, whereas the catheter only group shows the lowest ratio. No significant differences were detected.

6. Discussion

1. We observed no differences in relative collagen content of the bladders between the four groups of the pBOO study. 1 week of pBOO is probably not enough time for the fibrotic bladder remodelling to happen. This might be the reason no change in fibrosis was found between the 1 week pBOO and its control group. 7 weeks after pBOO, we found no significant increase in collagen deposition in the bladder tissue of pBOO mice. Collagen deposition upon pBOO is controversial among different studies, since some report increase, whereas others describe no change in collagen content.

The tightness of surgically induced obstruction is a limitation of the pBOO mouse model, as it is hard to control the tightness of the stitch around the urethra. Thus, comparison between studies needs caution since the degree of obstruction may vary. Moreover, there is no standard method to assess fibrosis.
Many studies use the manual grading of fibrosis and others use a similar approach like the here applied method using computer-based image processing. These differences in evaluation can lead to different results.

2. We did not see any statistical differences between the groups of the EUS-EMG study. We showed that the implantation of electrodes next to the EUS, in this short amount of time (3 weeks) does not affect the bladder morphology. Moreover, the implantation of a catheter into the bladder seems not to induce morphological changes of the bladder tissue, as no differences were seen compared to the healthy (non-operated) controls.

References
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Figures
Fig. 5.1 Ratio of collagen to total bladder tissue within the ROI. Displayed are group mean and standard deviation, including the value of each animal represented as dots.
P value < 0.05, own figure
Fig. 5.2 Ratio of collagen to total stained section of the ROI of each group. Displayed are group mean and standard deviation, including the value of each animal represented as dots. P value < 0.05, own figure