# THE EFFECT OF 5α REDUCTASE INHIBITOR AND ESTROGEN IN PROSTATE PROLIFERATION
## An Experimental Study in Rats

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**ABSTRACT**

*This was an experimental study using posttest control group design involving Wistar strain Rattus norwegicus as experimental animal. The purpose of this study was to explain the mechanism of BPH in elderly. Samples were randomly divided into 2 groups, 1- and 2-month group, each comprising 26 rats. Each group was divided further into two subgroups, one group received combined estrogen and finasteride, and the other, receiving finasteride only, served as control group. Each subgroup consisted of 13 rats. After treatment for 1 and 2 months, the prostate was removed and examined for TGF-ß1, EGF, FGF, and proliferation. Immunohistochemistry was used for examining TGF-ß1, EGF, FGF, and the examination of proliferation was carried out using graticulae. This study employed univariate analysis with 2 sample t test as TGF-ß1, EGF, and FGF had no correlation. Data analysis used in this research was univariate analysis with 2 sample t test. Analysis result showed that estrogen could reduce TGF-ß1 significantly in 1 month and 2 month groups ($p < 0.05$) and estrogen also stimulated significant increase of EGF in 2 month groups ($p < 0.05$). Estrogen also increased proliferation significantly in both 1 and 2 month groups ($p < 0.05$) but estrogen did not increase FGF significantly in both groups. Multiple regression analysis on the effect of TGF-ß1, EGF, FGF and estrogen to proliferation revealed that only TGF-ß1 had negative feedback. This indicated that TGF-ß1 decreased, so that the proliferation increased. Estrogen had positive impact in proliferation, indicating that increased estrogen would also increase proliferation. In conclusion, estrogen increased the proliferation of the prostate cell and EGF significantly and decreased the expression of TGF-ß1 significantly. This leads to inhibition of proliferation, and finally may cause the occurrence of BPH.*

***Keywords**: Prostate, estrogen, TGF-ß1, EGF, FGF, BPH*

## INTRODUCTION

Human life expectancy is increasing along with the progress of time, leading to the increase of the number of elderly. Elderly is often subjected to diseases, one of which is prostatic disorder called as benign prostatic hyperplasia (BPH). Prostatic abnormalities most commonly found in elderly comprises BPH (80%), prostate cancer (18%), and prostatitis (2%). The prevalence of BPH is positively proportional with the age of the patients. As reported by Kirby (1994), the rate of obstructive BPH in 40 years old individual is 14%, 60 years 24%, and more than 60 years 43%. In Dr Soetomo Hospital, the annual incidence rate of BPH requiring operation is 250, and most of the patients aged 60 - 70 years (Sunaryo, 1999). Although BPH is common among males, the cause of this diasease remains unclear. It is suggested that BPH results from the influence of androgen and estrogen during aging process, in which there is imbalance between estrogen and testosterone in the prostate (Weisser H et al., 1997). Until today, the mechanism of BPH is still debatable. This study was conducted to disclose the mechanism. The results will be useful for improving and developing BPH management.

As the prevalence rate of BPH in elderly (more than 60 years old) is high, which is around 67% (Sunaryo, 1999), the management of BPH is clearly imperative. Otherwise, the prevalence of obstructive BPH may sharply increase, subjecting the patients to the possibility of having urinary disorder, Lower Urinary Tract Symptoms (LUTS), sudden dysuria, recurrent urinary tract infection, recurrent hematuria, bladder stones, and renal abnormalities. If it is not adequately managed, it may result in a fatality.

In Dr Soetomo Hospital, the annual incidence of BPH requiring operation is 250 cases, occurring mostly in patients aged 60 - 70 years (Sunaryo, 1999). Similarly, Berry (1984) and Yamanouchi (1994) reported that the BPH incidence in patients more than 60 years old was 90%. Several hypothetical explanations have been suggested to explain the mechanism of BPH (Kirby et

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al., 1994). The first is dehydrotestosterone hypothesis, explaining an increase of 5α reductase and androgen receptor that causes hyperlpasia in stromal cells and epithelium. The second hypothesis is about the changed balance between estrogen and testosterone that results in stromal hyperplasia. Interaction of growth factors in the epithelium is another hypothesis, in which hyperplasia of stromal cells and prostate epithelium are induced by the increase of epithelial growth factor (EGF), and fibroblast growth factor (FGF) and the reduction of transforming growth factor beta (TGF-ß1). The fourth hypothesis suggests that the suppressed process of cell death explains the increase of estrogen, leading to enhanced growth of stromal and epithelial cells. Finally, stem cell theory explains the increasing number of stem cells passing through the prostate and proliferates.

In aging process, plasma and salivary testosterone level is decreasing along with age. In autopsy to 925 individuals, it was found that prostate weight increased along with age. This indicated that in aging process, testosterone is decreasing while, conversely, prostate weight is increasing (Coccket ATK et al., 1995). From those hypotheses and facts, it is apparent that the mechanism of BPH is still unclear. Further study and examinations is therefore needed to obtain results that can be used to manage prostate abnormalities, particularly Benign Prostatic Hyperplasia (BPH), in the presence of estrogen increase and testosterone decrease.

