Sustained Delivery Of DHT Via TCPL Delivery System To Develop A BPH Model In Rats

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Introduction: Fifty percent of men over the age of 50 develop benign prostatic hyperplasia (BPH). Benign prostatic enlargement can lead to lower urinary tract symptoms (LUTS) and renal disease. In 2007, 4.5 million new cases were diagnosed, costing the health care industry $3.9 billion/year in direct and indirect costs. Even with this information the literature lacks definition of pathogenesis and risk factors for disease, other than age and gender. The overall objective of this research project is to add to the body of knowledge concerning the developmental process of BPH specifically relating to morphologic and chemical changes that occur at the cellular level from very early stages of change through the transformation to classic clinical disease presentation using the rat as a model. The hypothesis of the project was the ventral prostate of the Sprague Dawley rat can be used as a valid animal model for benign prostatic hyperplasia showing the spontaneous development of BPH as a function of age and androgen use.

Material and Methods

TCP Ceramic Fabrication: The microcrystals of tricalcium-phosphate (TCP) powder were prepared by following standard laboratory protocols. The TCP material was calcined at 1150^{O}C for one hour. The calcined TCP was then placed on an automatic Tyler sieve stack to obtain particles 1-38 µm in size. The calcined material was then pressed into cylindrical form using a 5/16" die set (French pressure cell) at a compression load of 800 Kg. The pressed capsules were sintered at 1250^{O}C for 12 hours to achieve the appropriate mechanical strength. TCPL implants were fabricated in two different sizes (1.2 or 0.6 cm in length) using two different ceramic compositions (80 and 98%) and impregnated with 40 mg of dihydrotestosterone (DHT).

Experimental Design: A total of 80 male Sprague-Dawley rats were obtained from Harlan, Inc. (Indianapolis, IN). The animals were randomly divided into 2 groups. Group 1 served as an intact control group while group 2 was implanted with a TCPL delivery system containing 40 mg DHT.

Methods of Evaluation and Analysis: Surgical aseptic technique was employed according to standard laboratory protocol. Blood samples were collected semi-weekly during the entire investigation, and X-rays were performed weekly to ensure the integrity of the delivery devices. At 6 weeks, 10 weeks, 16 weeks, 1year, and 2 years eight animals/group were sacrificed. The prostates were harvested, processed, embedded, sectioned and screened for cellular changes.

Hormonal Analysis: DHT levels were measured by means of a radioimmunoassay according to standard procedures.

Data Analysis: Computer software programs (Sigma Stat Software, Jandel Inc., Slidewrite) were used to determine statistical significance and develop graphics. Analysis of variance was used to compare the groups, and significant differences were reported at $p<0.05$.

Results

DHT Serum Levels: Data obtained from this study revealed that TCPL capsules were capable of releasing DHT at a sustained level for 16 weeks in adult male rats without any noticeable side effects. The range of DHT released from TCPL capsules was 3.98 to 5.31 ng/ml serum.

Vital Organ Weights: No significant changes were observed in total wet weights or morphology of spleen, kidney, adrenals and heart between the experimental groups.

Prostate Data analysis of prostatic tissue obtained from rats implanted with 40 mg DHT TCPL showed microscopic variations from one area of the gland to another by 6 weeks. Generally, there was considerable hyperplasia, as evidenced by accentuation of glandular folds and an increase in the number and size of the epithelial cells, with some areas of the gland showing more severe hyperplasia than others (Figure 1). Meanwhile, cross-sections of prostate obtained from control rats were characterized by an essentially normal histological appearance of the gland (Figure 2). These data exemplify the complex interactions that occur between prostate components and exogenous sustained delivery of DHT that are reflected in cell structure and function.

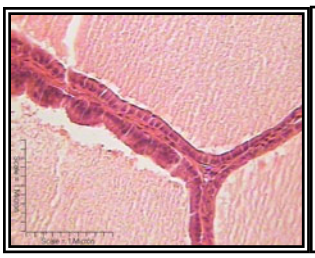

Figure 1: Hematoxylin and Eosin in prostatic tissue t=6 wks in DHT treated animals.

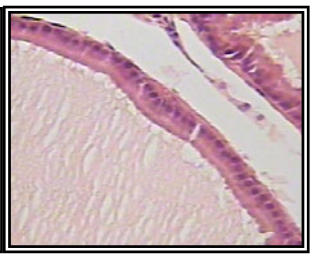

Figure 2: Hematoxylin and Eosin of prostatic tissue in control.

Control rats without DHT, BPH developed spontaneously after 1 year (Figure 3). At 2 years there is BPH, metaplasia and dysplasia (Figure 4a-c). "Wedge" cells can be seen in developing nodules. There are areas of BPH with metaplasia suggesting that these cells may become more disorganized with disease.

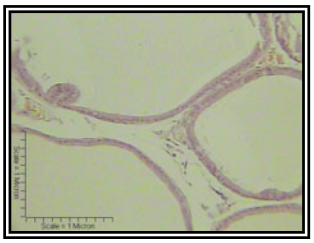

Figure 3: Hematoxylin and Eosin staining of prostatic tissue of control untreated rats at 1 year.

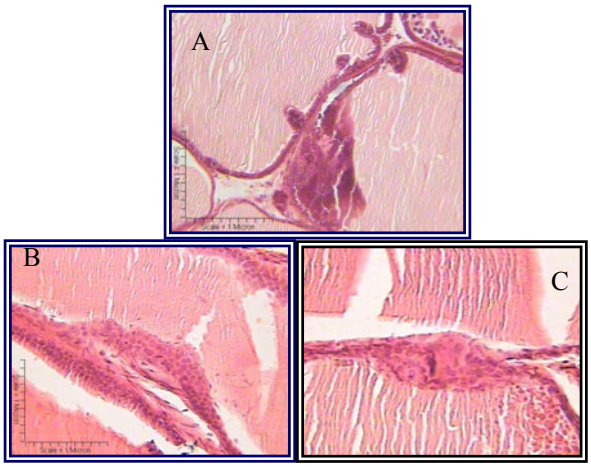

Figure 4: Hematoxylin and eosin staining of prostatic tissue from 2 year old rats. 4a shows evidence of BPH along with hyperplasia and metaplasia. 4b shows evidence of hyperplasia, and 4c shows metaplasia.

Conclusion: This study provides information that sustained delivery of DHT results in alterations prostate organ morphology after 6 weeks that resembles spontaneous BPH seen in the ventral prostate of animals at 1 year and 2 years.

References:

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