Effect of Dutasteride on Microvessel Density in Benign Prostatic Hyperplasia

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Abstract. *Aim: Dutasteride, a dual 5α-reductase inhibitor, is used to treat benign prostatic hyperplasia (BPH). However, its histopathological effects on the morphometrics of blood vessels and glands are still controversial. This study aimed to assess the histopathological effects of dutasteride in cases of BPH in a retrospective manner. Patients and Methods: Patients with BPH were administered 0.5 mg of dutasteride daily or left untreated prior to undergoing holmium laser enucleation of the prostate (HoLEP). After HoLEP, remaining prostatic peripheral tissue at the bladder neck and the apex was resected. Each specimen was subjected to hematoxylin/eosin and immunohistochemical staining for CD31, and microvessel density (MVD) was analyzed. Results: In the dutasteride-treated group (n=14), the mean duration of administration was 7.07±2.46 weeks. MVD was significantly lower at the bladder neck side in the dutasteride-treated group than in the control group (p=0.018). Conclusion: The present study, to our knowledge for the first time, assessed MVD by evaluating the bladder neck and apex sides of the remaining prostatic peripheral tissue after HoLEP, allowing evaluation of MVD in more detail without intraoperative damage of the peripheral tissue, such as through heat denaturation. Dutasteride reduces MVD in the bladder neck side of the prostate among patients with BPH and may lead to decreased risk of perioperative prostatic urethral bleeding.*

Benign prostatic hyperplasia (BPH) affects 50% to 80% of men older than 50 years of age (1). Out of these men, 50% will eventually require treatment for symptomatic relief of clinical

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BPH by the time they reach 80 years of age (1, 2). Although these epidemiological data indicate the clinical importance of BPH, surprisingly little is known regarding the molecular pathogenesis of the disease and its complications. Hematuria, for example, is a well-documented symptom of BPH, which can lead to significant morbidity, including anemia, clot retention, and need for transfusion of blood products. Although the natural history of bleeding from BPH is probably related to increased vascularity in the prostate, which is associated with friable prostatic tissue, it still remains not fully-understood.

As an initial treatment for symptomatic BPH, pharmacotherapy with α1-adrenergic blockers, 5α-reductase inhibitors, anti-muscarinic agents, or a combination thereof is often used (3). Type 1 and type 2 isoenzymes of 5α-reductase are present throughout the body (4). Dutasteride, a dual 5α-reductase inhibitor, acts competitively and specifically on type 1 and 2 isoenzymes to inhibit the conversion of testosterone to the more potent dihydrotestosterone (5-7). Dutasteride is a 45-fold greater inhibitor of type-1 5α-reductase, and a 2.5-fold greater inhibitor of type-2 5α-reductase compared to finasteride, a 5α-reductase inhibitor, which acts selectively on type 2 isoenzymes (8-11).

Several studies assessing the clinical and histopathological perioperative effects of dutasteride have been reported (12-14). In these studies CD31 or CD34 staining in the endothelium was used to detect microvessels. Furthermore, data delineating the histopathological effects of dutasteride administration on BPH tissue are still lacking.

In the present study, we performed an *in vivo* evaluation of microvessel density (MVD) in prostates with BPH treated with dutasteride. In particular, we separately evaluated the bladder neck and apex sides of the prostatic peripheral tissue remaining after holmium laser enucleation of the prostate (HoLEP) because it allows more detailed evaluation of MVD without intraoperative damage of the peripheral tissue such as through heat denaturation. Our study provides histochemical insight into the mechanism by which dutasteride may reduce prostatic urethral bleeding.

Patients and Methods

Ethics statement. The study was conducted in accordance with the Helsinki declaration, after approval by the Ethics Committee of Nozaki-Higashi Hospital (Approved number 011, August 2011). The committee approved the use of oral consent documented for each patient because the study was retrospective and non-randomized.

