

CELL THERAPY FOR NEUROGENIC DETRUSOR OVERACTIVITY: PROOF OF CONCEPT USING INTRAMURAL IMPLANTATION OF ADRENAL MEDULLARY CHROMAFFIN CELLS FOLLOWING CHRONIC SUPRASACRAL SPINAL CORD INJURY

Hypothesis / aims of study

Suprasacral spinal cord injury is often occasioned by the development of high pressure non-voiding contractions, referred to as neurogenic detrusor overactivity. Such high pressure contractions may result in significant morbidities, such as ureteral reflux, incontinence and autonomic dysreflexia. Previous work from our laboratory has demonstrated that β_3 -adrenergic receptor agonists are remarkably effective in reducing the numbers and amplitudes of NDO in chronic spinal cord injured (SCI) rats. We hypothesized that stimulation of β -adrenergic receptors by endogenous catecholamines produced by adrenal medullary chromaffin cells (AMCC) transplanted into the bladder walls of chronic SCI rats would similarly inhibit NDO. In order to test this hypothesis, we transplanted AMCC cells into the bladder walls of chronic SCI Lewis rats and examined both their survival and ability to inhibit NDO following 1-2 weeks post implantation.

Study design, materials and methods

Female Lewis rats were selected because the degree of inbreeding in this strain eliminates the need for anti-rejection therapy and the ease of study for female animals of this species. Recipient rats underwent spinal cord transection at the T9-10 vertebral level under isoflurane anaesthesia and their bladders were manually expressed by external crede 2X daily for 4-6 weeks prior to AMCC or media only (Media) bladder wall injections.

On the day of recipient rat bladder wall injections, adrenal medullas were harvested and AMCC were isolated from 41 donors (mean \pm SEM age = 70 \pm 1 days, body weight = 180 \pm 1.4 g). The cells were spun and resuspended in 50 μ l of culture medium and injected in 4-6 sites using a Hamilton Syringe with a 28G needle for total cell loads of 10.9 \pm 1.6 \times 10⁵ cells/bladder via a midline laparotomy in 10 recipient SCI rats. Media animals received 50 μ l of culture medium only at 4-6 sites. The abdomens were closed in layers and the animals were allowed to recover for 1-2 weeks (1.2 \pm .1) prior to cystometric investigation. During the recovery period, the animals were placed in Ballman restraint cages for 3 \times 1 hour sessions over 3 days in order to acclimate them to the cages.

On the day of cystometric investigation, the animals were anesthetized with isoflurane and transvesical catheters were inserted into the apex of the bladder dome. One femoral vein was catheterized for hydration and drug delivery, and the animals were mounted in Ballman restraint cages with food and water provided *ad libitum*. The bladder catheters were connected to an infusion pump and pressure transducers via 3-way stopcock. Following a 1 hour recovery period from surgery, saline infusion at 0.1 ml/min via the bladder catheter was initiated for 1 hour as a control. The animals then received an intravenous injection of normal saline vehicle (1 ml/kg) followed 30 minutes later by administration of a cocktail of β -adrenergic receptor antagonist (0.5 mg/ml propranolol + 1 mg/ml SR-59230A). Following cystometric evaluation, the animals were euthanized and transcardially perfused first with iced PBS and then with 4% paraformaldehyde and the bladders harvested for histological evaluation.

Fixed bladders went through successive sucrose washes to 30%, and were then frozen sectioned on a cryostat at 20 μ m thicknesses and stained for tyrosine hydroxylase (TH) using Rabbit anti-TH (Millipore AB152) and Rhodamine Red-tagged Donkey Anti-Rabbit (Jackson Immuno), both at 1:100 dilutions.

Cystometric data were analysed by comparison of Vehicle vs. β -adrenergic receptor antagonist treatment periods by 2-Way RM ANOVA. Histological processing is still underway, but analysis will be based on presence vs. absence of AMCC identified as TH-labelled cell bodies (which are absent from normal bladders).

Results

Treatment with β -adrenergic receptor antagonists resulted in an \sim 2.5 fold increase in the number of NDO events in AMCC treated animals ($P < 0.0001$ by Sidak's multiple comparison test; $P < 0.0001$ for Interaction by 2-Way RM ANOVA), while no effect was seen on this parameter in Media animals. While the mean values for the AMCC animals were \sim 40% lower and higher during the vehicle control and the β -adrenergic receptor blockade periods, respectively, these differences were not significantly different by post-test). While maximum bladder contraction amplitude increased 22 and 34% for Media and AMCC treated animals, respectively, following β -adrenergic receptor blockade ($P = 0.0108$ by 2-Way RM ANOVA), this trend was not significant in the post-test analysis. Finally, in the small number of processed bladders for TH immunofluorescence thus far, numerous AMCC cells were detected up to 1 week post-implantation.

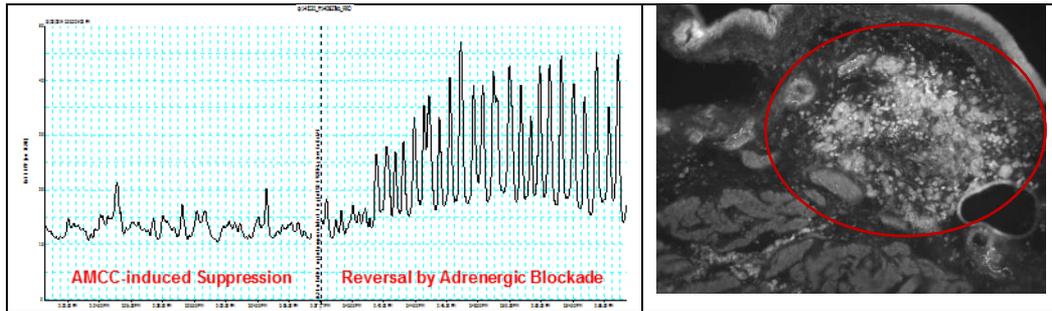


Figure: Left panel shows cystometric trace from an AMCC-treated animal before and after adrenergic blockade. Right panel shows injection site with brightly labelled AMCC one week following implantation.

Interpretation of results

These results provide proof of concept that transplanted AMCC are capable of surviving in the walls of suprasacral SCI animals and are functional, as evidenced by release of NDO inhibition following β -adrenergic receptor blockade.

Concluding message

Cell therapy using catecholamine (norepinephrine and epinephrine) producing cells holds promise for the treatment of NDO due to suprasacral SCI. AMCC may be harvested from the patients' own adrenal medullae, or autologous stem/progenitor cells may be converted into catecholamine producing cells for this purpose.

Disclosures

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Clinical Trial: No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Durham VAMC IACUC