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STRUCTURAL CHANGES IN THE BLADDER WALL AS A RESULT OF AGEING: A COMPARISON BETWEEN RATS AND MICE.

Hypothesis / aims of study

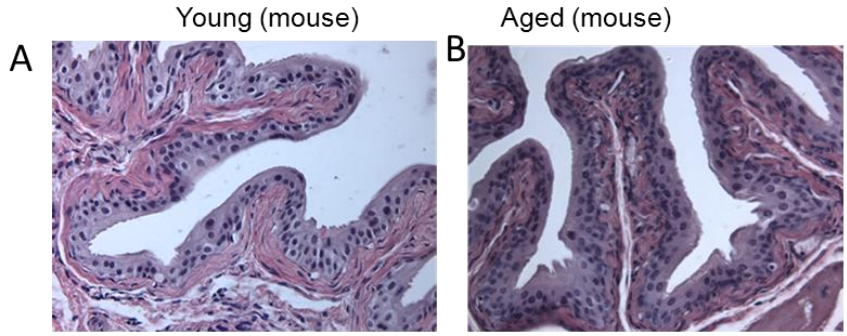
Overactive bladder syndrome (OABS) is a debilitating condition which poses serious adverse effects on quality of life. OAB is characterised by the symptoms of urgency, frequency and nocturia and in some patients is associated with urinary incontinence. Although OAB and urinary incontinence are not exclusively diseases of ageing, both have increased prevalence in the elderly, despite this relatively little is known about how the structure and function of the bladder changes with age. Previous histological studies in rodent and human bladder have produced contradictory reports with some investigators finding an age-dependent fall in contractile ability, detrusor muscle thinning and collagen deposition and other investigators seeing no functional change and increased muscle mass (1,2), moreover recent studies from our laboratory suggest that sensory nerve firing is increased in aged mice (24 months old) compared to young mice (3 months old) and that urothelial cells have altered responses to chemical and mechanical stimuli (i.e. bladder filling). The aim of this study was to examine how urothelial structure changes as a result of age in both rats and mice (3).

Study design, materials and methods

This study used young (3-5 months old) and aged (24 months old) male mice (n=5 and 6 respectively) and young (12 month old) and aged (24-28 months old) female rats. Structural features were examined by H&E staining. Mast cells were identified using a 0.1% toluidine blue stain and quantified using a light microscope. To assess urothelial integrity, western blot was performed. For these experiments the whole bladder mucosa (containing the urothelium, sub urothelium, lamina propria and basement membrane) was dissected from the detrusor muscle under a microscope and lysed in RIPA buffer. Protein levels were quantified using a Bradford assay (BioRad). 20 µg of protein were separated on an 8-10% SDS-PAGE gel and transferred to nitrocellulose membranes (Protran). After blocking the membranes were incubated with rabbit polyclonal anti-ZO1 (1:1000, Abcam), connexin 43 (1:1000, Abcam), rabbit monoclonal anti-heparanase (1:1000, Abcam), or anti-β-actin (1:1000, Abcam) at 4 °C overnight. Membranes were washed and incubated with HRP-conjugated secondary antibody. Protein bands were visualized using the western bright detection system (Avansta) and protein expression levels were evaluated by densitometry (Image J), and normalised to β-actin.

Results

In both aged rats and aged mice, bladder morphology and structure were unchanged (fig 1A B &C). To examine urothelial integrity we investigated expression of the tight junction protein ZO-1, the gap junction protein connexin-43 and heparanase (an enzyme responsible for cleavage of the GAG layer) using western blot. We identified some small changes in ZO-1 and connexin-43 expression but this did not reach significance. Interestingly, heparanase expression was not detectable in the young mouse urothelium but was clearly present in the aged urothelium indicating a significant increase in the level of expression. In the rat, heparanase was detected in both young and aged samples but its expression was also moderately increased by ageing (figure 1C). Toluidine blue staining indicated significantly more mast cells in both aged rats and aged mice compared to their respective young controls (figure 1D and E).



C

Marker	Rat	Mouse
ZO-1	↓ -0.26 fold	↑ 1.18 fold
Heparanase	↑ 1.41 fold	↑↑
Connexin 43	↑ 1.22 fold	↔ 1.065 fold
Mast cells	↑ 1.59 fold	↑ 2.05 fold**
Histological structure	Normal	Normal

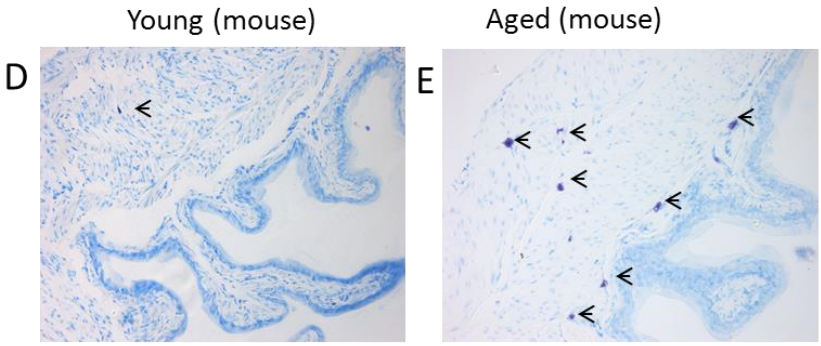


Figure 1: Age associated changes in the mouse and rat bladder
A) H&E staining in young mouse bladder. B) H&E staining in aged mouse bladder. C)Table summarising changes in mouse and rat urothelial expression as a result of ageing (** indicates statistical significance at $P<0.05$, Students T test). D) Toluidine staining in young mouse bladder, E) Toluidine staining in aged mouse bladder. Arrows indicate mast cell staining (20X objective)

Interpretation of results

Histological analysis found no obvious difference in bladder wall structure between aged and young rats and mice; however our western blot analysis revealed some subtle changes in urothelial junctional integrity associated with ageing. Whether these subtle changes are sufficient to alter urothelial function and cell-cell communication in the bladder wall remains to be determined. Of all of the parameters tested, the greatest change observed was an increase in mast cell number in aged animals compared to the younger controls (in both rats and mice). This may suggest an altered immune regulation or activation of the immune system as a result of ageing, however further studies are still required to fully identify the underlying mechanisms involved.

Concluding message

In these early observations we show similar subtle changes in urothelial expression and increased mast cell number between rats and mice as a result of ageing.

References

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Disclosures

Funding: LN was funded by a Marie Curie ITN **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat and Mouse **Ethics Committee:** Univeristy of Pittsburgh animal care and use committee (IACUC) University of Sheffield animla care and ethics committee