Study participants. This was a retrospective study to investigate the effects of dutasteride administered before HoLEP in Japanese patients with BPH. A total of 40 patients with BPH whose prostate volume, as determined by transrectal ultrasonography, was greater than 30 cm^3 were enrolled between December 2011 and June 2012. Patients with serum prostate-specific antigen greater than 4.0 ng/ml underwent preoperative transrectal prostate biopsy to rule out prostate cancer. The patients had not received luteinizing hormone-releasing hormone agonists, anti-androgens, estrogens, or 5α-reductase inhibitors, and had not undergone prostatic surgery or radiation therapy to the pelvis. Patients with severe cardiovascular disease, liver disease, renal failure, or bleeding tendency were excluded since they were not suitable for HoLEP.

Patients were administered 0.5 mg of dutasteride once daily (GlaxoSmithKline, Brentford, UK) for 4-10 weeks (n=14), or they were subjected to HoLEP without dutasteride treatment (n=23). A daily 0.5-mg tablet of dutasteride is the therapeutic dose approved for treating symptomatic BPH in Japan. Age and duration of administration of dutasteride were recorded preoperatively. HoLEP was performed by the typical procedure using continuous saline solution irrigation. The procedure was as follows. Firstly, we dissected the median and lateral prostatic lobes from the surgical layer of the prostate in a retrograde direction from the apex, and then released them into the bladder. Secondly, enucleation was completed and hemostasis was achieved. The enucleated tissue was evacuated from the bladder by mechanical tissue morcellator. At the end of the operation, a three-way 20-Fr Foley catheter was inserted, and the bladder was continuously irrigated. Patients were usually discharged after removal of the Foley catheter on the second postoperative day. Patients were not transfused with autologous or allogeneic blood in the perioperative period. The operation time for HoLEP and the weight of resected prostatic tissue were recorded as perioperative data. Pre- and postoperative blood hemoglobin (Hb) and hematocrit (Hct) levels were also determined. Furthermore, the International Prostate Symptom Score (IPSS), quality of life (QOL), and overactive bladder syndrome score (OABSS) were determined in the perioperative period for the evaluation of symptoms.

Histological analysis of BPH tissue. As shown in Figure 1, we resected specimens from the bladder neck and apex sides of the remaining prostatic peripheral tissue after HoLEP. Tissue specimens were fixed in 10% buffered formalin and embedded in paraffin. The tissue specimens were immunohistochemically-stained for CD31 using the standard three-step streptavidin-peroxidase technique, with relevant positive and negative controls. MVD was independently evaluated by two of the authors (SS and SM). Each specimen was examined in a blinded manner. We used a mouse monoclonal antibody (Leica Biosystemics, Wetzlar, Germany) against CD31 and determined the number of stained microvessels per high-power field. MVD was determined by quantifying the number of blood vessels positively stained for CD31 in the three most vascularized fields at ×200 magnification ('hotspots') for each slide (15, 16).

Statistical analysis. Statistical analysis was performed using the R i386 2.15.1 software package (Vienna University of Economics and Business Administration, Vienna, Austria). The significance of differences in the mean MVD among dutasteride-treated and control groups were determined by Student's *t*-tests. Statistical analysis was performed using the Mann–Whitney *U*-test for intergroup comparisons. A value of *p*<0.05 was considered statistically significant.

Results

Characteristics of the patients are shown in Table I. There were no significant differences in age and prostate volume at first visit between the control and dutasteride-treated groups. In the dutasteride-treated group, the mean period of dutasteride administration was 7.07±2.46 weeks.

Perioperative parameters of HoLEP are also shown in Table I. There were no significant differences in operation time, weight of resected prostate tissue, and perioperative changes in Hb and Hct. Furthermore, the IPSS, QOL, and OABSS were not significantly different between the groups in the perioperative period.

Pathological examination of resected specimens by HoLEP showed glandular and fibromuscular hyperplasia in all patients. Atrophic glands were frequently observed in the dutasteride-treated group. No significant inflammatory lesions were observed.

Using the CD31 antibody, vascular networks of the prostate were easily observed. Figure 2 shows a typical result of immunohistochemical staining for CD31, a marker of MVD, in prostate from dutasteride-treated (Figure 2A) and control patients (Figure 2B).

