The Effect of Tamsulosin, Dutasteride Monotherapy and Tamsulosin-Dutasteride Combination on Prostate Smooth Muscle Contractility in BPH Model Wistar Strain Rattus Norvegicus

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ABSTRACT

Background: Following the c In the management of BPH, Tamsulosin is an example of α-adrenergic receptor blocker drug that is usually used. In addition, dutasteride is also a BPH drug that works as a group of 5 α reductase inhibitor. However, the weakness of long-term administration of α1-adrenergic receptor antagonists can result in upregulation of prostate smooth muscle cell contractility and expression of α-adrenergic mRNA receptors, resulting in hyperactivity and supersensitivity to α-agonists. Objective: Our study aimed to determine the effect of long-term administration of tamsulosin, dutasteride and tamsulosin-dutasteride combination on the contractility of prostate smooth muscle cells in BPH model rats. Methods: This study was designed using an experimental post test only method, control group design. It measured the contractility of prostate smooth muscle cells from samples obtained from the prostatic stroma of experimental animals adult male Rattus norvegicus Wistar strain induced BPH and administered tamsulosin 1 mg/kg/day, dutasteride 0.5 mg/kg/day, and a combination of continuous administration for 1, 6 and 12 consecutive days. Data were analyzed using one way ANOVA if the data distribution was normal or Kruskall Walis if the data distribution was abnormal. Result: The effect of tamsulosin, dutasteride and the combination of tamsulosin and dutasteride on prostate smooth muscle cell contractility in experimental animals Rattus norvegicus Wistar strain showed that tamsulosin administration for six days, twelve days, and the combination of tamsulosin dutasteride for one day got statistically significant different result (p=0.016; p=0.006; p=0.029) compared to the negative control group. In addition, there was a difference between the tamsulosin and dutasteride combination group for 12 days compared to tamsulosin monotherapy for 6 days and 12 days (p=0.160; p=0.010). Conclusion: Continuous administration of monotherapy tamsulosin has an upregulation effect on the sixth to twelfth day. Decreased contractility of prostate smooth muscle cells occurs on the first day but will increase on the sixth to twelfth day. On the other hand, the results of our study also showed that the combination of tamsulosin and dutasteride gave the effect of reducing contractility and was most effective on day 12. Keywords: Dutasteride, Muscle contractility, Tamsulosin.

1. BACKGROUND

Benign Prostate Hyperplasia (BPH) is the most common benign disease in the prostate gland and is caused by benign hyperplasia of the smooth muscle cells of the prostate and the stromal cells of the prostate gland.1 Its prevalence increases by the age, occurring in 50-75% of men aged 50 years, and reaches the peak up to 88-90% in men aged > 81 years.2 BPH condition occurs in older men causing urinary tract disorder that correlate to a decrease in Health Related Quality of Life.2 BPH patients with Lower Urinary Tract Symptoms (LUTS) are not only caused by increased contractility of smooth muscle tone in the prostate gland, prostatic stroma, and bladder neck which is known as the dynamic component, but also due to an increase in prostate volume which causes blockage in the posterior urethra which is known as the static component.4 These symptoms of LUTS can increase patient morbidity.
and causes a decrease in quality of life which results in patients coming to health services for treatment.

Treatment of LUTS symptoms caused by BPH has progressed significantly based on research and there is a drug of choice which is widely known as a strategy for treating BPH with LUTS symptoms. There are two groups of drugs that are most often used, namely α-adrenergic receptor blockers and 5 α-reductase inhibitors (5-ARI).

Tamsulosin is α-adrenergic receptor blocker, while dutasteride is a class 5 α reductase inhibitor and reduces LUTS symptoms.1 The disadvantage of long-term administration of α1-adrenergic receptor antagonist is that it can result in upregulation of prostate smooth muscle cell contractility and α-adrenergic receptor mRNA expression, namely hyperactivity due to super sensitivity to α-agonists.5 This is supported by studies in experimental animals by giving one of the α blockers, namely doxazosin 2-4 mg/kgBW subcutaneously with an additional 4mg/kgBW orally every day for 8 to 12 weeks causes upregulation of mRNAα1-adrenergic receptor.6 Administration of drugs combination with 5-α Reductase Inhibitor (5-ARI) is expected to decrease the contractility of prostate smooth muscle cells. However, in previous animal studies, administration of one of the 5 ARI class drug, dutasteride at a dose of 0.5 mg/kgBW/day for 2 months, showed no statistically significant difference in the mRNA expression of -1A adrenergic receptor.

Based on the theoretical basis, it is necessary to further study regarding the long-term administration of tamsulosin, monotherapy dutasteride and tamsulosin-dutasteride combination in order to maintain their effectiveness in improving LUTS symptoms in BPH.

