

INFLAMMATORY RESPONSE AND URINARY HYALURONIC ACID MEASUREMENTS IN A SUB ACUTE RAT MODEL OF PROTAMINE SULFATE INDUCED CYSTITIS

Hypothesis / aims of study

The urinary bladder is normally impermeable to hostile environmental factors and toxic urinary wastes. This protection is provided by the integrity of the urothelium with the surface glycosaminoglycans (GAG) and the tight junctions. Impaired barrier function of bladder epithelium and subsequent infiltration of urine contents are the proposed initial events in the pathophysiology of interstitial cystitis. Protamine sulfate (PS) is a polycation quaternary amine which neutralizes the electronegative surface polysaccharide and leads to increased urothelium permeability. The aim of this study is to examine the evolution of inflammation on bladder injury following intravesical PS exposure and its relationship with urinary hyaluronic acid (HA), a GAG layer component, to provide a reliable experimental model of non-bacterial cystitis.

Study design, materials and methods

Cystitis was induced by transurethrally catheterizing adult female Wistar rats and instilling 200 µL of PS (10 mg). Controls were instilled with 200 µL of saline. After 30 minutes, bladders were drained and washed with 200 µL of saline. The rats were housed 6 hours before the sacrifice in a metabolic cage in order to collect urine. The rats were sacrificed 1 to 7 days after the PS instillation (7 groups of cystitis, 7 rats per day and 7 groups of controls, 5 rats per day) and their bladders were removed for histopathological analysis. Edema, congestion and inflammatory infiltrate were graded from 0 to 3, namely none, mild, moderate and severe, respectively. The urine was frozen for further HA measurement by a noncompetitive fluorescence-based assay [1]. The HA dosage was corrected by urinary creatinine.

Results

PS caused mild/moderate inflammation in all the 7 groups (day 1 to day 7), with different cell types according to the follow-up time. There was a predominance of polymorphonuclear neutrophil (PMN) infiltrate on the 3 first days and a raise in lymphomononuclear-plasmocitic (LMN) infiltrate by the 4th day. Mast cells were seen in all groups and were more prevalent on 4th to 6th day (Fig. 1). There were no differences in interstitial edema, congestion and mast cell occurrence between PS and controls. In PS groups, urinary HA level was maximum in the first day and decreased thereafter, although there was not statistic significance. HA level was higher in PS rats compared to saline controls on days 2, 3 and 4 ($p < 0, 05$) (Fig. 2).

Interpretation of results

Intravesical instillation of PS caused bladder inflammation and we could observe a sequential inflammatory infiltrate evolution through the days, from PMN to LMN. The urinary HA level was higher on the first days what probably correlates to inactivation of native bladder polysaccharides and increased epithelial permeability at this period.

Concluding message

This seems to be a reliable animal model for non bacterial cystitis. The 7 days observation leads to a better comprehension of the inflammatory response evolution caused by PS intravesical injection. The HA measurement provides more information about the cytodestruction of bladder epithelium and allows better differentiation between disease and control groups. This model could be useful for pathophysiology and treatment studies in bladder diseases in which a defective urothelium and/or GAG layer are a major suspected etiological factor such as interstitial cystitis.

[1] Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. *Analytical Biochemistry* 319 (2003) 65-72.

Figure 1. Inflammatory response after intravesical PS instillation

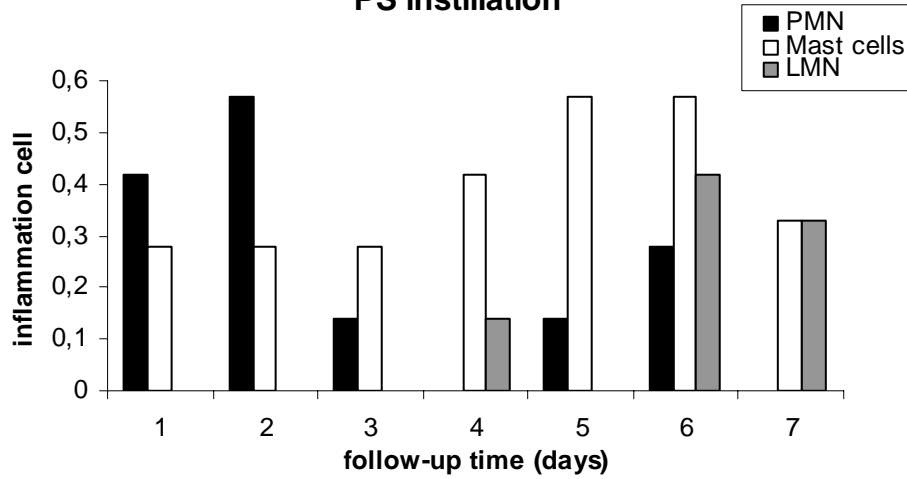


Figure 2. Urinary HA level according to follow-up time

