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REGENERATIVE CAPACITIES OF THE URINARY BLADDER DECLINE WITH AGE

Hypothesis / aims of study

Although models of organ regeneration in lower vertebrates (ie. Salamanders) have been used to identify important aspects of *de novo* regeneration, there are not as many opportunities in adult mammals. To this end, we have previously reported on a rodent model of bladder regeneration, whereupon removal of ~70% of the bladder (subtotal cystectomy; STC) induces a regenerative response in 12 week old female rats [1]. In this report, we presented that by 8 weeks post-STC normal volume and function are restored, despite reduced responses to muscarinic stimulation *in vitro*. It is common knowledge that healing capabilities are lost with age [2,3]. As such, we have begun to examine the impact of age on the remodeling/regenerative response of the bladder after STC in 12 month old females.

Study design, materials and methods

12 month or 12 week old Fisher F344 female rats underwent trigone-sparing STC with functional characterization performed at certain timepoints post-STC. *In vivo* filling cystometry studies were performed on conscious, freely-moving animals at 2, 4, and 8, and 26 wks post-STC. After euthanasia, some tissue was subjected to pharmacological analysis via muscarinic stimulation with carbachol. Cumulative dose response curves were generated in order to analyze maximal contractility (E_{max}), Hill Slope and EC_{50}. The remaining tissue was subjected to histological analysis. Routine H&E was performed on excised bladders and kidneys. Additionally, immunohistochemistry was performed to examine immune response (myeloperoxidase for neutrophils, and CD68 for macrophages) and smooth muscle maturation (Actin, Desmin, myosin).

Results

Cystometry revealed that bladder capacity 8 weeks post-STC was 0.54 +/-0.06mL, or 60% of age-matched controls (0.9+/-0.05mL). Pressures generated in bladders 8 weeks post-STC also remained low (33.28 +/- 4.46 cmH2O) compared to controls (56.72 +/- 4.75cmH2O). Maximal steady state contractions in response to muscarinic stimulation (ie. Carbachol) were drastically reduced after STC in older animals, and showed no improvement from 2 weeks post-STC (124.6 ± 8.84 g/g tissue) to 8 weeks (74.83 ± 5.38 g/g tissue) post-STC. Excised kidneys from older animals 8 weeks post-STC were significantly larger (1.10 +/-0.03g) than age-matched controls (0.99+/- 0.02g). Corroborating this finding, H&E staining of excised kidneys showed renal damage consistent with hydronephrosis. Immunostaining revealed an increased monocyte infiltration (neutrophils/macrophages) 2 weeks post-STC in older animals, which has not been seen in 12 week old animals. Systematic analysis of phenotypic muscle marker expression revealed time-dependent expression, with early smooth muscle actin expression, desmin expression by 4 weeks post-STC, and myosin heavy chain expression (as well as the other markers) by 8 weeks post-STC. *In vivo* filling cystometry showed that while residual volume remained low, bladder capacity was also still drastically reduced (0.47 ± 0.06 mL). Additionally, although complete recovery of contractile responses of bladder strips was not obtained in either age group, responses from older animals post-STC were significantly lower than those from young animals (Fig 1.)

Interpretation of results

These data suggest that bladder regeneration in older (12 month old) animals following STC is incomplete. Although older animals subjected to STC do remain continent, there are significant differences volume, contractility, and kidney morphology. Preliminary studies point to an increased inflammatory response, and/or a delayed maturation of bladder parenchyma (ie. detrusor smooth muscle) in older animals. Although there is an incomplete regenerative response in older animals, continence is achieved and appears durable, for at least up to 6 months post-STC. However, STC performed in older animals appeared to have serious adverse effects on kidney morphology, potentially due to hydronephrosis.

Concluding message

These studies demonstrate an age related decline in regenerative capacity characterized by significant reductions in bladder capacity, detrusor contractility and kidney morphology. Such findings are in stark contrast to the rather complete regeneration observed in younger animals. As such, this model may provide a valuable tool for elucidating the basic biology of organ regeneration, as well as age-related mechanisms responsible for impaired regenerative capacity. Ultimately we hope to leverage these findings to identify novel therapeutic targets and strategies for enhanced organ/tissue regeneration.

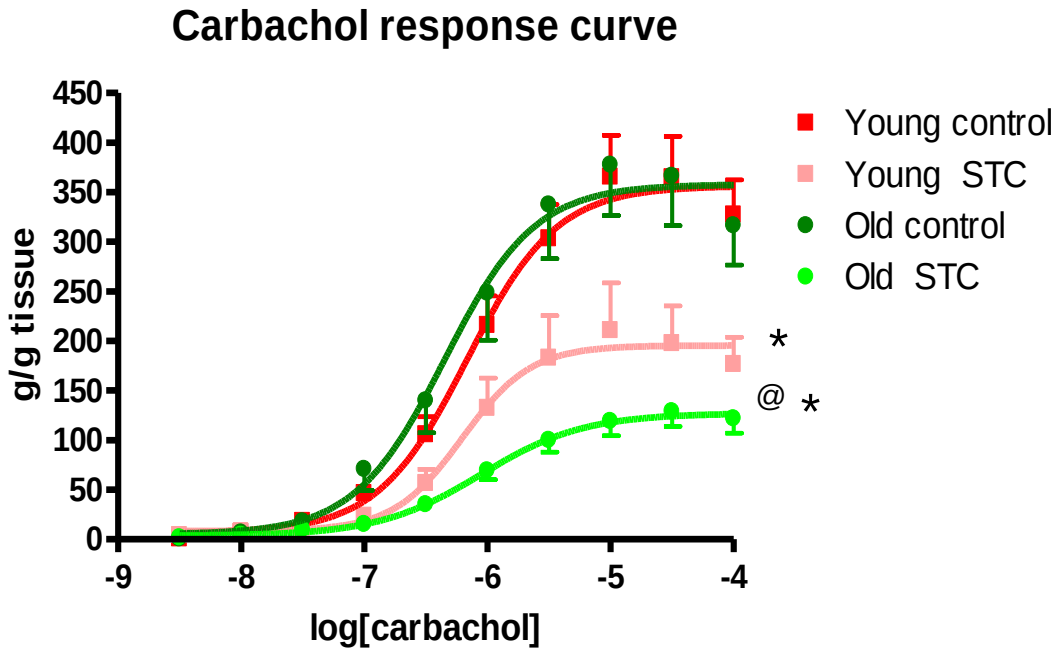

Figure 1. Generated carbachol response curves from *in vitro* contractility studies on cystectomized or age-matched control animals 6 months post-STC. While maximal contractility (E_{max}) is lower in both STC groups, E_{max} values in older animals is even further reduced compared to younger animals. *- E_{max} less than control values (P<0.001) @- E_{max} values less than young STC animals (P<0.01).

References

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