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The Effect of Long-Term Tamsulosin Monotherapy and Tamsulosin – Dutasteride Combination Therapy on PKC-α Enzyme Expression in Prostate Stromal Tissue

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ABSTRACT

Background: The α-adrenergic receptor antagonist is the most effective medical therapy to reduce the dynamic component in patients with BPH. However, long-term administration of receptor antagonists can cause upregulation of mRNA receptor expression, resulting in tolerance of drug effectiveness. PKC-α is involved in the process of prostate smooth muscle contraction through activation of the voltage-gated Ca2+ conducted canal, influenced by androgen hormones, especially testosterone, and has an isoform with Twist1, a transcription factor that plays a role in up-regulation of androgen receptors. **Objective:** The aim of the study was to compare the effect of long-term tamsulosin monotherapy and tamsulosin – dutasteride combination therapy in PKC-α enzyme expression in prostate stromal tissue of Rattus norvegicus rats of Wistar strain. **Methods:** Out of 80 samples of Rattus norvegicus rats were divided into 8 groups with different interventions: negative control group, positive control group, tamsulosin monotherapy administration for 1 day, 3 day, and 6 day groups, and tamsulosin – dutasteride combination therapy for 1 day, 3 day, and 6 day groups. BPH was induced with 3 mg/kg of testosterone proprionate for 3 weeks, continued with drugs administration according to intervention grouping. Prostate stromal tissue was taken and prepared for PKC-α enzyme measurement with ELISA method. **Results:** There was a significant difference ($p<0.05$) in the effect of tamsulosin monotherapy and tamsulosin-dutasteride combination therapy on the PKC-α expression. There was a strong positive relationship between the duration of tamsulosin–dutasteride combination therapy on the PKC-α expression, which means the longer the duration of the combination of tamsulosin–dutasteride combination the higher the PKC-α expression. **Conclusion:** Administration of long-term tamsulosin – dutasteride combination therapy causes upregulation PKC-α expression more than tamsulosin only.

Keywords: BPH, PKC-α, tamsulosin, tamsulosin – dutasteride combination, upregulation.

1. BACKGROUND

Benign prostatic hyperplasia (BPH) is a histological diagnosis that shows the proliferation of smooth muscle and epithelial cells in transitional zone of the prostate (1). The enlarged prostate causes the lower urinary tract symptoms (LUTS) in two ways: (1) direct obstruction to the bladder outlet (bladder outlet obstruction / BOO) due to tissue enlargement (static component); and increased smooth muscle tone and its resistance (dynamin component) (1).

The α-adrenergic receptor antagonist is an effective therapeutic agent for treating LUTS due to BPH because the prostatic smooth muscle basic tone mainly involves α-adrenergic receptors, especially α-1a. Tamsulosin as selective α-adrenergic antagonist is very effective in treating BPH (8, 10). While 5-α reductase inhibitor (5-ARI) inhibits the 5-α reductase enzyme (5-AR) which converts testosterone into dihydrotestosterone (DHT). Dutasteride as a second generation of 5-ARI could inhibit both of 5-AR isoenzymes and reduce static component of BPH (1, 2). However, long-term administration of

α-adrenergic receptor antagonists can cause upregulation of receptor mRNA expression. This causes difficulty in receptor inhibition in the process of smooth muscle contraction, resulting in a decrease of effectiveness of the drugs (3).

Protein kinase C (PKC) is a family of serine / threonine kinase proteins that are mainly involved in intracellular signal transduction, one of its isoforms is PKC-α (5). PKC-α is involved in the process of prostate smooth muscle contraction through activation of the voltage-gated Ca2+ conducted canal. PKC-α is also influenced by androgen hormones, especially testosterone, and has an isoform with Twist1, a transcription factor that plays a role in up-regulation of androgen receptors, so that inhibiting PKC could decrease Twist1 expression, blocking the AR marker and decrease AR induction (6, 7).