The occurrence of BPH in elderly is suggested as being strongly affected by the activity of adrenal gland, since in advanced age there is a decrease of Leydig cells function, so that the production of testosterone is also decreasing. To maintain homeostasis, in such condition the adrenal gland plays an important role to express the protein androstenedion. Androstenedion is an adrenal androgen, the post-menopausal precursor of male and female estrogen (Ganong WF, 2003). Estrogen has a capacity to suppress the expression of TGF-ß1 (Matsuda, 2001). TGF-ß1 itself is produced by prostate cells that have an important role in the inhibition of cell growth or epithelial proliferation in various tissues (Story, 1995; McConnel, 2002). Additionally, it may also trigger cell death in the prostate (Kirby, 1994). Estrogen suppresses TGF-ß1 expression that inhibits proliferations and triggers apoptosis, so that it stimulate epithelial proliferation, stromal cell proliferation, and prostatic stromal cells hyperplasia, leading to a condition of BPH (Griffiths, 2002). BPH therapy has been developed by using 5α reductase inhibitor (finasteride) to reduce existing BPH symptoms. The administration of 5 mg finasteride for 12 months could reduce serum DHT and prostatic volume as much as 75% and 19%, respectively, and improve urinary flow for 1.6 ml/seconds (McConnel, 2002).

As it is not possible to carry out this study in human subjects, Wistar strain white rats were used as experimental animals. To enhance the aging process of prostate in rats, they were given with 5α reductase inhibitor (finasteride), so that dyhydrotestosterone (DHT) reduced, which was further decreasing TGF-ß1, EGF, and FGF. They were also given with estrogen that suppressed the expression of TGF-ß1, triggering the expression of EGF and FGF, and enhancing epithelial and prostatic stromal proliferation.

This study was conducted to address the problems whether the administration of 5α reductase inhibitor and estrogen could reduce the expression of Transforming Growth Factor beta-1 (TGF- beta1), increase the expression of Epidermoid Growth Factor (EGF), and Fibroblast Growth Factor (FGF), as well as the proliferation of prostate epithelial cells in Wistar strain white rats. The general objective of this study was to disclose the mechanism of BPH in elderly, and the particular objectives were to prove the reduction of TGF-ß1, the increase of FGF, and the increase of prostatic epithelial cells proliferation in Wistar strain white rats after the administration of 5α reductase inhibitor and estrogen. The benefit of this study was to provide insights on the mechanism of BPH in elderly as a basis for managing BPH in this age group.

## METHODS

This was an experimental study involving 52 male three-month old Wistar stain white rats, obtained using the formula from Higgins and Klingaum (1985), with the bodyweight of 150 - 200 gr. This study used posttest control group design (Zainuddin, 1995), with the consideration that since the experimental animals were homogeneous, the difference between control and treatment group was present only due to the treatment in this experiment. The study was conducted at Experimental Animal Laboratory, Department of Biochemistry, and at the Department of Pathology, Airlangga University School of Medicine, from October 2003 to July 2004. The rats were divided into two groups, 1 month and 2 month-groups. Each group was further divided into two sub-groups, one group received finasteride, and another received finasteride and estrogen. Group 1 received treatment every day for 30 days and group 2 for 60 days. After the treatment was over, the groups were subjected to the examination of the levels of TGF-ß1, EGF, FGF and proliferation in the prostate.

TGF-ß1 was determined by the fibroblast count in the prostatic tissue incision that showed positive response against TGF-ß1 monoclonal antibody/0.01 mm2 using

immunohistochemistry. Similarly, EGF and FGF were also determined by fibroblast count with positive response against the EGF and FGF monoclonal antibodies/0.01 mm2 using the same method. Epithelial proliferation was determined according to the epithelial thickness measured using micrometer. Estrogen of 0.0011 and finasteride of 0.090 mg/day was given every day for 1 month in one group and 2 in another. The assessment of finasteride conversion from human to rats was based on the table from Laurence & Bacharach (1964) as cited by Donatus and Nurlela (1986). Collected data were processed using univariate statistical test (t test).

## RESULTS

Prior to statistical analysis on the observed variables, pre-treatment bodyweights of the rats in each group was determined, since the difference of bodyweight might influence the difference in observed variables. According to the results of variant analysis, there was no significant difference in the bodyweight of the rats, so that the bodyweight of the experimental animals prior to the study had no influence on the results of further treatment and statistical analyses.

