

LONG-TERM ADMINISTRATION OF TAMSULOSIN INCREASE APOPTOSIS ON PROSTATE CELL OF BPH PATIENT: STUDY ON TGF- β 1, SMAD-4, AND NF- κ B

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ABSTRACT

Objective: In this study we aimed to know further mechanism and relation between the long-term usage of tamsulosin with the apoptotic mechanism on prostatic cells of BPH patients qualitatively. **Material & Methods:** A Case-control study from prostate resection specimens. Subjects were 20 male who underwent the TUR-P procedure, were divided into two groups equally, a group who got long-term administration of tamsulosin 0.4 mg, and one without tamsulosin. TGF- β 1 and Smad-4 expression was measured by Enzyme-Linked Immunosorbent Assay (ELISA), meanwhile expression of NF- κ B measured by immunohistochemistry assay. **Results:** There was a significant difference on the Independent T-Test that showed an increase in expression of TGF- β 1 and Smad-4 expression and a decrease in expression of NF- κ B $p=0.003$ ($p<0,05$) from a group of patients who got long-term oral administration of tamsulosin 0.4 mg, compared with a group of patients who didn't get any treatment. **Conclusion:** Long-term tamsulosin would induce an apoptotic process in the prostate cells shown by the activity of TGF- β 1, Smad-4, and NF- κ B.

Keywords: BPH, tamsulosin, TGF- β 1, Smad-4, NF- κ B, apoptosis.

ABSTRAK

Tujuan: Penelitian ini bertujuan untuk mengetahui mekanisme dan hubungan antara penggunaan tamsulosin jangka panjang dengan mekanisme apoptosis pada sel prostat penderita BPH secara kualitatif. **Bahan & Cara:** Penelitian kasus-kontrol dari spesimen reseksi prostat. Subjek penelitian adalah 20 orang laki-laki yang menjalani prosedur TUR-P, dibagi menjadi dua kelompok secara merata, yaitu kelompok yang mendapat tamsulosin 0,4 mg jangka panjang dan kelompok yang tidak mendapat tamsulosin. Ekspresi TGF- β 1 dan Smad-4 diukur dengan Enzyme-Linked Immunosorbent Assay (ELISA), sedangkan ekspresi NF- κ B diukur dengan uji imunohistokimia. **Hasil:** Terdapat perbedaan bermakna pada uji Independent T-Test yang menunjukkan adanya peningkatan ekspresi TGF- β 1 dan Smad-4 serta penurunan ekspresi NF- κ B $p=0,003$ ($p<0,05$) pada kelompok pasien yang mendapat tamsulosin 0,4 mg oral jangka panjang dibandingkan dengan kelompok pasien yang tidak mendapat pengobatan apa pun. **Simpulan:** Pemberian tamsulosin jangka panjang akan menginduksi terjadinya proses apoptosis pada sel prostat yang ditunjukkan oleh aktivitas TGF- β 1, Smad-4, dan NF- κ B.

Kata kunci: BPH, tamsulosin, TGF- β 1, Smad-4, NF- κ B, apoptosis.

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INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a disease that commonly affects men above 50 years old and manifests with Lower Urinary Tract Symptoms (LUTS). BPH occurred in 50% of men above 60 years old and 80% of men above 80 years old, enlargement of the prostatic gland will make disturbance of urinary flows so that it will make

disturbance of micturition.¹ Symptoms that might happen is LUTS, polyuria, sleep disturbance, and several systemic conditions that are not related to the bladder even irritative or obstructive symptoms might also occur.²⁻³ Prolonged obstruction will create acute urinary retention, recurrent urinary tract infections, hematuria, bladder stones, and renal insufficiency.⁴ The therapeutic approach to BPH consists of watchful waiting, medical therapy, and surgery.

The therapeutic approach using medical therapy on BPH patients nowadays uses α -adrenergic-blocker and 5 α - reductase inhibitor (5-ARI) drugs. The selective α 1-adrenergic-blocker such as Tamsulosin works by decreasing the resistance of smooth muscle cells of the prostate as dynamic components. Tamsulosin will create a significant therapeutic effect without the need for dose titration.⁵

A previous study on cell line culture NRP-152 from mice prostate cells by Yun Hee Youm et.al,2006 showed that antagonist receptor adrenergic- α tamsulosin would make cells death by giving pro-apoptotic effect on NRP152 cells line, but in a higher dose, it would make necrosis.⁶ Whereas in the other study conducted by Sagala, et al, we got information that long-term administration of adrenergic- α tamsulosin would increase the apoptotic effect on prostate cells, but there were no significant differences in apoptotic effect toward the duration of drug administration.⁷ Therefore in this study, we qualitatively measure whether or not there was an apoptotic process on prostate cells from BPH patients who were administered tamsulosin and underwent the transurethral resection of the prostate (TURP) procedure. In this study apoptotic process of prostate cell resection was measured by the apoptotic kit.⁷