Based on this background, it is necessary to conduct a research on the administration of long-term α-adrenergic receptor antagonist (tamsulosin) as monotherapy and in combination with 5-α reductase inhibitor (dutasteride) on PKC-α expression in prostate stromal tissue.

2. OBJECTIVE

The aim of this study was to compare the effect of long-term tamsulosin monotherapy and tamsulosin – dutasteride combination therapy in PKC-α enzyme expression in prostate stromal tissue of Rattus norvegicus rats of Wistar strain.

3. MATERIAL AND METHODS

This study was designed using a controlled, prospective post-test only experimental method. This study measured the PKC-α expression from prostate stromal tissue of BPH. Ethical clearance number 400/144/K.3/302/2019.

Study Design

80 samples of Rattus norvegicus rats of Wistar strain were acclimatized for 2 weeks and then divided into 8 groups (10 samples per group) with different interventions: negative control group, positive control group, tamsulosin monotherapy administration for 1 day, 3 day, and 6 day groups, and tamsulosin – dutasteride combination therapy for 1 day, 3 day, and 6 day groups. All groups, except negative control group, were castrated and induced to BPH with 3 mg/kg of testosterone proprionate for 3 weeks. Drugs administration were given by gastric lavage according to intervention grouping: negative and positive control group were given 5 mg/kg of distilled water, tamsulosine monotherapy groups were given 1 mg/kg of tamsulosin for 1, 3, and 6 days, and tamsulosin – dutasteride combination therapy group were given combination of 1 mg/kg of tamsulosin + 0,5 mg/kg of dutasteride for 1, 3, and 6 days. After drug administration was done, samples were terminated and prostate stromal tissue was taken.

Measurement of PKC-α Enzyme

Prostate stromal tissue samples were prepared with vertical laminary air flow. Prepared prostate tissue then rinsed off one time with phosphate buffered saline (PBS), homogenized in 1 ml PBS, and stored overnight in -20oC temperature. After two freezing-thawing cycles to break down cell membrane, homogenate were centrifuged for 5 minutes in 2-8oC temperature. Supernatant were taken and assayed (11). PKC-α enzyme expression were measured with ELISA tehchnique using Rat p-PKC ELISA Kit from Abbexa® product serial number CSB-E12801r.

Statistical analysis

Results data is shown in mean ± SD. All data were analyzed using SPSS version 2.1 software using Kruskall Wallis parametric test if fulfilling data normality Kolmogorov-Smirnov test and homogeneity Levene test. If data normality isn't met, Chi Square test is preferred. Correlation was analyzed using Spearman test. Data were analyzed with confidence rate of 95% and α=0.05.

4. RESULTS

Based on descriptive analysis of all 8 groups of interventions, it was found that negative control group (without castration) showed the highest mean of PKC-α enzyme level in prostate stromal tissue. The group which was given tamsulosin – dutasteride combination therapy for 1 day showed the lowest mean of PKC-α enzyme level in prostate stromal tissue of Rattus norvegicus of Wistar strain.

Figure 1 illustrates the mean levels of PKC-α in the group giving tamsulosin and tamsulosin-dutasteride combinations have significant differences. It was found

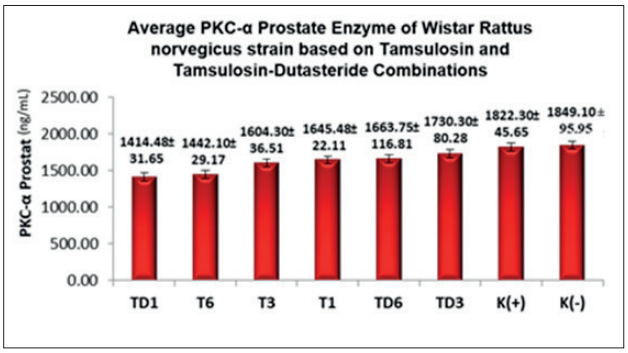

Figure 1. Average PKC-α Prostate Enzyme of Wistar Rattus norvegicus strain based on Tamsulosin and Tamsulosin-Dutasteride Combinations

