Histologic Evaluation of Human Benign Prostatic Hyperplasia Treated by Dutasteride: A Study by Xenograft Model With Improved Severe Combined Immunodeficient Mice

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OBJECTIVE To evaluate histologic change in human prostate samples treated with dutasteride and to elucidate direct effects of dutasteride on human prostate tissue, the present study was conducted by using a xenograft model with improved severe combined immunodeficient (super-SCID) mice, although it is well known that dutasteride reduces prostate volume.

METHODS After establishment of a xenograft model of human benign prostatic hyperplasia in morphology and function, samples implanted into super-SCID mice with and without dutasteride were evaluated pathohistologically at 2 and 6 months after initiation of dutasteride administration.

RESULTS The proliferative index evaluated by Ki-67 staining was significantly lower in the dutasteride group than the control at 2 and 6 months after administration. Apoptotic index evaluated by the terminal transferase TdT-mediated dUTP-biotin nick end labeling staining was higher in the dutasteride group than the control at 2 and 6 months after administration. Quick scores in the dutasteride group for staining of both cyclooxygenase-2 (Cox-2) and Ras homolog gene family, member A (RhoA) were significantly lower than those in the control group at 2 and 6 months after administration.

CONCLUSION Dutasteride inhibits cell proliferation and induces apoptosis of prostatic cells, causing a reduced prostate volume. Furthermore, decreased expression of Cox-2 and RhoA within benign prostatic hyperplasia tissue by dutasteride may induce an early effect on improvement of lower urinary tract symptoms, probably by attenuating inflammation reaction of the prostate and decreasing intraurethral pressure, other than the mechanism of reduced prostate volume. UROLOGY 85: 274.e1–274.e8, 2015. © 2015 Elsevier Inc.

Benign prostatic hyperplasia (BPH), a very common disease related to aging and a cause of lower urinary tract symptoms (LUTS), is a condition histopathologically characterized by hyperplasia of stromal and epithelial elements in the transition zone of the prostate. The goal of medical treatment is to alleviate LUTS secondary to prostate enlargement and obstruction. Because contraction of the human prostate is mediated predominantly by α1A-adrenoceptors, the first-line drug treatment for male LUTS has been considered to be α1-blockers.[1] It is also well known that circulating testosterone influences prostatic growth in its active form, dihydrotestosterone (DHT), mediated by 5α-reductase enzymes. Recently, 5α-reductase inhibitors (5-ARIs) have been a recommended treatment option for men with moderate to severe LUTS and an enlarged prostate.[2] The study of the 4-year extended monotherapy trial with dutasteride, which is a selective inhibitor of both the type 1 and type 2 isoforms of steroid 5-α-reductase, showed an 80% better symptom response and 27% better prostate volume reduction than those in the placebo group.[3] Recently, the 4-year combination of dutasteride and tamsulosin study (the CombAT study) also showed that the adjusted mean percentage change from baseline in total prostate volume was −28.0% for dutasteride.[4] This reduction in prostate volume by dutasteride was also

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found in Japanese subjects.[5] In terms of cell proliferation and apoptosis, it was reported that dutasteride had no significant effect on the progression to G_2M phase in any primary epithelial cultures from patients who underwent radical prostatectomy for prostate cancer, whereas some cultures treated with dutasteride underwent apoptosis.[6] However, an in vivo study with histologic evaluation of the effect of dutasteride on human prostate tissue has not been reported, probably because of the difficulty of establishing an appropriate animal model.

In improved severe combined immunodeficient (super-SCID) mice, normal human organs and tissues are considered to be well maintained in morphology and function for a long period by the consecutive transplantation of these tissues.[7-11] In the present study, after establishing a xenograft model of human BPH implanted subcutaneously in super-SCID mice, we evaluated histologic changes in human prostate tissue caused by dutasteride.

METHODS

Human Prostate Tissue

Human prostatic tissue obtained by surgical operation from the patient with BPH (69 years old) was used for heterotransplantation into super-SCID mice. Although the serum levels of prostate-specific antigen (PSA) of the patient before the operation were 5.37 ng/mL, no adenocarcinoma had been proven by preoperative prostatic biopsy. Use of human tissues was permitted by the ethics committees of the Osaka University, Graduate School of Medicine and the National Institute of Biomedical Innovation, and all experiments were performed following the guidelines of the Ministry of Education, Science, and Culture and the Ministry of Health and Labor.

