The effectiveness of pirfenidone and halofuginone in the reversal of prostatic fibrosis and BPH in mice

Riley J. Medenwald[1], Teresa Liu[1, 2], Will Ricke[1, 2]

[1]University of WI – Madison, [2]Department of Urology

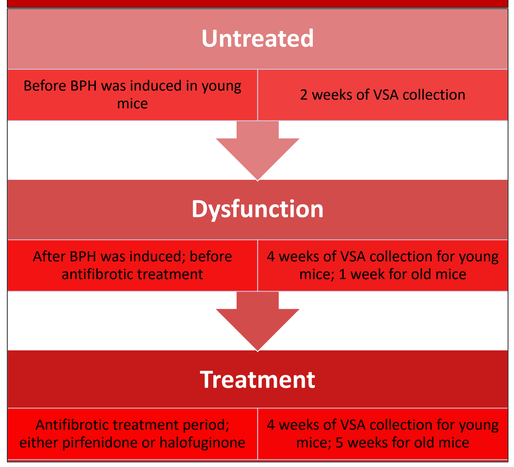
Abstract

Benign prostatic hyperplasia (BPH) is a very prominent disease among aging men and is characterized by the enlargement of the prostate, resulting in an obstructed urethra and the inability to urinate properly. Recent studies have suggested the enlargement of the prostate seen in BPH could be the result of prostatic fibrosis. In response to these claims, we decided to test the effectiveness of two antifibrotic drugs in treating BPH and resolving the obstruction of the urethra. We observed old mice with BPH and induced BPH in young mice and measured how many times they urinated per hour by collecting void spot assays (VSAs) for each mouse. After we began treatment with the antifibrotics, pirfenidone and halofuginone, we continued to collect VSAs to determine if there was a reduction in void spots. The halofuginone seemed to have a minimal effect on the void spot counts initially, while the pirfenidone caused an immediate decrease in void spots. This could mean that pirfenidone could be a more viable candidate for BPH treatment in humans and improve the lives of millions of BPH patients while potentially saving the U.S. billions of dollars in ineffective treatments.

Introduction

Benign prostatic hyperplasia (BPH) is a common condition of the prostate, characterized by prostatic enlargement as a result of the aging process (Liu *et al.*, 2019). This enlargement of the prostate causes it to press against the urethra, preventing complete and effective urination. Sequelae resulting from the development of BPH include urinary retention, urinary tract infections, bladder calculi, and renal impairment or failure (Rodriguez-Nieves & Macoska, 2013). Approximately 70% of men in their sixties and 90% of men in their eighties have developed BPH (Nicholson *et al.*, 2012) and treatment for BPH in the U.S. costs around $4 billion annually (Liu *et al.*, 2019). Recent research suggests that the buildup of collagenic fibers around the prostate, known as prostatic fibrosis, may also be a significant contributor to the enlargement of the prostate seen in BPH and by extension the blockage of the urethra associated with BPH (Bushman & Jerde, 2016). Based on these findings, we decided that it may be worth investigating whether drugs that reverse fibrosis may be effective in treating BPH and lower urinary tract dysfunction (LUTD). We tested the ability of two drugs with known antifibrotic properties, pirfenidone and halofuginone, to reverse the fibrosis found in the prostate after the development of BPH. By testing the effectiveness of these drugs on mice with both natural and hormonally-induced BPH, we were able to determine whether it would be feasible to proceed with clinical trials of halofuginone and pirfenidone to treat BPH in humans. We hypothesized that after the administration of either pirfenidone or halofuginone to mice with hormonally-induced BPH, the mice would urinate significantly fewer times per hour in comparison to the time period between BPH development and antifibrotic treatment.

Experimental Timeline



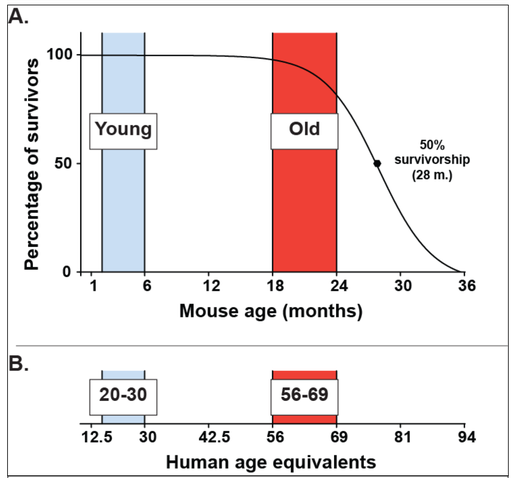


Figure 1. A. Average survival curve of C57Bl6 mice in months. **B.** Human age equivalents in years. Blue = 2-6 month old mice/20-30 year old human; Orange = 18-24 month old mice/ 56-69 year old human. Adapted from "The mouse in biomedical research" in James G. Fox (ed.), American College of Laboratory Animal Medicine series (Elsevier).

