Unravelling the time course of acute cyclophosphamide-induced rat bladder inflammation

Acute cyclophosphamide-induced rat bladder inflammation rapidly leads to tissue damage and functional changes. Within four days, the function is restored, despite persistent inflammation.

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Background & Aim

Chemotherapy with cyclophosphamide (CYP) can give rise to hemorrhagic cystitis in humans. This knowledge was used to establish animal models for cyclophosphamide-induced cystitis to study bladder inflammation, functional changes and effects of interventions. In an effort to improve methodological rationale, this study aimed to investigate the correlation of bladder inflammation and bladder overactivity over time in a rat model of acute cyclophosphamide-induced cystitis.

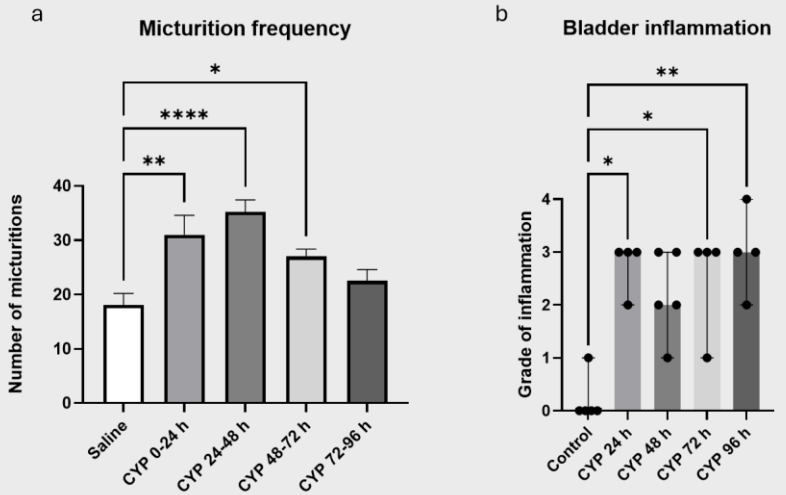

Figure 1. Micturition frequency and grade of inflammtion. (a) the number of micturitions over a 16 h period in saline-treated controls (n=12), as compared to animals with bladder inflammation (CYP) at different time points after injection with cyclophosphamide (n=6 in each group). (b) the grade of bladder inflammation for saline-treated controls (n = 5), compared to animals that were intraperitoneally injected with cyclophosphamide (CYP; n = 4-5 in each group). The time (24-96 h) indicates time after cyclophosphamide injection. Statistical comparisons were made by (a) one-way ANOVA or (b) non-parametric Kruskal-Wallis test. * p<0.05, ** p<0.01, **** p<0.0001

Study design

Thirty-six male Sprague-Dawley rats were included in the study. Each rat received a single intraperitoneal injection with either saline (n=12), serving as control, or cyclophosphamide (n=24; 100 mg/kg). After 0, 24, 48 or 72 hours the animals were placed in a metabolic cage for 16h, measuring micturition parameters, water consumption and urine production. Subsequently, the animals were euthanized and their bladders were excised and used for immunohistochemical evaluation of inflammation (H&E stain and inflammatory markers) and expression of functional receptors.

Results

Induction of bladder inflammation resulted in a transient significant increase of micturition frequency (Fig 1a). The H&E staining showed signs of bladder inflammation throughout the studied time period (0-96 h; Fig 1b). Expression of the inflammatory marker IL-6 gradually increased after cyclophosphamide injection (Fig 2a). The expression of antioxidant enzymes was either unaltered (glutathione peroxidase; Fig 2b) or significantly decreased (superoxide dismutase, SOD-1; Fig 2c). The expression of functional receptors (muscarinic M3, β3 & α1 adrenoceptor, P2X3 purinoceptor) was unaltered at all time points (data not shown). Previous studies in animal models of cyclophosphamide-induced cystitis have occasionally demonstrated alterations in expression of functional receptors. In this study, we could not detect such changes.

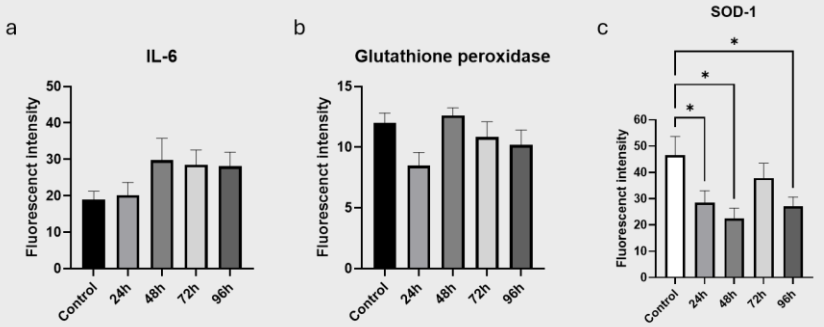

Figure 2. Expression of inflammation-associated proteins. The graph displays the expression of proteins often used as indicators of inflammation or oxidative stress for saline-treated controls (n = 7-8), compared to animals that were intraperitoneally injected with cyclophosphamide (CYP; n = 5-6 in each group). The time (24-96 h) indicates time after cyclophosphamide injection. * p<0.05

Conclusion

The current study describes how induction of rat bladder inflammation with cyclophosphamide correlates with functional changes. Tissue damage and invasion of inflammatory cells occur rapidly, leading to bladder overactivity. As normal bladder function returns, the inflammation still persists, albeit in a later-stage form.