Autologous Mesenchymal Stem Cell Transplantation in Male Stress Urinary Incontinence

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Radical prostatectomy is the most traditional treatment for prostate cancer, which affects one out of every seven men. One of the most common side effects of a radical prostatectomy is stress urinary incontinence. [1] A 2010 study states that 6 weeks after surgery 59% of men were incontinent, defined as any pad use. At 58 weeks after surgery 22% (46%) of men were incontinent. At 58 weeks incontinence was more prevalent in men who were obese and physically inactive (59% incontinent). Physical activity may offset some of the negative consequences of being obese because the prevalence of incontinence at 58 weeks was similar in the obese and active (25%) and, nonobese and inactive (24%) group. The best outcome was in men who were nonobese and physically active (16% incontinent). Men who were not obese and were active were 26% less likely to be incontinent than men who were obese and inactive (RR 0.74, 95% CI 0.52-1.06). [2]

Furthermore, almost 10% of patients experience urinary incontinence permanently. Conservative treatment for stress urinary incontinence in men consists of pelvic floor exercises. In cases where conservative treatment is unsuccessful, “urethral bulking agents, male slings, and the artificial urinary sphincter” can be used. Stem cells have proven to have regenerative effects and have been looked into as a treatment for urinary incontinence in women, by using stem cells to repopulate missing smooth muscle in the pelvic floor muscles. [3] Therefore, it is necessary to look into stem cells as a possible treatment for male urinary incontinence.

An animal trial is a necessary step in accessing how an effective and possible drug can be. Generally, it is difficult to find a mouse model that displays incontinence. Previous animal models used to test stem cell treatments for urinary incontinence involved artificially creating incontinence in mice. [3] In order to determine if a mouse is urinary incontinent, one can observe the urination pattern of a mouse model in a cage. Generally, mice urinate in one or two discriminate locations, which can be observed by placing filter paper on the bottom of the cage. When a mouse is urinary incontinent, there would be spots on the filter paper indiscriminately. [4] There is an animal model that is currently being used in a bladder cancer study associated with New York Medical College and other institutions that display the incontinent urination pattern, and have been determined to be an excellent model of urinary incontinence. The goal of the summer was to determine how to consistently produce smooth muscle cells from mouse bone marrow.

Following review of literature, Transforming Growth Factor Beta (TGF-ß) was decided to be the cytokine of choice. It has been proven that TGF-ß can cause smooth muscle cell differentiation. TGF-ß can discriminate locations, which can be observed by placing filter paper on the bottom of the cage. Following aspiration of IMDM/20% FBS from one 60 mL dish of MSCs, cells were resuspended with trypsin/EDTA. Cells were then spun at 1000 RPM, 31°C for 10 minutes, and the supernatant was removed. The remaining pellet was placed in 4 ml of IMDM/20% FBS. 2 ml of the serum mixed with cells were placed in one column (4 chambers) of a Lab-Tek chamber slide. TGFß-1/TGFß-3 was then added at a concentration of 1 ng/ml. The remaining mix was then added to the other columns in the Lab-Tek chamber slide. Allow the chamber to incubate at 4°C for 72 hours.

Bone marrow derivation.

Animal protocols were approved by the Institutional Animal Care and Use Committee at New York Medical College. Standard protocol was followed to remove the bone marrow from the mice. [5] Following collection of bone marrow, a Ficoll Paque gradient was used to separate the cells in bone marrow by density. The smooth muscle cells were collected from the gradient and resuspended in Iscoves Modified Dulbecco’s Media (IMDM) with 20% FBS at a concentration of 10-7 cells per ml of serum in petri dishes. The cells were cultured for three weeks and were fed every three days.

The following figure demonstrates the process by which TGFß can stimulate smooth muscle cell differentiation. TGFß can both directly stimulate smooth muscle cell differentiation and upregulate the expression if myocardin which assists in the differentiation of smooth muscle cells.

Mesenchymal stem cells may be useful in the treatment of post prostatectomy stress urinary incontinence by populating sphincteric scar with viable smooth muscle cells.

RESULTS

Bone marrow from 5 mice was used. The following animal study was conducted in order to determine the ideal exposure for the treatment of MSCs with TGFß-3. The methods above were followed until the treatment time for the cells. Half of the results, a second experiment was conducted in order to determine the ideal exposure for the treatment of MSCs with TGFß-3. The methods above were followed until the treatment time for the cells. Half of the chambers were treated for 24-hours while the bottom half of the chambers were treated for 48-hours. One column was then stained for calponin while the other column was stained for smooth muscle actin.

The images above were captured using a confocal microscope. The blue Dapi stained the nuclei of the cells while the green FITC stain was the MHC marker.

CONCLUSIONS

The study found that while TGFß-1 is not an optimal cytokine to consistently produce smooth muscle cells, TGFß-3 is. Additionally, it was determined that though the previous study that used TGFß-3 treated the cells for only 24-hours, it is more optimal to treat the cells for 48-hours. It was determined that in the 48-hour treatment the cells were stained for smooth muscle actin.

BIBLIOGRAPHY