After 1 month was over, observation revealed that the mean of TGF-ß1 in group receiving estrogen and finasteride was lower than that in finasteride group. In contrast, the means of EGF, FGF, and proliferation in group receiving estrogen and finasteride was higher than those in finasteride group. In observation after 2 months, it was found that the mean of TGF-ß1 in group receiving estrogen and finasteride was lower than that in group receiving finasteride only, while the mean of proliferation is group receiving estrogen and finasteride was higher that that in group with finasteride only. From the observation, it was apparent that TGF-ß1 in 2 month group was lower than that in 1 month group, while EGF, FGF, and proliferation were higher in 2 month group than in 1 month group. The p values of all variables in 1 and 2 month groups receiving estrogen and finasteride and finasteride only were more than 0.05. This indicates that all data were normally distributed, meeting the criteria of using parametric tests, such as independent two sample t test, Pearson correlation test, and multiple linear regression test.

Results of Pearson correlation test showed that p value of more than 0.05 was found in all variables receiving finasteride only and combination of estrogen and finasteride in 1 and 2-month groups. This indicated that there was no correlation between TGF-ß1, EGF and FGF. There were two p values that were less than 0.05 in 2-month group between TGF-ß1 and FGF in animals receiving estrogen and finasteride and between TGF-ß1 and EGF in those receiving finasteride. It was apparent that TGF-ß1, EGF and FGF had no correlation, so that we used univariate statistical test.

### Analysis of TGF-ß1

Table 1. TGF-ß1 in each treatment group and observation time in group receiving estrogen and finasteride and that receiving finasteride only.

| Time of Observation | Treatment Groups | | P value of 2 sample t test (between groups) |
|---|---|---|---|
| | Estrogen Finasteride ( $\bar{X} \pm SD$ ) | Finasteride ( $\bar{X} \pm SD$ ) | |
| 1 Month | 2.31 ± 1.44 | 4.00 ± 1.58 | 0.009 |
| 2 Months | 1.69 ± 1.32 | 2.85 ± 1.07 | 0.022 |
| P value of 2 sample t test (between observations) | 0.266 | 0.039 | |

In observation for one month, the mean of TGF-ß1 in group receiving estrogen and finasteride was lower than that of group receiving finasteride only. Results of t 2 sample test showed that the mean of TGF-ß1 had $p < 0.05$, indicating significant difference between group receiving estrogen and finasteride and that receiving finasteride only. In observation for 2 months, the mean of TGF-ß1 in group receiving combination of estrogen and finasteride was lower than that receiving finasteride only. Results of t 2 sample test revealed that TGF-ß1 had $p < 0.05$, indicating significant difference between group receiving estrogen and finasteride and that receiving finasteride only.

The mean of TGF-ß1 in groups receiving combined treatment and finasteride only in 2 months observation

was lower than that in 1 month observation. The difference was observed using t 2 sample test since the subjects of observation in 1 and 2 month groups were different. The results of the test only demonstrated that the mean of TGF-ß1 in finasteride group was significantly different ($p < 0.05$) between observation after 1 month and that after 2 months. However in group receiving combined estrogen and finasteride, no significant difference was found ($p > 0.05$). Analysis of TGF-ß1 indicated that both estrogen and finasteride could significantly reduce TGF-ß1 concentration.

**Analysis of EGF**

Table 2. EGF in each treatment group and observation time in group receiving estrogen and finasteride and that receiving finasteride only.

| Time of Observation | Treatment Groups | | P value of 2 sample t test (between groups) |
|---|---|---|---|
| | Estrogen Finasteride ( $\bar{X} \pm SD$ ) | Finasteride ( $\bar{X} \pm SD$ ) | |
| 1 Month | 5.62 ± 2.33 | 4.23 ± 1.74 | 0.099 |
| 2 Months | 14.54 ± 6.08 | 7.08 ± 3.97 | 0.001 |
| P value of 2 sample t test (between observations) | 0.0001 | 0.030 | |

In observation after 1 month, the mean of FGF in group receiving estrogen and finasteride was higher than that in group receiving finasteride only. Results of t 2 sample test showed that the mean of FGF was less than 0.05, indicating no significant difference between both treatment groups. In observation after 2 months, the mean of EGF in group was higher than that found in observation after 1 month, either in group receiving both therapies or receiving finasteride only. Results of t 2 sample test showed that in group receiving estrogen and finasteride and group receiving finasteride only, there was significant difference in EGF after 1 and 2 month observations, each with $p < 0.05$. Analysis of EGF proved that estrogen and finasteride could both increase EGF significantly.

**Analysis of FGF**

Table 3. FGF in each treatment group and observation time in group receiving estrogen and finasteride and that receiving finasteride only.

| Time of Observation | Treatment Groups | | P value of 2 sample t test (between groups) |
|---|---|---|---|
| | Estrogen Finasteride ( $\bar{X} \pm SD$ ) | Finasteride ( $\bar{X} \pm SD$ ) | |
| 1 Month | 7.08 ± 4.97 | 5.08 ± 2.66 | 0.217 |
| 2 Months | 8.23 ± 4.83 | 6.62 ± 3.01 | 0.317 |
| P value 2 sample t test (between groups) | 0.554 | 0.180 | |

In observation after 1 month, the mean of FGF in group receiving estrogen and finasteride was higher than that in group receiving finasteride only. Results of t 2 sample test showed that the mean of FGF had $p > 0.05$, showing no significant difference in both treatment groups. In observation after 2 months, the mean of FGF in group receiving estrogen and finasteride was higher than that in group receiving finasteride only. The results of 2 sample t test showed that the mean of FGF had $p > 0.05$, indicating no significant difference in both treatment groups.