Super-SCID Mice

C3H/HeJ-*scid/scid* male mice ($N_{16}F_{4\text{-}6}$) were used for the experiment. C.B17-*scid*/+ male and female mice were provided by Dr. M.J. Bosma,[7] Institute of Cancer Research, Philadelphia, in 1986, and then C.B17-*scid/scid* mice were maintained by selective sister-brother inbreeding of C.B17-*scid/scid* homozygote showing undetectable serum IgG and IgM (<1 μg/mL) by T. Nomura to diminish the leaky and leukemic mice.[9,10] C.B17-*scid/scid* male (N_1F_3) was mated with C3H/HeJ female (F_{153}; provided by E. S. Russell, Jackson Laboratory at F_{129} in 1976 and inbred by sister-brother mating for further generations). Progeny was crossed, and *scid* homozygote mouse was repeatedly back-crossed to C3H/HeJ to make congenic strain of C3H/HeJ-*scid/scid* ($N_{14}F_{4\text{-}6}$) by T. Nomura.[10] Mice were maintained in the complete barrier condition, light from 4 AM to 6 PM at 23 ± 1°C and 50%-70% in the humidity with autoclaved mouse diet CRF-1 (Charles River Laboratories Japan, Kanagawa, Japan) and acidified, chlorinated, and filtrated (by Millipore) water. Serum IgG and IgM were examined at 4-6 weeks after birth by enzyme-linked immunosorbent assay, and 2-month-old C3H/HeJ-*scid/scid* mice showing undetectable serum IgG and IgM (<1 μg/mL) were used for the heterotransplantation of human BPH. Animal experiments were carried out in the barrier section of the National Institute of Biomedical Innovation following the Guidelines for Animal Experimentation.

In Vivo Experiment With Super-SCID Mice to Evaluate the Effect of Dutasteride on BPH Tissue

Procedures for the heterotransplantation of human tissues into the super-SCID mice were performed as previously described.[7-9] Resected human BPH tissues were cut into 5-6 mm cubic masses in Dulbecco's Modified Eagle's Medium (high glucose) with L-glutamine and phenol red (Wako, Osaka, Japan) culture medium on containing high concentrations of antibiotics. Eight super-SCID mice were anesthetized with Isoflurane inhalation (Mylan pharmaceutical Co, Osaka, Japan) by Isoflurane Inhalation Anesthetic System (DS Pharma Biomedical Co, Osaka, Japan), and BPH tissue pieces were implanted subcutaneously into the back (both right and left sides) of the mice. Testosterone pellet (12.5 mg; Sigma-Aldrich Co) with sustained release of testosterone at least for 2 months was also implanted into the central part of the back by surgical operation because there was concern that endogenous testosterone produced by mice testes was not sufficient to maintain human prostate tissue. Six days after transplantation, 5 mice were treated twice a week with 0.5 μg/g bodyweight of dutasteride dissolved in 0.5% hydroxypropyl methylcellulose solution (provided by GlaxoSmithKline, United Kingdom) by esophageal tube, and 3 mice were untreated. If the mice died or were dying, transplanted BPH tissues were removed and transferred to other super-SCID mice by the same procedure. To investigate whether implanted BPH tissues are maintained in morphology and function, the original BPH tissues from the patient and tissues taken at 2 and 6 months after implantation were evaluated by hematoxylin and eosin staining and immunohistochemical staining for p63, cytokeratin 8 (CK 8), androgen receptor (AR), PSA, Ki-67, cyclooxygenase-2 (Cox-2), and Ras homolog gene family, member A (RhoA). The incidence of apoptosis in situ was also evaluated by terminal transferase TdT-mediated dUTP-biotin nick end labeling (TUNEL) staining using the ApoTag kit (Oncor, Inc, Gaithersburg, MD).

Immunohistochemistry and Histopathologic Assessment

Procedures for immunohistochemical staining were performed as previously described.[12] Briefly, sections were incubated with primary antibody of 1:1000 mouse anti-p63 monoclonal antibody (Dako, Carpinteria, CA), 1:200 mouse anti-CK8 monoclonal antibody (Progen, Heidelberg, Germany), 1:200 mouse anti-AR monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 1:200 mouse anti-PSA monoclonal antibody (Dako), 1:5000 rabbit anti-Ki-67 polyclonal antibody (Novocastra, New Castle, United Kingdom), 1:200 rabbit anti-Cox-2 monoclonal antibody (Cell Signaling Technology, Danvers, MA), and 1:200 mouse anti-RhoA monoclonal antibody (Dako) in 0.1-M phosphate buffered saline with 10% normal goat serum for 90 minutes at 37°C. Thereafter, the sections were incubated at 4°C overnight with Horseradish Peroxidase-labeled polymer-conjugated secondary antibody against mouse or rabbit IgG (Dako EnVision+ System-Horseradish Peroxidase [DAB]). Finally, sections were counterstained with hematoxylin for 10 minutes.

At both time points (2 and 6 months), 3 sections from each sample were examined immunohistochemically. The proliferative index of each sample was determined using Ki-67 immunostaining as previously described.[13] Briefly, precise quantitative evaluation of the positive staining of cells was performed by manual counting. For each specimen, 5 areas were randomly selected, and at least 300 cells were counted, making