2. OBJECTIVE

The aim of this study was to examine the effect of long-term administration of tamsulosin, dutasteride and tamsulosin-dutasteride combination on the contractility of prostate smooth muscle cells in prostate stromal tissue of BPH model rats.

3. MATERIAL AND METHODS

Research methods

Experiment Design and Experimental Animals

This study was designed using an experimental post test only method, control group design. The method used was a completely randomized design because the experimental animals, experimental sites, and other research materials were homogeneous. It measured the contractility of prostate smooth muscle cells from samples obtained from the prostatic stroma of experimental animals adult male Rattus norvegicus Wistar strain induced BPH and administered with tamsulosin 1 mg/kg/day, dutasteride 0.5 mg/kg/day, and administration combination via sondase (gastric lavage) with a total oral administration volume of 5 mL/kg and evaluated on days 1, 6 and 12, and observations were made.7

The experimental animal were 10-week-old male Rattus norvegicus Wistar strain weighing 250-300 g which was obtained from the Laboratory of the Biosciences Institute, Faculty of Medicine, Universitas Brawijaya. This study uses the Federer’s formula (T-1) (N-1) > 15 sample calculation formula.8

Based on this formula, the samples used in each group have a minimum sample size of 3 rats. It used a sample of 55 rats divided into 11 groups, in each group there were 5 male Rattus norvegicus Wistar rats.

The experimental animal, male Rattus norvegicus Wistar strain, aged 10 weeks weighing 250-300 g which had been selected according to the inclusion criteria, were divided into 11 groups. Then the T1, T3, T6, D1, D3, D6, TD1, TD3, TD6 groups were castrated first and prophylactic antibiotics were given using penicillin (7.14x104 IU/kgbw).9 After 7 days, testosterone propionate was administered. After that, it was terminated with Pento-barbital 100 mg/kg intraperitoneally and continued by measuring the contractility of the smooth muscle of the prostate. Tissue was removed by prostatectomy.

Immediately after the results of the prostatectomy of the prostate are taken, the blood and urine are washed with physiological saline solution. Then the tissue sample was inserted into the tube transport medium for examination of contraction using thyrod fluid and checked before 48 hours, then sent to the Pharmacology Laboratory of Mulawarman University.

Evaluation

The prostate stromal tissue was removed from the transport medium and then washed with physiological fluids. Then the prostate stromal tissue was cut into smaller pieces with sterile scissors with a size of + 0.5 cm, separated from the necrotic tissue. The cuts were made on a cup filled with Tyrod's fluid. The resulting tissue pieces were immediately placed in the organ bath in the form of 10 cc of Tyrod buffer fluid which was fed with oxygen at 37°C. The results of the preparation were connected to the organ bath system. The tissue sections were stabilized for + 1 hour beforehand. The piece of tissue is slowly stretched with the most optimal resting pull with a weight of 0.5 g. After the resting stress stabilizes, the organ bath fluid is added with phenylephrine at a dose of 1 mg/L or 10-6 mg/mL, 10 mg/L or 10-5 mg/mL, and 100 mg/L or 10-4 mg/mL to obtain rapid tone and followed by steady contraction (tonic contraction). The cumulative contraction results in the form of a curve were recorded using the MLS023 chart software.

Statistical Analysis

Data were analyzed using One Way ANOVA if the data distribution was normal or Kruskall Walis and Mann-Whitney if the data distribution was not normal. Data analysis was performed using SPSS version 22. (IBM Corp. Released 2013. IBM SPSS Statistics for 4 Windows, Version 22.0. Armonk, NY: IBM Corp).

4. RESULTS

Descriptive analysis was carried out to see the mean, standard deviation, minimum and maximum values of the prostate weight of experimental animals. Treatment with dutasteride for 1 day had the highest average prostate weight in animals. Meanwhile, the negative control
had the lowest average prostate weight in mice, Rattus norvegicus Wistar strain. The normality test of prostate weight data of rats in the negative control group and prostate weight of BPH rats was carried out using Kolmogorov Smirnov with $p = 0.200$ so that the data were normally distributed. The statistical test was continued with an independent t test between the negative control group and the BPH group, the results were $p = 0.000$ so it can be concluded that the prostate weight of BPH rats was statistically significantly higher than the negative control group and BPH induction was successful. This was also evidenced by prostate histology preparation in the positive control group which showed an enlargement of stromal cells and an increase in epithelial cells.