The mean of FGF in 2-month group was higher than that in 1 month group, both in group receiving estrogen and finasteride and group receiving finasteride only. The results of t 2 sample test showed that both groups after 1 and 2-month observation showed no significant difference ($p > 0.05$). The analysis of FGF after 2 months treatment showed no significant increase of FGF expression in group treated either with estrogen or finasteride, although there was an FGF increase after receiving estrogen.

**Analysis of epithelial proliferation and prostatic stroma**

Table 4. Proliferation in each treatment group and observation time in group receiving estrogen and finasteride and that receiving finasteride only.

| Time of Observation | Treatment Groups | | P Value of 2 sample t test (between groups) |
|---|---|---|---|
| | Estrogen Finasteride ( X ± SD ) | Finasteride ( X ± SD ) | |
| 1 Months | 33.35 ± 4.51 | 30.62 ± 4.01 | 0.116 |
| 2 Months | 43.31 ± 5.68 | 27.69 ± 2.88 | 0.0001 |
| P value of 2 sample t test (between observations) | 0.0001 | 0.043 | |

In observation after 1 month, the mean of proliferation in group receiving estrogen and finasteride was higher than that finasteride group. The results of t 2 sample test showed that the mean of proliferation in both groups had no significant difference ($p > 0.05$). After two month observation, the mean of proliferation in group receiving estrogen and finasteride was higher than that in group receiving finasteride. The results of t 2 sample test showed that the mean of proliferation had $p < 0.05$, indicating significant difference between both groups.

The mean of proliferation in 2 month observation was higher than that in 1 month in group receiving combined estrogen and finasteride, while in group with finasteride only, the mean was lower. The results of t test showed significant difference ($p < 0.05$) in groups receiving estrogen and finasteride and that receiving finasteride only. Analysis of proliferation proved that estrogen administration could increase proliferation, while, contrastingly, finasteride administration reduced proliferation.

**Multipe linear regression analysis of TGF-ß1, EGF, FGF and estrogen in proliferation**

To find the role of TGF-ß1, EGF, FGF and estrogen in prostate epithelial and stromal proliferation, multiple linear regression analysis was undertaken. The results of multiple linear regression analysis can be seen in the following table.

Table 5. Multiple linear regression analysis of TGF-ß1, EGF, FGF and estrogen in proliferation

| Variables | B | T | P value |
|---|---|---|---|
| 1 Month observation | | | |
| TGF-β1 | – 1.035 | – 2.153 | 0.042* |
| Constant | 35.244 | 20.525 | 0.0001 |
| 2 Month observation | | | |
| Estrogen Finasteride | 15.615 | 8.838 | 0.0001* |
| Constant | 27.692 | 22.164 | 0.0001 |

Note:
* = significant t = results of t test
B = beta/slope

Multiple linear regression analysis using stepwise method revealed that in 1 month only TGF-ß1 had negative effect on proliferation. In this study, the lower level of TGF-ß1 played a role in the increase of proliferation. After two month observation, it was only estrogen that had positive effect on proliferation.

**Results of immunohistochemical staining**

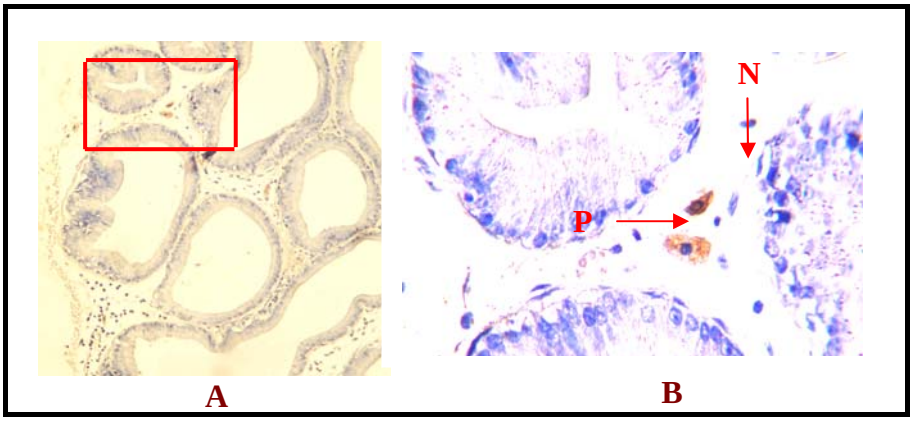


Figure 1. Incision of Wistar white rats prostatic tissue stained immunohistochemically using monoclonal antibody TGF ß in group treated with estrogen and finasteride for 2 months.
A : magnification 100x B : magnification 400x
N : negative, no stain reactions against monoclonal antibody TGF ß
P : positive, showing stain reactions against monoclonal antibody TGF ß