There was no significant difference in MVD between bladder neck and apex of the prostatic peripheral tissue of the groups (dutasteride-treated group: *p*=0.351, control group: *p*=0.396). However, the MVD at the bladder neck side was significantly lower in the dutasteride-treated group compared with the control group (*p*=0.018) (Figure 3).

The incidence of prostate cancer between the two groups was not significant. Prostate cancer was diagnosed by resected tissue of HoLEP in only one patient in the dutasteride-treated group (6.7%) and in two patients in the control group (8.0%). These three patients were excluded from this study.

Discussion

BPH-associated hematuria is a well-recognized sequela of benign prostatic disease, which at times can be difficult to manage. Significant morbidity from this condition may result, and eventual need for surgical treatment is likely if left untreated. Therefore, many researchers have focused on the natural history of BPH-induced hematuria in order to provide more conservative treatment options. In the past decade, studies using androgen deprivation, especially
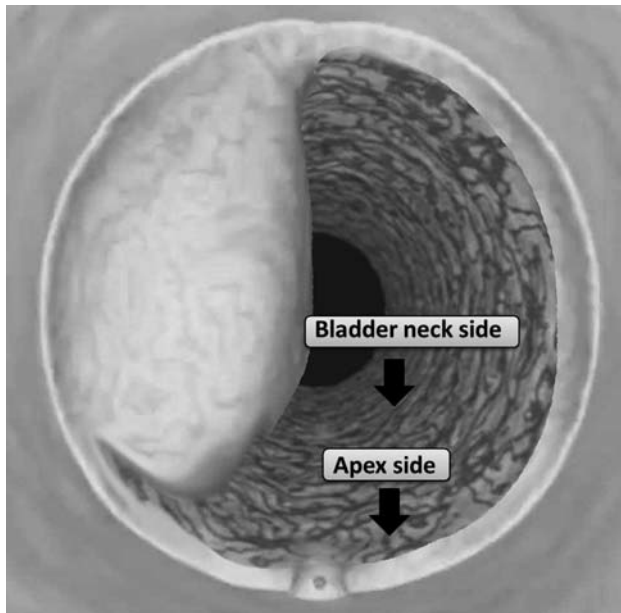
Figure 1. *Specimen areas intraoperatively. Bladder neck and apex sides of the postenucleated prostatic peripheral tissue are indicated.*

Table I. *Patients' characteristics and overall study results.*

	Dutasteride	Control	*p*-Value*
Age, years mean±SD	73±8	73±7	0.78
PV at first visit (ml)	81.5±40.6	76.4±33.5	0.68
Dutasteride duration (week)	7.07±2.46	–	
PSA (ng/ml) mean±SD			
Pre	11.2±8.9	15.7±25.3	0.58
Post	0.83±0.83	1.54±1.94	0.22
IPSS			
Pre	21.5±7.8	21.5±8.3	0.98
Post	6.5±4.9	5.6±2.9	0.54
QOL			
Pre	4.9±0.9	5.1±1.0	0.41
Post	1.7±1.3	2.0±1.4	0.60
OABSS			
Pre	8.1±3.8	7.5±3.0	0.60
Post	4.9±2.3	5.3±3.5	0.77
Hb (g/dl)			
Pre	14.6+1.6	14.4±1.6	0.80
Post	12.7±1.5	12.8±1.6	0.78
Ht (%)			
Pre	42.5±4.6	42.2±4.5	0.86
Post	37.1±4.3	37.8±4.9	0.65
Surg time (min)	110.6±32.1	105.9±35.9	0.69
Resected weight (g)	57.5±24.4	53.7±27.9	0.67
Total	14	23	

PSA: Prostate specific antigen; PV: prostate volume; SD: standard deviation; IPSS: International prostate symptom score; QOL: quality of life; OABSS: over active bladder syndrome score; HB: hemoglobin; Hct: hematocrit; Surg: surgical; *Based on Mann-Whitney *U*-test.

finasteride, a type 2 5α-reductase inhibitor, have consistently shown reduced prostatic bleeding in patients with BPH and in those undergoing BPH-related surgery (17, 18).