The mean and standard deviation of the contractility of prostate smooth muscle cells in experimental animals based on the administration of tamsulosin, dutasteride and the combination of tamsulosin with dutasteride can be seen in Figure 1. The treatment group showed that the tamsulosin group on the twelfth day had an average contractility of prostate smooth muscle cells that was higher. While the administration of tamsulosin-dutasteride on the twelfth day had the lowest mean contractility of prostate muscle cells in experimental animals. Dutasteride on the first 6th and 12th days had a lower mean prostate smooth muscle contractility than the BPH group but could not approach the normal condition (negative control). The decrease in contractility occurred slowly from day to day until on the 12th day the highest decrease was found, as shown in Figure 2. Meanwhile, the Dutasteride group showed no different results of prostate smooth muscle contractility on days 1, 6, and 12. The tamsulosin-dutasteride combination treatment group had lower prostate smooth muscle contractility than the BPH group even on the twelfth day of administration, close to normal conditions (negative control), as can be seen in Figure 3.

Testing the difference between the administration of tamsulosin, dutasteride and the combination of tamsulosin and dutasteride on the contractility of prostate smooth muscle cells in experimental animals was carried out using the Kruskal Wallis test because the data distribution was not normal (based on the Kolmogorov Smirnov test) ($p=0.001$).

The test results with Kruskal Wallis were obtained; $p=0.017$ indicated that there was a significant difference in the administration of tamsulosin, dutasteride, and the combination of tamsulosin and dutasteride on smooth muscle contractility in experimental animals Rattus norvegicus Wistar strain. Furthermore, the Mann Whitney test was carried out to determine the difference between the 2 groups. Based on the Mann Whitney test, there was a significant difference in smooth muscle contractility between the negative control group without BPH and the positive group with BPH ($p=0.001$), taking tamsulosin on day 6 and day 12 ($p=0.012$; $p=0.007$). and administration of tamsulosin dutasteride combination on the first day ($p=0.042$).

There was a significant difference between the positive control group induced by BPH and the negative group, day 1 tamsulosin, day 6 dutasteride and the combination of tamsulosin dutasteride on day 1, day 6, and day 12 ($p=0.004$; $p=0.034$; $p=0.026$; $p=0.049$; $p=0.049$; $p=0.011$). In addition, administration of tamsulosin in experimental animals for 1 day (equivalent to a month in humans), dutasteride for 6 days and the combination of tamsulosin dutasteride for 1, 6, 12 days can reduce the contractility of prostate smooth muscle in this study. The results of our study also showed that there was a statistically significant difference between the tamsulosin-dutasteride combination group for 12 days compared to tamsulosin monotherapy for 6 days and 12 days indicating that the tamsulosin-dutasteride combination was more effective than tamsulosin alone or dutasteride monotherapy ($p=0.160$; $p=0.010$). In addition, administration of a combination of tamsulosin and dutasteride for 12 days could significantly reduce prostate smooth muscle contractility ($p=0.001$) even close to normal prostate smooth muscle contractility (negative control without BPH) with $p=0.722$. The results of our Mann Whitney test are shown in Table 1.

5. DISCUSSION

Based on the results of this study, there was a statistically significant difference in contractility of prostate smooth muscle cells between the BPH-positive control group receiving 1 day of tamsulosin therapy, but contractility increased until there was no significant difference between the BPH group and the group receiving tamsulosin 6 and 12 day.

This is in accordance with the research of Daryanto et al. in 2019, where the administration of tamsulosin which acts as an alpha blocker can decrease the contractility of prostate smooth muscle cells in the short term, but actually increase the contractility due to the mechanism of upregulation of alpha receptors in the long term.

Increased contractility is the final outcome of the upregulation process characterized by an increase in 1 receptor mRNA, PKC-α, and intracellular calcium. Increased m-RNA at prostate adrenergic receptors causes an increase in 1 receptors due to increased sensitivity of the prostate gland itself. Theoretically, the receptor density becomes higher which in turn causes an increase in the activity of the protein kinase C-alpha (PKC-α) enzyme and intracellular calcium levels. Increased levels of DHT will also increase cAMP in the prostate gland which together with calcium ions causes PKC-α activation. PKC-α enzyme activation shares a common marker pathway with various aspects of smooth muscle biology ranging from increased contractile activity to proliferation.

There were differences in prostate smooth muscle contractility in the BPH positive control group compared to the group receiving dutasteride treatment for 1 day, where the prostate smooth muscle contractility in the group receiving dutasteride for 1 day was lower than the BPH group but no statistically significant results were obtained. This is in accordance with the research of Wang et al., 2014 where it was found that there was no significant difference in contractility between the
group receiving dutasteride therapy compared to the BPH group. This is possible because the mechanism of action of dutasteride which is a class 5 alpha reductase inhibitor does not affect contractility. However, on long-term administration, there was a significant decrease in contractility between the BPH positive control group compared to the group receiving dutasteride therapy for 6 and 12 days. This is in accordance with clinical studies where improvement in LUTS symptoms only occurred after long-term administration of dutasteride, and LUTS symptoms were pathophysiologically related to prostate smooth muscle cell contractility. Research by Suharibawa et al. (2021) found that there was no effect of dutasteride monotherapy which is a class 5 ARI drug as a long-term DHT-lowering drug on PKC alpha. PKC alpha induces relaxation and does not induce contraction of prostate smooth muscle despite overexpression of PKC-potentiated protein phosphatase-1 inhibitory protein (CPI-17).