Methods

We employed a repeated measures study design, meaning the same mice were measured during each phase of treatment in order to control for different urination habits among the mice due to factors other than those associated with BPH and LUTD. The old mice that received pirfenidone treatment were given a daily dosage of approximately 3 mg. After hormone pellet implantation, the young mice that were treated with halofuginone were given 4 µg every 3 days. Once a week, the mice were placed in individual cages containing only the VSA paper (no food, water, etc.). They were each left in the cage for one hour to urinate and were then returned to their usual cages. After the papers were dried, they were imaged using a Bio-Rad ChemiDoc Imager with Trans-UV light. The images were manually color-corrected using Adobe Photoshop. Color corrected images were cropped using ImageJ, and were analyzed using the VoidWhizzard ImageJ plugin, which counts the void spots in each image. After the data for each phase of the experiment were collected, they were compiled into GraphPad Prism 8, where they were modelled graphically and analyzed. The dataset was analyzed using a one-way ANOVA test to determine significant relationships between phases of the experiment.

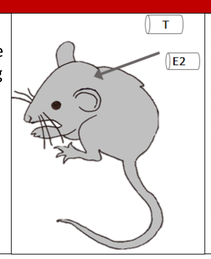


Figure 2. Model of hormone pellet implantation (T = testosterone, E = estradiol). Incision made in back near tail and pellets are placed in the back of the neck.

Results

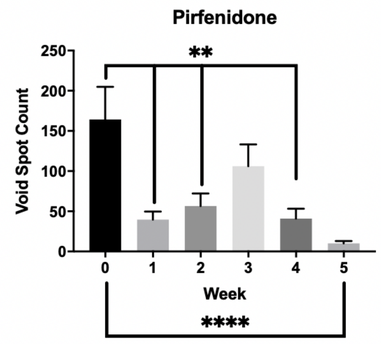


Figure 3. This figure shows mean VSA data ± SEM for each week following treatment with pirfenidone. Week 0 is the VSA data collected the week before treatment began. Brackets indicate a significant difference with week 0 only. (* = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001)

- After only one week of treatment, void spot count dropped markedly
- Void spot count experienced a slight rebound in weeks 2 and 3
- Void spot count dropped again in the 4th week
- By the 5th week, a significant difference with a p-value of less than 0.0001 emerged

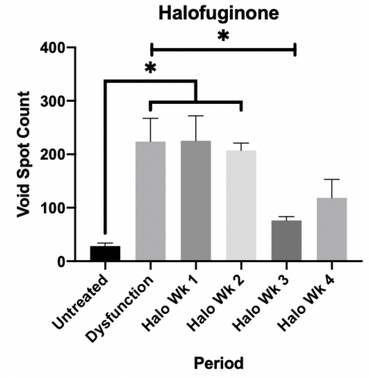


Figure 4. This figure shows mean VSA data ± SEM for the pre-surgery (untreated) period, the dysfunction period, and each week following treatment with halofuginone. Brackets indicate a significant difference. (* = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001)

- Unlike the pirfenidone counts, there was no visible decrease after the 1st or even 2nd week of treatment
- By the 3rd week, a significant difference from the dysfunction period emerged
- Similar to the pirfenidone trend, a slight rebound occurred during week 4, which no longer differed significantly from the dysfunction period

Discussion

- Based on the results of the experiment, it appears that pirfenidone may be a viable candidate for treating BPH
  - Upon further investigation, it may be beneficial to proceed with human clinical trials of pirfenidone to treat BPH and LUTD
- Halofuginone seems to be less effective than pirfenidone in reducing LUTD as a result of BPH, and further research is required to determine the feasibility of clinical trials for halofuginone
  - It may also be worth investigating the antifibrotic properties of halofuginone further to provide an explanation for the results seen in this experiment, or perhaps repeat the halofuginone treatment with old mice instead
- The next phase of this experiment will be to analyze the histology of the prostate tissues from the mice in this experiment to determine if the resolution of LUTD seen in the pirfenidone mice and the slight resolution in the halofuginone mice was actually the result of the reversal of fibrosis rather than an off-target effect of the drugs
  - To do this, we will stain collagen in the prostate tissues with PSR and image the tissues, then analyze the images to determine if a reduction in collagen occurred

Acknowledgements

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References

Bushman, W.A., and T.J. Jerde. 2016. "The Role of Prostate Inflammation and Fibrosis in Lower Urinary Tract Symptoms." *American Journal of Physiology - Renal Physiology* 311 (4): F817–21. https://doi.org/10.1152/ajprenal.00602.2015.

Liu, T.T., S. Thomas, D.T. Mclean, A. Roldan-Alzate, D. Hernando, E.A. Ricke, and W.A. Ricke. 2019. "Prostate Enlargement and Altered Urinary Function Are Part of the Aging Process." *Aging* 11 (9): 2653–69. https://doi.org/10.18632/aging.101938.

Nicholson, Tristan M., Emily A. Ricke, Paul C. Marker, Joseph M. Miano, Robert D. Mayer, Barry G. Timms, Frederick S. vom Saal, Ronald W. Wood, and William A. Ricke. 2012. "Testosterone and 17β-Estradiol Induce Glandular Prostatic Growth, Bladder Outlet Obstruction, and Voiding Dysfunction in Male Mice." *Endocrinology* 153 (11): 5556–65. https://doi.org/10.1210/en.2012-1522.

Rodriguez-Nieves, J.A., and J.A. Macoska. 2013. "Prostatic Fibrosis, Lower Urinary Tract Symptoms, and BPH." *Nature Reviews Urology* 10 (9): 546–50. https://doi.org/10.1038/nrurol.2013.149.