A Histological Analysis of Bladder Structure and DNA Damage Across EAE Severity Grades in Mice.

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1. Summary

Multiple sclerosis (MS) is frequently associated with lower urinary tract dysfunction (LUTD), but its effects on the bladder remain poorly understood. This study investigated bladder changes in the EAE mouse model of MS. Increasing EAE severity was associated with greater collagen deposition and DNA damage (γH2AX), while PAR-associated DNA repair activity showed no significant differences. Human MS tissue showed similar trends. Overall, the findings suggest that MS-related neuroinflammation may contribute to structural and molecular changes in the bladder.

2. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system characterized by inflammation and damage to myelin and axons. Around 80% of people with MS develop lower urinary tract dysfunction (LUTD), including urgency, incontinence, retention and incomplete bladder emptying. [1,2]

Although LUTD is highly prevalent in MS, structural and molecular changes within the bladder wall remain poorly understood. Previous research has demonstrated DNA damage and bladder remodelling following spinal cord injury, [6] raising the question of whether MS-associated neuroinflammation may affect the bladder through similar mechanisms.

Experimental autoimmune encephalomyelitis (EAE) is a widely used mouse model of MS and allows disease progression to be classified according to neurological severity. [3] In this study, γH2AX was used as a marker of DNA double-strand-break signalling, while PAR was used to assess PARP-associated DNA-repair activity. [4,5]

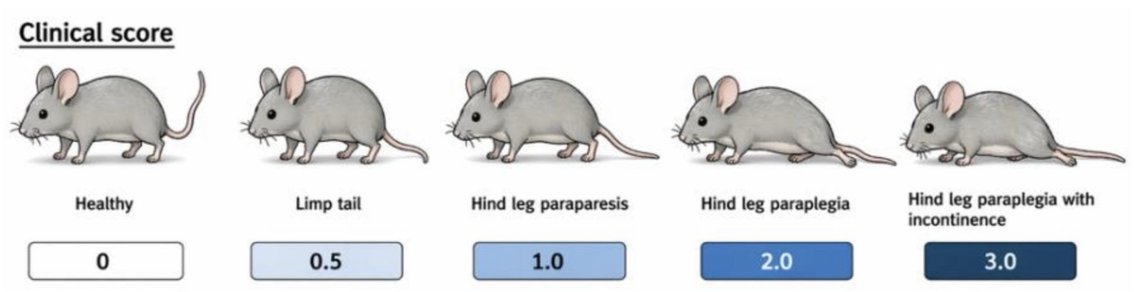

Fig. 1: EAE Score Sheet

3. Aims and Leading Questions

The primary aim was to investigate whether DNA damage and DNA-repair pathways in the bladder are associated with increasing EAE severity

- Does neuroinflammation induce DNA damage in bladder cells?
- Does DNA damage increase with higher EAE scores?
- Are DNA-repair pathways, such as PARP-associated activity, activated in response to DNA damage?

A secondary aim was to assess structural remodelling of the bladder wall, including fibrosis, collagen deposition and tissue morphology.

- Does EAE lead to fibrosis or extracellular matrix remodelling in the bladder?
- Is bladder wall architecture altered with increasing disease severity?

Due to its relevance to the main research question, the primary aim was the main focus of this study.

Figures

4. Material and Method

Bladder tissue from healthy control mice and EAE mice with clinical scores of 0, 0.5, 1 and 2 was analysed. Human bladder tissue from one healthy control and one patient with MS was additionally examined as an exploratory qualitative comparison. Frozen bladder tissue was cryosectioned at 5 µm. H&E staining was used to assess general bladder-wall morphology, while Masson's Trichrome staining was used to evaluate collagen and muscle. [7] Indirect immunofluorescence was performed using γH2AX as a marker of DNA double-strand-break signalling and PAR as a marker of PARP-associated DNA-repair activity. [8] Images were acquired by confocal microscopy and quantified using QuPath.