The combination of tamsulosin and dutasteride can significantly reduce the contractility of prostate smooth muscle cells on the first, sixth, and twelfth days. This is in accordance with the study of Roehrborn et al., which stated that the combination of tamsulosin-dutasteride could significantly reduce peak urinary flow compared to tamsulosin monotherapy or dutasteride alone. The number of samples of 4844 also proved that tamsulosin-dutasteride combination therapy was superior to tamsulosin monotherapy alone or dutasteride monotherapy.

Another research stated that the combination of tamsulosin dutasteride can reduce all parameters of BPH including IPSS, prostate volume, and PSA with administration for 24 months. Another study conducted by Suharibawa et al. (2021) it was found that the combination of tamsulosin-dutasteride gave an increase in PKC-alpha enzyme expression when compared to the administration of tamsulosin and dutasteride alone on days 1, 3 and 6.

Dutasteride is a drug that prevents testosterone from turning into dihydrotestosterone (DHT). The DHT hormone works to provoke the mechanical potentiation of smooth muscle contraction through calcium sensitization. The hormone DHT (10 nM) increased phosphorylation of the 20-kDa myosin light chain (LC20), [regulatory myosin light chain, (MLC)] and CPI-17 (an endogenous inhibitor of MLC phosphatase). In addition, inhibition of Rho-associated Rho kinase (ROK) and kinase C (PKC) as well as Y27632 and chelethrine, prevents phosphorylation of LC20 phosphorylation reducing calcium sensitization. Inhibition of ROK prevents phosphorylation of CPI-17 thereby preventing the CPI-17-mediated PKC signaling cascade. DHT induces rapid activation of RhoA and its translocation to the plasma membrane to activate ROK. DHT hormone induces smooth muscle sensitization to calcium through the ROK activation pathway by activating PKC to induce phosphorylation of CPI-17. Activation of this pathway induces stimulation of LC20 by inhibiting MLC phosphatase and altering the equilibrium of the regulatory subunits under phosphorylation conditions. Smooth muscle is a physiological target of DHT and DHT increases prostatic smooth muscle contractility via a non-genomic calcium sensitization pathway.

This research can be the basis for further research in humans or research on mice with a larger scale and a longer period of time. The results of this study can be used as the basis for an understanding of the mechanism regarding the effect of tamsulosin, dutasteride or the combination of tamsulosin-dutasteride on the improvement of clinical symptoms. In this study, the duration of administration of tamsulosin, dutasteride or the combination of tamsulosin and dutasteride in rats required a longer time to cause an increase in prostate smooth muscle contractility.

Therefore, further research is needed with treatment in rats for more than 12 days simultaneously which can minimize the confounding factors that affect the variability of smooth muscle contractility by of course following the stages of laboratory and clinical trials. In addition, further research is needed on other upregulation markers such as intracellular calcium, alpha receptor mRNA, and PKC Alpha.

The implication of the results of the study on the relationship with the time line in humans is that one day of drug administration in experimental animals is equivalent to 26.7 days of drug administration in humans. The use of the drug for 6 days in mice is equivalent to giving it for six months in humans, and for 12 days it is equivalent to giving it for a year in humans (Sengupta, 2013). therefore, the use of tamsulosin in the first month will decrease the contractility of the smooth muscle of the prostate but on long-term administration of up to 6 months and even one year there is an upregulation of alpha receptors so that the receptors become resistant and the drug no longer works. Administration of the drug dutasteride can reduce contractility but not significantly on the first day and will begin to appear in the sixth month and one year in humans. The combination of tamsulosin and dutasteride can reduce the upregulation of alpha receptors so that it is effective in reducing contractility until the first human year.

6. CONCLUSION

Our study showed that there were differences in prostate contractility in a group of Rattus norvegicus Wistar rats given tamsulosin 1mg/kg/day, dutasteride 0.5mg/kg/day, and the combination continuous administration for 1, 6 and 12 days, respectively. There was a significant difference between the positive control group induced by BPH and the negative group, day 1 tamsulosin, day 6 dutasteride and the combination of tamsulosin dutasteride on day 1, day 6, and day 12. In addition, our study also showed that administration of a combination of tamsulosin and dutasteride for 12 days could significantly reduce prostate smooth muscle contractility (p=0.001) even close to normal prostatic smooth muscle contractility (negative control without BPH) with p=0.722.
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- **Author’s contribution**: All data underlying the results are available as part of the article and no additional source data are required.
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