On a normal prostate, the main function of TGF- β was to maintain tissue homeostasis by regulating the apoptotic process of epithelial prostatic cells. TGF- β will inhibit the proliferation of prostatic epithelial and induce differentiation of basal cells into luminal cells. TGF- β will stimulate the agregation of prostatic stromal smooth muscle.⁸⁻⁹

Smad-4 protein has an important role in signal transduction of TGF- β superfamily to induce antimitogenic and pro-apoptotic effects.¹² Smad-4 protein bonds with specific areas in DNA to control transcription factors and role in cell proliferation. Since the activation of TGF- β receptor phosphorylated R-Smad transported into the cell nucleus, creating an active complex between smad4 and another transcription factor. Smad4 signaling was needed to induce the apoptotic process induced by TGF- β .¹³

Another mechanism that figured out the role of TGF- β to induce cell apoptotic was by downregulating from NF- κ B activity by inducing I κ B α . I κ B α was a specific inhibitor from NF- κ B, NF- κ B is one of the transcription factors involved in the mediation of survival signal.¹⁴ NF- κ B is a

transcription factor that has an important role in the inflammation process, immunity, cell proliferation, and apoptotic. Activation of NF- κ B occurred by I κ B –Kinase phosphorylation from inhibitory molecule mediated by I κ B.¹⁵ In mammals, there are several families of NF- κ B There are RelA (P65), NF- κ B1 (p50,p105), and NF- κ B2 (p51,p100), This protein is conserved by N-terminal 300 region amino acid, which contains dimerization, core localization and DNA binding domain.¹⁶⁻¹⁷

The canonical pathway involved I κ B –Kinase, a main substance in NF- κ B activation, there will be I κ B –Kinase degradation in the NF- κ B activation process. The second pathway is the non-canonical pathway, where the activation process is induced by the signaling of LT-bm BAFF and CD40.¹⁸ Transduction from TGF- β signaling is supposed to have any relation with activation of NF- κ B. NF- κ B itself, known could inhibit the signaling pathway from TGF- β /Smad by transcriptional activity from Smad7 inhibitor.

OBJECTIVE

In this study we aimed to know further mechanism and relation between the long-term usage of tamsulosin with the apoptotic mechanism on prostatic cells of BPH patients qualitatively.

MATERIAL & METHODS

The sample of this study consists of 20 voluntary patients, divided into a group that administered tamsulosin 0.4, once a day and a group without any medication previously. The inclusion criteria for this study were men above 50 years old and indicated to undergo the TURP procedure. Meanwhile, the exclusion criteria were patients who were diagnosed with adenocarcinoma of the prostate. Prostate tissues collected during TURP were then preserved and measured according to parameters that were studied. This study was doing after the ethical number No:400/031/K.3/302/20119 was released and approved by Dr. Saiful Anwar General Hospital, Malang, Indonesia.

Prostate tissues were collected from the TURP procedure done by a urologist. The researcher collected prostate tissue from the TURP procedure in the operating room. Prostate tissue was then washed with normal physiological saline 0,9%, to ensure those tissues were free from blood and part of necrotic tissue. Parts of prostate tissue were

collected into a tissue container that contained a solution of formalin 10 % and was kept at room temperature (24-29°C), it would undergo an immunohistochemistry assay. Meanwhile, some other prostate tissue was preserved below 10°C, processed into supernatant before 24 hours, and then examined by ELISA.

Enzyme Linked Immunosorbant Assay (ELISA) were performed in Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya. The supernatant of specimens was examined in an ELISA spectrophotometry reader after a sequence of processes. Administration of 50-100µL standard buffer in every sample well and incubate for two hours, aspirate the buffer, and then washout. The process continued with the administration of 100 µ antibodies of each TGFβ1 and Smad4. The antibody of TGFβ1 was from the Human ELISA kit manufactured by Abcam Laboratory, United States (Catalogue No. AB100647) and the Smad4 protein using ELH-Smad4 Human ELISA kit manufactured by RayBiotech, Peachtree Corners, Georgia, United States (Catalogue No. ELH-Smad4-1). 100 µL liquid of HRP conjugate was administered in every well and incubated for 30 minutes, thus washout and aspirate. The next process was using the chromogenic substance and stop solution. Microplates were analyzed in 450 nm and 550 nm wavelength in an ELISA reader.