5. Results

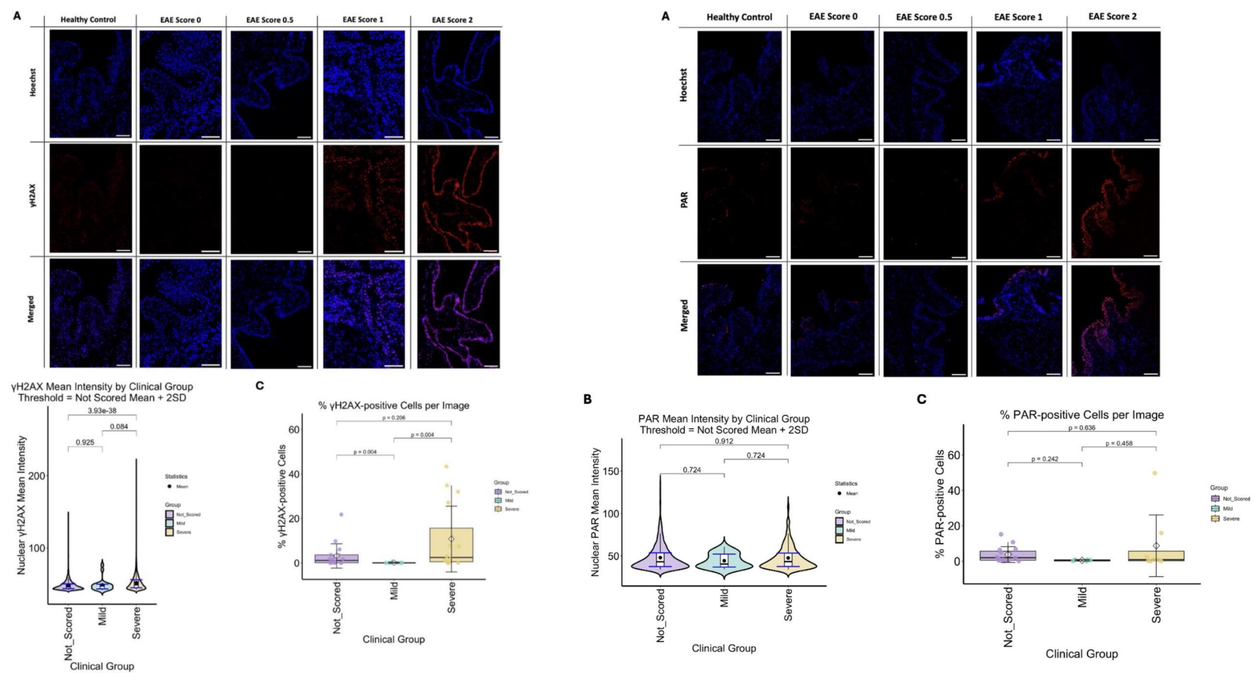

Fig. 2: γH2AX expression in bladder tissue across EAE severity groups.

Fig. 3: PAR expression in bladder tissue across EAE severity groups.

γH2AX staining became stronger with increasing EAE severity. Healthy controls and lower EAE scores showed only limited staining, whereas scores 1 and 2 showed more widespread γH2AX-positive nuclei. Quantification confirmed the highest DNA-damage signal in the severe group. PAR-positive nuclei were detected in all groups, but no significant severity-dependent differences were found in PAR intensity or the percentage of PAR-positive cells. In the exploratory human comparison, the MS bladder sample showed stronger γH2AX and PAR staining than the healthy control.

6. Discussion

The increase in γH2AX with higher EAE scores suggests that DNA damage in the bladder becomes more pronounced as disease severity progresses. This supports the idea that neuroinflammation may affect peripheral organs such as the bladder. Although PAR-associated repair activity was present in all groups, an increase with disease severity could not be confirmed. Future studies should include larger cohorts, more advanced EAE stages, additional DNA-damage and repair markers, functional bladder assessments, and a larger number of human control and MS samples.

References

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