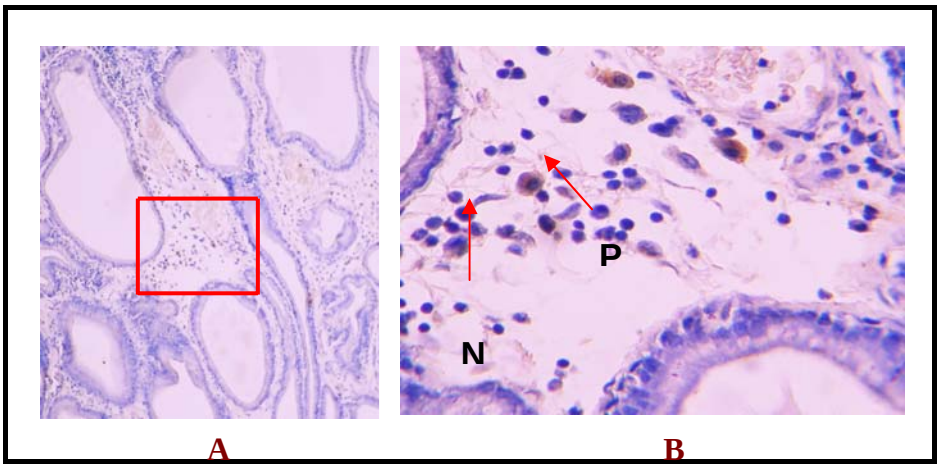


Figure 2. Incision of Wistar white rats prostatic tissue stained immunohistochemically using monoclonal antibody EGF in group treated with estrogen and finasteride for 2 months.
A : magnification 100x B : magnification 400x
N : negative, no stain reactions against monoclonal antibody EGF
P : positive, showing stain reactions against monoclonal antibody EGF

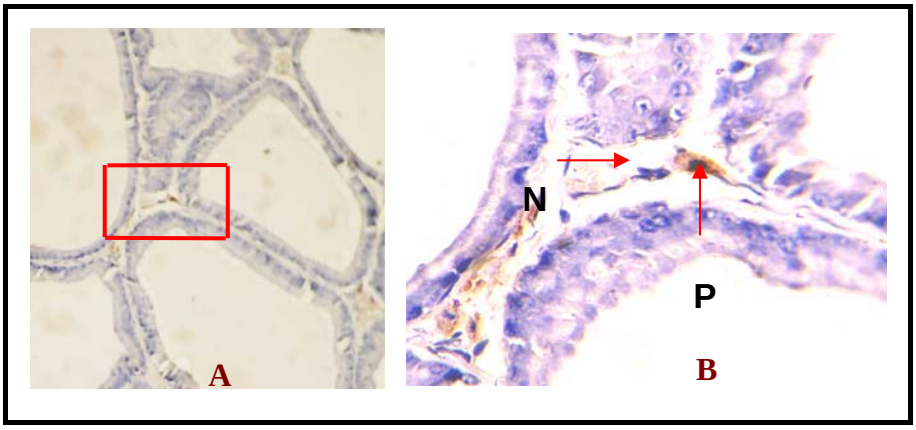


Figure 3. Incision of Wistar white rats prostatic tissue stained immunohistochemically using monoclonal antibody EGF in group treated with estrogen and finasteride for 2 months.
A : magnification 100x B : magnification 400x
N : negative, no stain reactions against monoclonal antibody EGF
P : positive, showing stain reactions against monoclonal antibody EGF

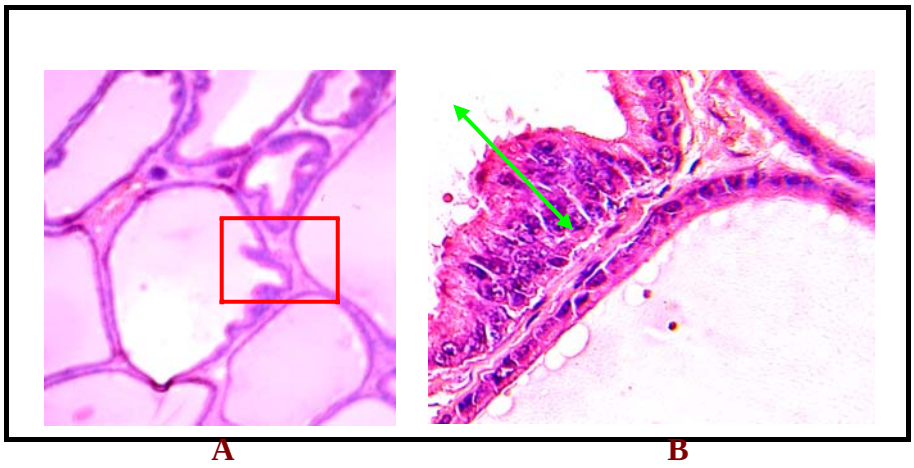


Figure 4. Incision of Wistar white rats prostatic tissue stained using HE to identify prostatic epithelial thickness (proliferation) in group treated with estrogen and finasteride for 2 months.
A : magnification 100x B : magnification 400x

## DISCUSSION

### Correlation between TGF-ß1, EGF and FGF

All variables receiving combined estrogen and finasteride treatment and receiving finasteride only showed no correlation between TGF-ß1, EGF and FGF. In general, it was found that TGF-ß1, EGF and FGF had no correlation among each other. However, TGF-ß1 and FGF in group receiving combined estrogen and finasteride was found to have correlation, although in the prostate TGF-ß1 induced downregulation of prostatic cells growth. However, TGF-ß1 also induced upregulation bFGF/FGF2 production, an autocrine growth factor for prostatic stromal cells (Lokeshwar BL et al., 1992).