Immunohistochemistry assay performed on Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya. Prostate tissue preserves to be embed section of paraffin blocked. Embed sections were on the slide 3-aminopropyl-triethoxy-silane (APES)-coated. That section must be dried on the airflow for between 30-60 minutes to

ensure those sections were dried entirely. Mix the fixation solution for 15 minutes at room temperature. Wash these slides using PBS. Then the fixation process using acetone. Then continued with the immunohistochemistry assay protocol. Measurement of NF-κB p50 using antibody kit manufactured by Santa Cruz Biotechnology, Dallas, Texas, United State (Catalogue No. SC-8414). Stained slides are then observed using a binocular light microscope Olympus-CX21 series manufactured by Olympus Optical Co.LTD., Tokyo, Japan, and then it undergoes to be scanned using Olympus Viewer Imaging Application. Cell countered using the hCell counting application.

The result of this study was analyzed by IBM Statistical Products and Services Solutions (SPSS) Statistics, version 20.0 for Windows as a statistical analysis program. Data analysis of this study using confidential interval 95% ($\alpha=0.05$). Data was analyzed using T-Test (Unpaired-T Test), and the result was statistically significant when $p<0.05$.

RESULTS

From BPH patients who underwent the TUR-P procedure after being administered with tamsulosin orally compared to without tamsulosin, there was significant value $p=0.034$ ($p<0.05$) using unpaired t-test. Therefore it could be concluded that there is significant difference in TGF-β1 in patient with BPH who administered with tamsulosin. The average level of TGF-β1 was 154 ng/ml in the group with tamsulosin, meanwhile in the group without tamsulosin was 87.2 ng/ml, as described in Table 1 below.

Table 1. Result of differential test of activity of TGF-β1 on ELISA from BPH patient who administered with tamsulosin and without tamsulosin, during 3 months, using unpaired t-test.

Group	N	Mean±SD	P
Group of patients with administration of tamsulosin	10	765.06±275.560	0.020
Group of patients without administration of tamsulosin	10	499.05±277.031	

Table 2. Result of differential test of activity of Smad4 protein level on ELISA from BPH patient who administered with tamsulosin and without tamsulosin, during 3 months, using unpaired t-test.

Group	N	Mean±SD	p
Group of patients with administration of tamsulosin	10	154.00±82.436	0.034
Group of patients without administration of tamsulosin	10	87.200±40.430	

From BPH patients who underwent the TUR-P procedure after being administered with tamsulosin orally compared to without tamsulosin, there was significant value $p = 0.020$ ($p < 0.05$) using unpaired t-test. Therefore it could be concluded that there is a significant difference in Smad4 Protein levels in BPH patients who administered tamsulosin to the group without tamsulosin. The average level of Smad4 protein was 765 ng/ml in the group with tamsulosin, meanwhile on the group without tamsulosin was 499 ng/ml, as described in Table 2 below.

From BPH patients who underwent the TURP procedure after being administered with tamsulosin orally and without tamsulosin, there was a significant value $p = 0.003$ ($p < 0.05$). Therefore it could be concluded there was a significant difference in NF- κ B activity in the group of patients who were

administered with tamsulosin and without tamsulosin. The average of NF- κ B was 173 ng/ml in the group with tamsulosin, meanwhile on the group without tamsulosin was 1219 ng/ml, as described in Table 3 below. The activity in BPH patients showed by the number of positive cells on immunohistochemistry staining on BPH patients who were administered with tamsulosin lower than the group of patients without tamsulosin, as described by Figure 1.

On immunohistochemistry staining NF- κ B activity of prostate cells showed by changes in cell color, even on cytoplasm or nucleus of cells become brown. Microscopic findings of immunohistochemistry staining showed that NF- κ B activity was higher in the group of patients who took long-term administration of tamsulosin compared with the group of patients without tamsulosin.

Table 3. Result of differential test of activity of NF- κ B on immunohistochemistry staining from BPH patient who administered with tamsulosin and without tamsulosin, during 3 months.

Group(s)	N	Mean $\pm$ SD	P
Group of patients with administration of tamsulosin	10	173.30 $\pm$ 183.003	0.003
Group of patients without administration of tamsulosin	10	1219.80 $\pm$ 943.966	