Human fetal and animal studies have demonstrated that testosterone is a stimulator of vascular endothelial growth factor, and androgen deprivation leads to decreased blood flow in the prostate (19-21). The effects of androgens are mediated by dihydrotestosterone-regulated production of vasoactive substances in stromal and epithelial cells, which contain androgen receptors. Puchner and Miller (22) first observed the effect of finasteride in the treatment of hematuria associated with BPH, and long-term follow-up results confirmed the efficacy of finasteride (17). A putative mechanism that explains the effects of finasteride on prostate angiogenesis is that it blocks the conversion of testosterone to dihydrotestosterone, and thus reduces the activity of the androgen-controlled growth factors.

Additionally, previous histopathological studies investigated the association between dutasteride and BPH. Hahn *et al.* (12) and Ku *et al.* (14) showed that perioperative administration of dutasteride had no effect on MVD in the prostatic tissue of patients with BPH given preoperative dutasteride for two or four weeks compared to placebo-treated controls in a prospective, randomized, multicenter study. In their study, MVD was counted by the number of microvessels immunostained with antibody to CD34 within chips removed by trans-urethral resection of the prostate.

However, Andriole *et al.* reported that patients with prostate cancer administered 10 mg of dutasteride daily for one week followed by 5 mg daily for greater than 45 days showed a decrease in MVD by 45% in cancer tissue (23). They also observed a trend toward increased cancer cell apoptosis compared to controls without dutasteride administration (23). In their study, the effect of a decrease in MVD by dutasteride was detected only in prostate cancer tissue, this effect was not observed in BPH.