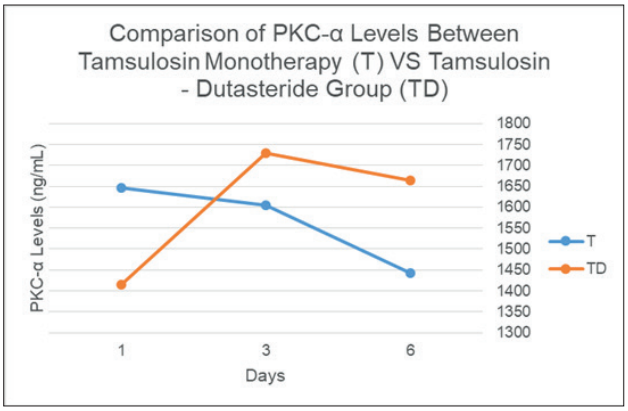

Figure 2. Comparison of PKC-α levels of the Tamsulosin Monotherapy Group and the Tamsulosin-Dutasteride Combination

that long-term alpha PKC levels would increase in the group with the combination of tamsulosin-dutasteride with level in day 3 was 1822.30 + 45.65 compared with tamsulosin alone with level 1604.30 + 36.51.

In Figure 2 shows the levels of PKC-α monotherapy compared with a combination. It was found that long-term alpha PKC levels would increase in the group with the combination of tamsulosin-dutasteride with level in day 3 was 1822.30 + 45.65 compared with tamsulosin alone with level 1604.30 + 36.51. In day 6, tamsulosin-dutasteride combination at level of 1663.75 + 116.81 while tamsulosin alone at level of 1442.10 + 29.17.

According to Chi Square test, there was a significant difference between administration of tamsulosin monotherapy and tamsulosin – dutasteride combination therapy on PKC-α enzyme expression in prostate stromal tissue of Rattus norvegicus rats of Wistar strain. The negative control group, which showed the highest PKC-α level, was significantly different with tamsulosin – dutasteride combination for 1 day group, tamsulosin monotherapy for 6 day group, tamsulosin monotherapy for 3 day group, tamsulosin monotherapy for 1 day group, tamsulosin – dutasteride combination therapy for 6 day group, and tamsulosin – dutasteride combination therapy for 3 day group in PKC-α level. But it was not significantly different from positive control group.

While tamsulosin – dutasteride combination therapy for 1 day group, which showed the lowest PKC-α level, was significantly different from tamsulosin monotherapy for 3 days group, tamsulosin monotherapy for 1 day group, tamsulosin – dutasteride combination therapy for 6 day group, tamsulosin – dutasteride combination therapy for 3 days group, positive control group, and negative control group in PKC-α level. But it was not significantly different from tamsulosin monotherapy for 6 days group.

Correlation Coefficient	Probability
0.608	0.000

Table 1. Spearman correlation study

Based on Spearmen correlation test, we found a strong negative correlation (opposite), which means the longer the administration of tamsulosin monotherapy, the lower the PKC-α level in rats' prostate stromal tissue. We also found a strong positive correlation, which means the longer the administration of tamsulosin – dutasteride combination therapy the higher the PKC-α level in rats' prostate stromal tissue.

5. DISCUSSION

Human prostate stroma consists of smooth muscle cells that have α-1A, α-1B, and α-1D adrenergic receptor subtypes responsible for smooth muscle tone. Activation of α1 adrenergic receptors will stimulate phospholipase C to produce diacylglycerol second messenger (DAG) and inositol triphosphate (IP3), which will activate protein kinase C (PKC), including the PKC-α subtype, and trigger the release of calcium deposits that are sensitive to IP3. Prostate tissue contraction is sensitive to the release of intracellular Ca2 + inhibitors and type L calcium channel blockers. In its contraction, PKC activation is involved in prostatic cell contractility (4).