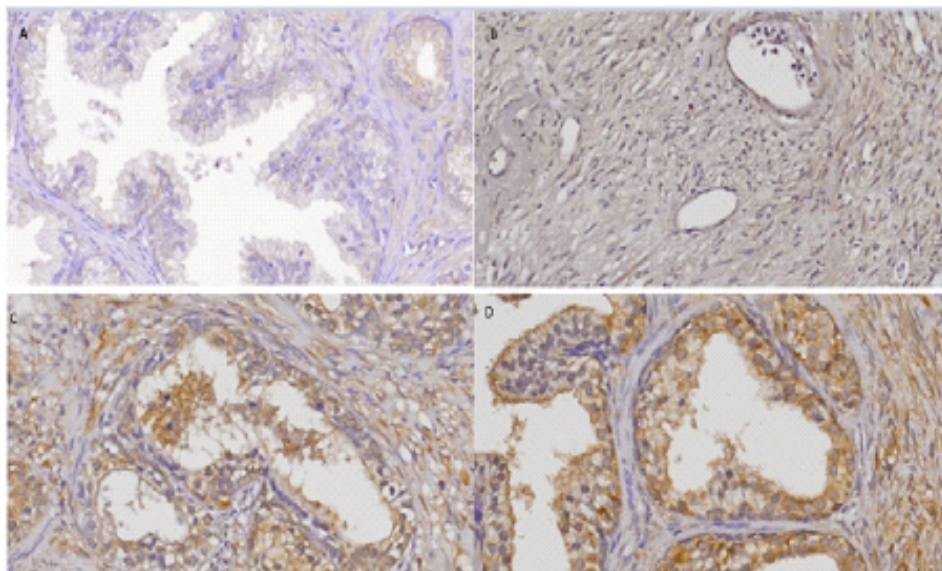


Figure 1. Microscopic findings of immunohistochemistry staining from the group of patients who got long-term administration of tamsulosin (3 months), pictures A and B figure out the group of patients who got tamsulosin, meanwhile, pictures C and D figure out the group of patients without administration of tamsulosin. Activity NF- κ B showed color changes to become brown on the nucleus and cytoplasm of prostatic cells. Scale bar are 100 μ m, in 100 fold magnification.

DISCUSSION

In our study, long-term administration of tamsulosin would increase the level of TGF- β and Smad 4 Protein. TGF- β 1 and Smad4 levels were higher in the group of patients who were administered with long-term administration of tamsulosin 0.4 mg daily. TGF- β 1 has a significant role in apoptosis and involution of prostate cells by its signaling pathway.¹⁰ Smad4 protein is part of the TGF- β signaling pathway. TGF β initiating intracellular signaling by smad protein Smad as co-activator and transcription factor. Those signaling process, increase expression of nuclear gene effector such Smad7, TIEG1, p21 and I κ B α and then stimulate the apoptotic process. On the other hand, those signaling process perform cross-talk mechanism through inhibition of NF- κ B by I κ B α .¹¹

NF- κ B expression measure based on immunohistochemistry result. The Mean of NF- κ B expression in the patient group was 173.30 cells, meanwhile in a group that was not administered with tamsulosin was 1219.80. From T Unpaired T-Test we got a significant value of 0.034 ($p < 0.05$). It shows that there is a significant difference that shows NF- κ B expression from the group of patients who were administered with tamsulosin was lower than that of a group of patients without tamsulosin. NF- κ B is a transcription factor that is controlled by activation of cascade signal. NF- κ B regulates several genes involved in immune response and inflammation, progressivity of cell cycle, inhibition of apoptotic and cell adhesion, and also induces carcinogenesis and progression of cancer. NF- κ B activates several gene expressions including genes that code the cytokine, molecule of cell adhesion, cell cycle regulator, and inhibition of apoptotic. Increasing NF- κ B cell expression on cells could be a means of inhibiting on apoptotic process.

Tamsulosin could induce activity such as the tyrosine kinase pathway. TGF- β ligand bound with TGF- β I and TGF- β II receptors. Those bonds would activate downstream signaling. TGF- β initiated intracellular signaling by medium Smad protein that was a co-activator and transcription factor. Those signals increase the expression of nucleus gene effectors such as Smad4, TIEG1, p21, and I κ B α , which will induce the apoptotic process. Another mechanism showing that TGF- β will induce programmed cell death is by downregulating NF- κ B by inducing I κ B α . A specific inhibitor from NF- κ B. NF- κ B will tie with and deactivated by I κ B α .¹⁰

NF- κ B is a transcription factor that is involved in the mediation process from survival signal.^{10,19} Therefore, increased levels of TGF- β I and Smad4 protein and a decrease of NF- κ B expression on the group of patients who long term administered with tamsulosin, show the process of apoptosis.

For further study that want to observe long term administration of tamsulosin, we recommend to considering the variables of differences dose as 0.2 mg and 0.4 mg and differences in the duration of tamsulosin administration as 0 month, 1 month, 3 months, 6 months, 9 months and 12 months

CONCLUSION

Long-term administration of tamsulosin 0.4 mg would increase apoptosis activity in prostate cells of BPH patients showing by increased level of TGF- β 1, Smad-4 protein, and a decrease of NF- κ B expression.

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