In the human prostate, there is more blood flow at the bladder neck than at the apex. However, the differences in the action of 5α-reductase inhibitors on MVD on BPH tissue have yet to be adequately clarified. Therefore, we designed the current study to evaluate MVD as a marker of angiogenesis in human prostates treated with dutasteride. We assessed MVD, for the first time as far as we are aware, by evaluating the bladder neck and apex side of the remaining prostatic peripheral tissue after enucleation. This enables more detailed evaluation of MVD without intraoperative damage of the peripheral tissue, such as is caused by heat denaturation. We compared these results with controls and found that MVD significantly decreased in the dutasteride-treated group compared to the control group, especially in the bladder neck side.
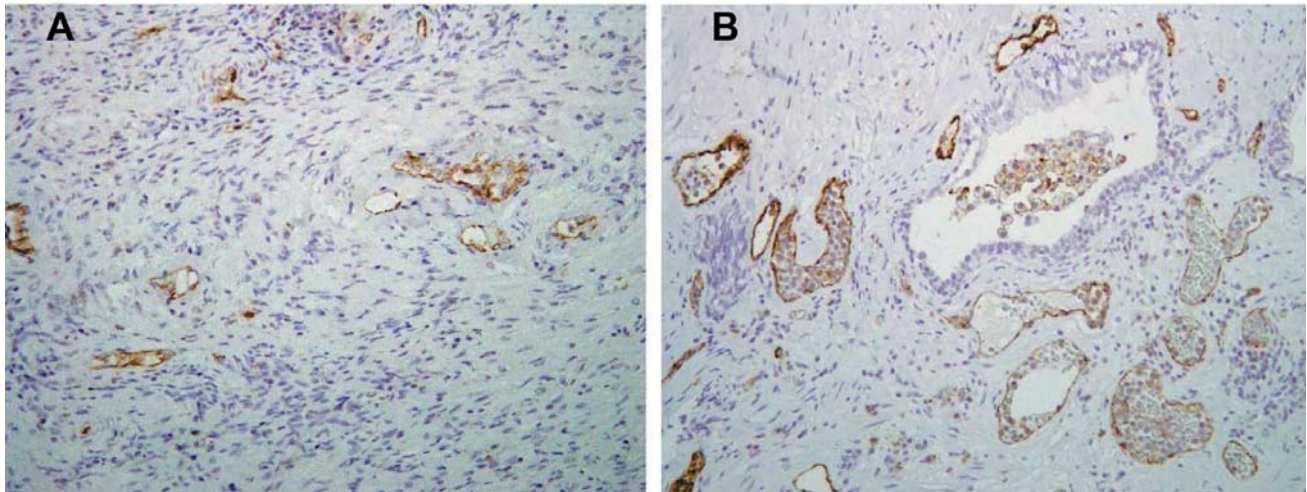
Figure 2. *Immunohistochemistry of CD31-stained sections shows sub-urethral prostatic microvessels in dutasteride-treated (A) and control (B) prostates. Original magnification, ×200.*
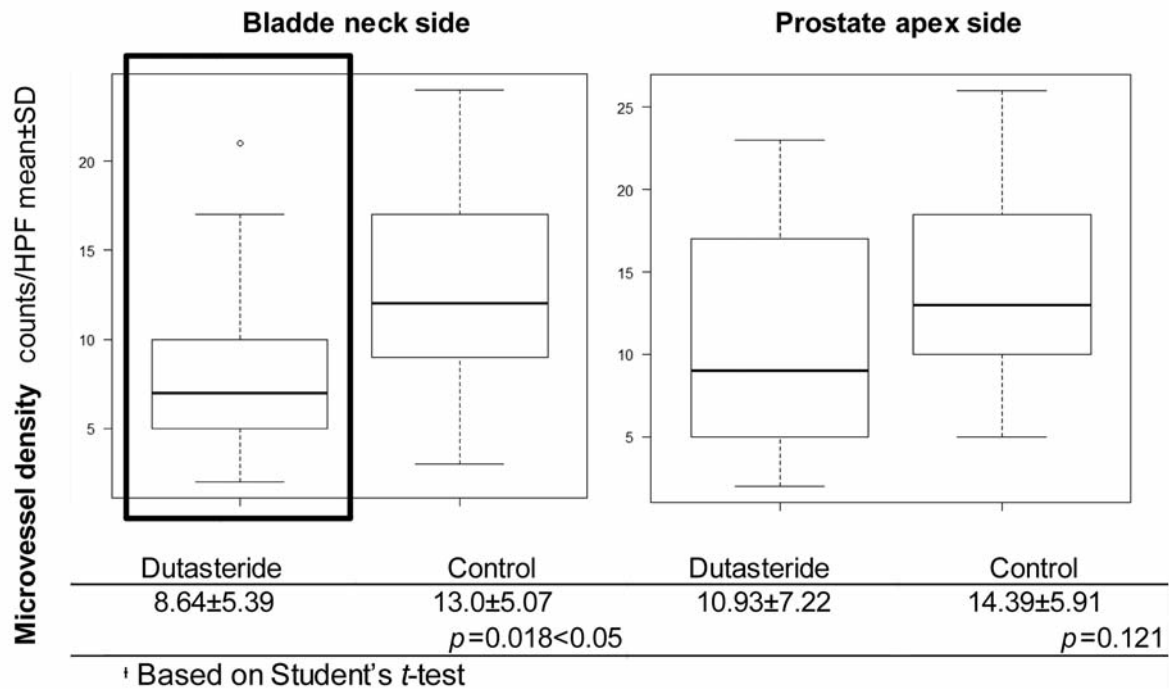

Figure 3. *Microvessel density in dutasteride-treated prostates and controls.*

Previous studies have shown that 4-6 weeks' perioperative administration of dutasteride had no effect on reducing blood loss during or after trans-urethral resection of the prostate (12, 13). However, in one of these studies (13), two patients in the control group required a blood transfusion postoperatively, while no patients required a blood transfusion in the dutasteride-treated group. Similarly, in our study, blood loss was comparable between the two groups as estimated from the perioperative changes in blood Hb and Hct. Therefore, the reduction in density of blood vessels in BPH did not directly lead to decreased blood loss in the perioperative period. However, our findings support the hypothesis that a reduction in MVD at the bladder neck side of the prostatic tissue, where blood flow is abundant, reduces the frequency of postoperative bleeding and hematuria in patients with BPH.

In conclusion, dutasteride administration for 7.07±2.46 weeks before HoLEP causes a reduction in MVD in the tissue of patients with BPH, particularly in the bladder neck side. This finding provides insight into the mechanism by which dutasteride reduces prostatic urethral bleeding. However, this was merely a pilot study, further studies in larger cohorts are required to confirm our results.

Conflicts of Interest

None.

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