PKC isoforms α, β1, β2, and γ require DAG and Ca2 + for activation. Previous study showed that activation of α1 adrenoreceptors stimulates PKC-α activity in human prostate cells. Because PKC-α is a conventional PKC isoform that requires DAG and Ca2+ for its activation, this proves the activation of the second messenger PLC pathway by prostate adrenoreceptors. In that study, contractions induced by phenylephrine caused PKC-α translocation (4). Thus, activation of the α 1 adrenergic receptor stimulates the PLC, then produces IP3 and DAG, and translocates PKC-α and opening the L type calcium channel. Therefore, blockade of contraction by alpha blocker agents can decrease PKC-α (4).

However, previous studies have shown that prolonged administration of alpha blockers can cause upregulation of α1 adrenergic receptors, which is characterized by a decrease in the effectiveness of alpha blocker agents after more than six months of use. Long-term use of alpha blockers can cause increased expression of α1 type mRNA in the ventral and dorsolateral prostate (12). Increased expression of α1 type adrenergic receptor mRNAs causes hypersensitivity of agonist agents and resistance to antagonistic agents (3, 13, 14).

Expression of α1 adrenergic receptors varies in each organ. The α1 receptor protein is most abundant in the prostate, with the dominance of the α1a receptor subtype, followed by α1b and α1d. The difference in location and activity of the α1 receptor causes different resistance patterns due to upregulation of the α1 receptor. α1a subtype receptors are present on the surface and intracellular, α1b subtypes on the cell surface, and α1d on intracellular (3). Long term administration of alpha blockers makes the receptors inactive so that phosphorylation is not easy to occur, so the density of the adrenergic receptor α becomes more abundant. Due to the dominance of the prostate receptor type, tamsulosin will provide upregulation especially in the α1a subtype (14).

Protein kinase C (PKC) family consists of 12 serine–threonine kinase isoenzymes that play an important role mainly in the transmembrane signaling pathway. Therefore, the PKC regulates cell proliferation and differentiation, cell-cell interactions, secretions, cytoskeletal function, gene transcription, apoptosis, and drug resistance. The PKC isoenzyme family is divided into three main categories based on its cofactor needs: classic, novel and atypical. Classical PKC (α, β1, β2, and γ) and the new PKC δ, ε, η, and θ) can be activated by the second messenger DAG produced by the receptor-ligand binding. Classical PKC isozyme requires calcium in its activation, while atypical PKC (ζ, λ, and μ) are not calcium dependent (15, 16).

The PKC isoenzymes initially belong to different subcellular compartments, and are regulators of intracellular homeostasis, intracellular communication, and the exchange of various protein and peptide molecules through their interactions with receptors for activated C kinases (RACK) at specific sites. PKC activation in-

volves translocation from the cytosol to the binding site of specific proteins in the cell membrane. After translocation from the cytosol to the cell membrane, activated PKC forms a complex with receptor proteins, phosphatidylserine, and several sitoactive proteins including drug-resistant glycoprotein p170. This can involve four basic types of interactions: forming membrane complexes that are dependent on Ca2+ ions which are catalytically inactive, forming two membrane contacts at C1 and C2, involving molecular conformational changes, and additional changes after bonding with DAG / phorbol diester. Each isoenzyme is characterized by its ability to phosphorylate certain intracellular protein spectrums, depending on the subcellular location, specificity of the substrate, and its activation requirements (15–18).

PKC in the cell nucleus is in an inactive form and undergoes phosphorylation. The first phosphorylation involves phosphoinositide-dependent protein kinase I (PDK1), then PKC undergoes two more autophosphorylation. After undergoing phosphorylation, PKC enters the cytosol even though it is still in an inactive form. PKC activation occurs if the C1 region binds to DAG and the C2 region binds to Ca2 + and phosphatidyl serine (16-18). Adrenergic receptor mRNA upregulation causes an increase in α1 receptor activity due to increased sensitivity. Thus, PKC-α activity also increases as a result of stimulation of adrenergic receptor α (3).