**Transforming Growth Factor (TGF-ß1)**

TGF-ß1 is a cytokine produced by fibroblasts, condrocytes, osteocytes, thrombocytes, monocytes, and T cells. TGF-ß1 is one of growth factors in the prostate. It has a strong effect to inhibit proliferation in prostatic epithelial or stromal cells, and it also triggers apoptosis in prostatic epithelial cells. In stromal cells, at lower dose TGF-ß1 triggers proliferation, while at higher dose it inhibits stromal cell proliferation (Chatelain C, 2001).

In some literatures, it is appropriately mentioned that TGF-ß1 can play a role as inhibitor and stimulator, depending on cell type, differentiation status, and cell condition, although, in general, it has inhibitory characteristics (Emberton, 1999; Itoh N, 1989). Estrogen has a role in TGF-ß1. It can suppress the expression of TGF-ß1 (Matsuda, 2001), and it can also activate other growth factors, EGF and FGF, leading to the occurrence of prostatic proliferation. Finasteride is a 5α reductase inhibitor type II that inhibits testosterone alteration to become dehydrotestosterone (DHT) in nucleus membrane, so that the DHT cannot bind to receptor androgen in the nucleus to form DNA, and being transcripted into RNA to form other proteins and growth factors, TGF-ß1, EGF, and FGF. Since finasteride inhibits DHT, TGF-ß1, as well as EGF and FGF will also reduce. Finasteride has been used in individuals with benign prostatic hyperplasia (BPH). It can reduce prostatic volume and restore BPH symptoms (Kirby, 1991). In previous study, the administration of 5 mg finasteride for 12 months reduced DHT to 75% and prostatic volume to 19%, restore urinary flow to 1.6 ml/second. In another study for 2 years, 34% of the patients experienced the reduction of ejaculation volume, libido, and erectile function. Finasteride can reduce serum PSA expression to 50% (McConnel, 2002). Finasteride can also induce apoptosis in prostatic cells, causing the reduction of growth factors, including TGF-ß1. Finasteride is a competitive inhibitor of 5α reductase enzyme. It can reduce intraprostatic serum dihydrotestosterone level. Finasteride is a type-2 isoenzyme-selective inhibitor, so that it cannot reduce the level of dihydrotestosterone to castration level since testosterone in circulation is altered to become dihydrotestosterone by type-1 isoenzyme present in skin and liver (Lepor AC and Lowe F, 2002; Wein AJ and Rovner ES, 2001).

From the results of observation in 1 month group using immunohistochemistry on TGF-ß1 in both treatment groups, it was found that in group receiving combined estrogen and finasteride the mean of TGF-ß1 was lower than in that receiving finasteride only. The mean of TGF-ß1 in group receiving estrogen and finasteride was significantly different from that in group receiving finasteride only ($p < 0.05$). These findings were in line with the literatures since estrogen has a role in suppressing TGF-ß1 expression, while finasteride inhibits or prevents the change of testosterone into dihydrotestosterone (DHT). Because DHT was not formed, DNA formation in the nucleus of prostate cells was disturbed, with the result that growth factor-forming protein was not produced. Consequently, the mean of TGF-ß1 was reduced. Therefore, the mean of TGF-ß1 was lower in group receiving combined estrogen and finasteride compared to that in group receiving finasteride only.

In 2 month groups, the mean of TGF-ß1 in group receiving estrogen and finasteride was lower than that in group receiving finasteride. This is because estrogen has a role in suppressing TGF-ß1 expression, while finasteride inhibits or prevents the change of testosterone into dihydrotestosterone (DHT) that will trigger the growth factor to produce TGF-ß1. It was therefore reasonable that the mean of TGF-ß1 in group receiving estrogen and finasteride was lower than that in group receiving finasteride only. If the mean of TGF-ß1 in group receiving estrogen and finasteride for 2 months was compared to that in group receiving estrogen and finasteride for 1 month, that in 2 month group was lower, although it was statistically not significantly different ($p > 0.05$). The mean of TGF-ß1 in finasteride group for 2 months, compared to that for 1 month, was lower, and the difference was significant ($p < 0.05$). In the analysis of TGF-ß1, it was proved that both estrogen and finasteride could reduce TGF-ß1 level significantly.