Based on statistical results, it is shown that the tamsulosin-dutasteride combination therapy for 1 day gives lower PKC-α results than the tamsulosin-dutasteride combination therapy for 3 days and 6 days. This is consistent with previous research that long-term administration of alpha blocker will cause upregulation thereby increasing PKC-α levels. The results of PKC-α levels obtained by the combination of Tamsulosin Dutasteride are higher than Tamsulosin alone in the long run. This refers to the study of Andreollo et al, 2012 which says that the ratio of human age to rats is 13.2 rat days is 1 human year, and in other words 1 rat day is equal to 1 human month (3).

However, the results of this study do not show any regularity in increase of PKC-α regarding the duration of tamsulosin monotherapy nor the tamsulosin – dutasteride combination therapy administration. This could be due to other factors that influence PKC-α levels in rat prostate stroma, for example initial PKC-α levels and inflammatory conditions that occur during treatment. In addition, the duration of giving tamsulosin to provide upregulation effects in humans is approximately 6 months (3), which in experimental rats was not long enough to induce upregulation. Therefore, further research with minimum confounding factors and longer duration of therapy administration is needed to expose the upregulation process clearer.

A previous study also concluded on the sixth to the twelfth day, the continuous monotherapy tamsulosin administration has an upregulation impact (20,21). On the first day, the prostate's smooth muscle cells are less contractile; however, by the sixth to the twelfth day, they are more so (22, 23). On the other hand, this study's findings also indicated that tamsulosin and dutasteride had the impact of reducing contractility, which peaked on day 12 (21-23).

A limitation of this study is that the duration of dutasteride in rats takes longer to induce increased PKC-α expression. Therefore, further research is needed with treatment on rats for more than six days simultaneously. Further research is also needed regarding the negative feedback mechanism by the PKC-α enzyme on phospholipase C activity.

6. CONCLUSION

Administration of long-term tamsulosin – dutasteride combination therapy causes upregulation PKC-α expression more than tamsulosin only.

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- **Conflict of interest** : None declared.
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REFERENCES

1. Bruskewitz RC, Foster HE, Mcnally T, Chan B, Zuckerman M. American Urological Association Guideline : Management of Benign Prostatic Hyperplasia (BPH). Am Urol Assoc Educ Res int. 2010;
2. Traish AM, Hassani J, Guay AT, Zitzmann M, Hansen ML. Adverse side effects of 5α-reductase inhibitors therapy: Persistent diminished libido and erectile dysfunction and depression in a subset of patients. J Sex Med. 2011;
3. Daryanto B, Ali M, Purnomo BB, Mintaroem K. The effect of long-term treatment with an alpha-adrenergic receptor antagonist on contractility of smooth muscle, mRNA, calcium level, and protein kinase C alpha enzyme of prostate in men with benign prostatic hyperplasia. Drug Invent Today. 2019; 12(4): 663–669.
4. Preston A, Haynes JM. a1-Adrenoceptor effects mediated by protein kinase C a in human cultured prostatic stromal cells. Br J Pharmacol. 2003; 138: 218–224.
5. Loegering DJ, Lennartz MR. Protein kinase C and toll-like receptor signaling. Enzyme Research. 2011.
6. Shiota M, Yokomizo A, Takeuchi A, Imada K, Kashiwagi E, Song YH, et al. Inhibition of Protein Kinase C/Twist1 Signaling Augments Anticancer Effects of Androgen Deprivation and Enzalutamide in Prostate Cancer. Clin Cancer Res. 2013; 20(4): 951–961.
7. Maddali KK, Korzick DH, Tharp DL, Bowles DK. PKC Delta Mediates Testosterone-induced Increases in Coronary Smooth Muscle voltage-gated calcium channels 1.2. J Biol Chem. 2005; 280(52): 43024–43029.
8. Chan SW. Pathology and medical therapy of benign prostatic hyperplasia. Med Bull. 2011;
9. Lee C-L, Kuo H-C. Pathophysiology of benign prostate enlargement and lower urinary tract symptoms: Current concepts. Tzu Chi Med J. 2017;
10. Roehrborn CG. Efficacy of -Adrenergic Receptor Blockers in the Treatment of Male Lower Urinary Tract Symptoms. Medreviews Urol. 2009; 11(1): S1–S8.