**Epidermoid Growth Factor (EGF)**

In finasteride group, the mean of EGF was lower than that in group receiving combined estrogen and finasteride, because finasteride prevented the formation of DHT from testosterone, so that the formation of DNA, RNA, and proteins, including the growth factor, EGF, reduced. This lead to the lower mean of EGF. In addition, finasteride also induced apoptosis of prostatic cells, so that the formation of growth factors, including EGF, lessened (McConnel, 2002; Kirby, 2002). In group receiving combined estrogen and finasteride, the mean of EGF was higher compared to group receiving finasteride only. Although the difference was not significant, this resulted from the fact that finasteride had a role in the inhibition of growth factor formation, including EGF. Estrogen receptor (ER ß) is commonly found in the prostate. By the presence of estrogen receptor in cytoplasma and nulceus, estrogen was bound toDNA, and performed translation to form mRNA, and subsequently underwent transcription to form proteins, including the growth factor EGF (Griffths et al., 2002; Miksicek R, 1994), so that the EGF in group receiving

combined estrogen and finasteride was higher. Sinergic administration of estrogen and androgen results in prostatic hyperplasia by triggering the growth factor to grow. Estrogen also increases androgen receptor in the prostate, facilitates binding between dehydrotestosterone (DHT) and androgen receptor, resulting in the increase of growth factor, including EGF (Kirby, 1997; Griffith et al., 2002).

In group treated for 2 months, the mean of EGF in group receiving treatment with finasteride only was lower than that in group receiving combined estrogen and finasteride, and the difference was significant ($p < 0.05$). This was also found in group receiving finasteride only ($p < 0.05$). The mean of EGF in 2 month group in all treatments was higher than that in 1 month group. The mean of EGF in group receiving finasteride for 2 months should have been lower, as it was suggested that there remained some testosterone that could not be inhibited by finasteride. Finasteride belongs to selective inhibitor of type II isoenzyme, while in the circulation, testosterone is altered to become DHT by type I isoenzyme in liver and skin, so that DHT level cannot be as low as that in castration (McConnel JD, 1996; Ganong WF, 2003; Lepor A et al., 2002). Consequently, the mean of EGF in finasteride group remained high. This could also result from aging rats and relatively enlarged prostatic volume, even though its growth had been inhibited by finasteride. For group receiving combined estrogen and finasteride for 2 months, the mean of EGF was higher compared to that in group treated for 1 month, because estrogen had a role to trigger the growth factor, including EGF, to be more active (Griffith et al., 2002). Therefore, the mean of EGF increased in those receiving estrogen. This study proved that estrogen could increase EGF significantly.

**Fibroblast Growth Factor (FGF)**

In groups receiving finasteride, the mean of FGF was lower than in that receiving combined estrogen and finasteride, because in finasteride group FGF production was inhibited by finasteride by blocking the alteration testosterone to become DHT. Since the level of DHT was low, protein formation, including the growth factor FGF, was also low (Kirby, 1997; McConnel, 2002). In addition, finasteride also triggers apoptosis in prostatic cells, leading to the reduction of FGF. In group receiving estrogen and finasteride, the FGF was higher than in group receiving finasteride only, although the formation of FGF had been inhibited by finasteride. However, estrogen plays a role in the prostate by triggering the formation of growth factor, including FGF, much higher than that in group receiving finasteride only (Griffith, 2002; Kirby, 1997). It also trigger the proliferation of prostate cells, inhibits the apoptosis of prostatic cells, resulting in the formation of FGF. The mean of FGF in group receiving finasteride for 2 months was also lower compared to that in group receiving estrogen and finasteride, although, using 2 sample t test, it was not statistically significant ($p > 0.05$).

If 2 month group was compared to 1 month group, it was found that the mean of FGF in groups receiving estrogen and finasteride and finasteride only for 2 months was higher compared to that in 1 month group. In group receiving finasteride for 2 months, the mean of FGF was higher compared to the mean of FGF in 1 month. This could result from the presence of remaining testosterone that could not be totally inhibited by finasteride, because finasteride is a selective inhibitor of type 2 isoenzyme, while testosterone in the circulation could be altered to become DHT by type 1 isoenzyme presented in liver and skin, so that DHT level was not as low as that after castration (Lepor AC and Lowe F, 2002; Wein AJ and Rovner ES, 2001). As a result, FGF still remained. Additionally, in a study it was found that finasteride reduced DHT level in the prostate, but it also increased testosterone (George FW, 1997). In preliminary study, it was proved that finasteride could reduce FGF significantly in observation after two months. In group receiving combined estrogen and finasteride for 2 months, the mean of FGF was higher compared to that in 1 month observation, although it was statistically not significant ($p > 0.05$). In this study, estrogen could not increase FGF significantly ($p > 0.05$), which was likely due to the need of longer time of observation or the use of estrogen with lover sensitiveness to FGF.

**Proliferation**

Group receiving the combination of estrogen and finasteride had a higher mean of proliferation because estrogen had a role in triggering proliferation directly in prostate cells. It triggered growth factor through EGF and FGF to undergo proliferation, prevented the expression of TGF-ß1 and apoptosis, so that many prostate cells, either in epithelium and stoma, proliferated (Story, 1995; McConnel, 1998; Kirby, 1998; Matsuda, 2001). However, in 1 month group the increase of proliferation was not statistically significant compared to that in finasteride group. This was also likely due to less longer time of observation. In 1 month group receiving combined estrogen and finasteride, finasteride played a role as an inhibitor of DHT formation, so that the growth factors, particularly EGF, FGF, and KGF, could not proliferate. However, the effect of estrogen was higher than finasteride, so that the mean of proliferation was high.

In 2 month group, the mean of proliferation in combined estrogen and finasteride group was higher than that in finasteride group because estrogen had a function to trigger proliferation in prostate cells, inhibit TGF-ß1 expression, which inhibited proliferation and triggered apoptosis, resulting in the increase of estrogen proliferation. Compared to that in 1 month group. the mean of proliferation in 2 month group receiving combined estrogen and finasteride was higher, and this was also statistically significant ($p < 0.05$). This was because estrogen triggered proliferation in epithelial cells and prostatic stromal cells, as well as triggering growth factors, the EGF and FGF, to proliferate. In groups receiving finasteride, the mean of proliferation in 2 month group was lower compared to 1 month group, because finasteride had a role in preventing the formation of DHT. In consequence, growth factor was less formed, and proliferation decreased. Additionally, finasteride treatment resulted in the apoptosis of prostate cells, so that the mean of proliferation in finasteride group reduced after 2 months. This study proved that estrogen administration could increase proliferation in prostatic cells significantly, and finasteride administration could also result in significant reduction of proliferation in those cells.

Multiple linear regression test revealed that in observation for 1 month, only TGF-ß1 had negative effect on proliferation, since TGF-ß1 was one of growth factors that inhibits proliferation, either in epithelial cells or prostatic stromal cells (Itoh N, 1989). TGF-ß1 also triggers the occurrence of apoptosis in prostatic cells, and it is suggested that higher dose of TGF-ß1 in prostatic stromal cells played a role in inhibiting proliferation, while, contrastingly, the lower dose would trigger proliferation (Chatelain, 2001). Therefore, by the reduction of TGF-ß1, proliferation in epithelial and prostatic stromal cells increase. Conversely, cancer cells demonstrate overexpression of TGF-ß1 and resistant against the inhibitory effect of TGF-ß1 (Wollf JM, 1998). In this study, the reduction of TGF-ß1 played a role in the high concentration of proliferation. In observation for 2 months, it was estrogen that had positive effect on proliferation, since it triggered proliferation in all tissues, particularly prostatic cells, inhibited TGF-ß1 expression and apoptosis in all tissues, including prostatic cells. Conclusively, if there is an increase of estrogen, proliferation will also increase, and vice versa.

In aging process, testicular function, particularly the Leydig cells, is decreasing, with the result that testosterone also decreases. To maintain homeostasis, therefore, there will be an increase of estrogen from adrenal tissue, aromatization of peripheral lipid, and aromatization of adrenal androstenedion. Estrogen has a function to trigger epithelial and prostatic stromal cells proliferation, and inhibit apoptosis by suppressing TGF-ß1 expression. By the increase of estrogen, TGF-ß1, playing a role in inhibiting proliferation and triggering apoptosis, is decreasing, resulting in higher level of proliferation, which finally lead to the occurrence of BPH.

This study had successfully disclosed the mechanism of BPH due to the increase of estrogen in aging process, the reduction of TGF-ß1, the increase of estrogen in aging process, the reduction of TGF-ß1, the increase of EGF, as well as the increase of proliferation. The longer the estrogen is administered, the more the reduction of TGF-ß1, the more the increase of EGF, and the more the enhancement of proliferation. If the increase of estrogen lasts longer, BPH may finally occur.

## CONCLUSIONS AND SUGGESTIONS

### Conclusions

The administration of combined 5α reductase inhibitor (finasteride) and estrogen can reduce the expression of TGF-ß1, increase the expression of EGF, increase prostatic epithelial cells proliferation, but cannot change FGF expression in prostatic tissue of Wistar strain white rats. After estrogen administration for 2 months, it was found that TGF-ß1 became lower, EGF became higher, and proliferation was enhanced. This proved that the increase of estrogen lead to enhanced proliferation, and eventually resulted in BPH. The administration of estrogen increases EGF, reduces, TGF-ß1 expression, and increase proliferation, while finasteride reduced the expression of TGF-ß1, EGF, and FGF, so that epithelial and stromal cells proliferation inhibition reduce, and, in contrast, epithelial and stromal cells proliferation increase, which finally lead to BPH.

### Suggestions

Further studies are warranted on medical therapy for BPH using combined 5α reductase inhibitor and anti-estrogen. The development of such medical therapy is also worth to be investigated. Studies on the level of estrogen that is able to trigger the BPH deserves thorough examination as well.

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