11. Soetikno V, Watanabe K, Sari FR, Harima M, Thandavarayan RA, Veeraveedu PT, et al. Curcumin attenuates diabetic nephropathy by inhibiting PKC-a and PKC-b1 activity in streptozotocin-induced type I diabetic rats. Mol Nutr Food Res. 2011; 2011(55): 1655–1665.
12. Setyawati A. Farmakologi dan Terapi Universitas Indonesia Edisi ke-5. 5th ed. Jakarta: Fakultas Kedokteran Universitas Indonesia; 2012. 13–14 p.
13. Imamura T, Ishii K, Kanda H, Sugimura Y. Structural Changes in a1-adrenoreceptor antagonist-treated human prostatic stroma. Clin Exp Med. 2009; 10(2): 99–106.
14. Drummond PD, Drummond ES, Dawson LF, Mitchell V, Finch PM, Vaughan CW, et al. Upregulation of a1 adrenoceptors on cutaneous nerve fibres after partial sciatic nerve ligation and in complex regional pain syndrome type II. Pain. 2014; 155: 606–616.
15. Cornford P, Evans J, Dodson A, Parsons K, Woolfenden A, Neoptolemos J, et al. Protein Kinase C Isoenzyme Patterns Characteristically Modulated in Early Prostate Cancer. Am J Pathol. 1999; 154(1): 137–145.
16. Noh J, Wende A., Olsen CD, Kim B, Bevins J, Zhu Y, et al. Phosphoinositide dependent protein kinase 1 is required for exercise-induced cardiac hypertrophy but not the associated mitochondrial adaptations. J Mol Cell Cardiol. 2015; 89: 297–305.
17. Wu-Zhang AX, Newton A. Protein kinase C pharmacology: refining the toolbox. Biochem J. 2013; 452(2): 195–209.
18. Brooks AJ, Dai W, O'Mara ML, Abankwa D, Chhabra Y, Pelekanos RA, et al. Mechanism of Activation of Protein Kinase JAK2 by the Growth Hormone Receptor. Science (80-). 2014; 344(6185): 1249783.
19. Budaya TN, Daryanto B. A To Z BPH (Benign Prostatic Hyperplasia). Universitas Brawijaya Press; 2019 Sep 30.
20. Prasetyo ZA, Budaya TN, Daryanto B. Characteristics of benign prostatic hyperplasia (BPH) patients undergoing transurethral resection of the prostate (TURP). Jurnal Kedokteran Brawijaya. 2021 Aug 31; 31(4): 220-223.
21. Daryanto B, Naim HY, Budaya TN. The Effect of Tamsulosin, Dutasteride Monotherapy and Tamsulosin-Dutasteride Combination on Prostate Smooth Muscle Contractility in BPH Model Wistar Strain Rattus Novergicus. Medical Archives. 2023 Feb; 77(1): 13.
22. Daryanto B, Sagala AE. Long term effect of alpha-adrenergic antagonist tamsulosin on prostate smooth muscle apoptosis in BPH patients. In: BJU INTERNATIONAL 2017 Apr 1 (Vol. 119, pp. 40-40). 111 RIVER ST, HOBOKEN 07030-5774, NJ USA: WILEY.
23. Daryanto B, Soeharto S, Purnomo BB, Arliyansyah FK. The effect of long term alfa adrenoceptor antagonist treatment on prostatic muscle contraction response after exposure to alfa adrenoceptor agonist (in vitro) in men with BPH. INTERNATIONAL JOURNAL OF UROLOGY 2016 Jul 1 (Vol. 23, pp. 122-122). 111 RIVER ST, HOBOKEN 07030-5774, NJ USA: WILEY-BLACKWELL