Effects of Celecoxib (A Cyclooxygenase-2 Inhibitor) on Benign Prostatic Hyperplasia in Rat

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ABSTRACT

For many years, use of nonsteroidal anti-inflammatory drugs (NSAID) has been common for the treatment of cancer. On the other hand, it has been shown that celecoxib (CLX), a NSAID, can cause apoptosis by inhibiting cyclooxygenase 2 (COX-2). We aimed to describe the effects of CLX on COX-2 expression and cell apoptosis in the prostate tissues of rats with benign prostatic hyperplasia (BPH).Thirty-two Wistar rats were allocated to sham, control, BPH, and BPH+CLX groups. During 4 weeks, 3 mg/kg/day of testosterone propionate (TP) was subcutaneously administered for BPH induction. CLX or distilled water was administrated via oral gavage for 30 days, besides TP injection. After the last day, the animals sacrificed and the prostates removed and weighed. The ventral lobes of the prostates were carefully removed and placed in paraffin blocks, followed by the assessment of mast cell count. Cell apoptosis was assessed by a TUNEL assay, and COX-2 expression was evaluated via immunohistochemistry. Administration of CLX reduced PI and number of mast cells in rats with BPH. Also, treatment with CLX caused apoptosis in prostate cells, compared to the BPH groups. In addition, following treatment with CLX, expression of COX-2 diminished in prostate tissue, compared to the BPH groups. The findings revealed that CLX can be effective in treating rats with BPH. It may be has positive effects in the treatment of BPH patients.

Key words: Benign Prostatic Hyperplasia, Celecoxib, Apoptosis, Cyclooxygenase-2

INTRODUCTION

Benign prostatic hyperplasia (BPH) involves the uncontrollable growth of the prostate gland. It is recognized as a common disorder among older men [1] and normally affects the central region of the prostate gland [2]. With gradual enlargement of the prostate, the urethra is pressured, causing symptoms such as weakening of the urine flow, lack of adequate emptying of the bladder, nocturia, dysuria, and obstruction of the bladder.

Different options are available for the treatment of BPH. Treatment is preferred in patients with mild to moderate symptoms of BPH, which do not normally cause uneasiness. Medical or surgical approaches can be applied in more severe cases with respect to the patient's condition. Recently, use of 5α-reductase inhibitors, as well as 1α-adrenergic receptor antagonists, has reduced the prevalence of surgeries and diminished their negative effects. Due to their diminishing effects on the smooth muscle tone of the urethra and prostate gland, 1α-adrenergic receptor antagonists are recognized as effective agents for BPH treatment, with rapid effects and high safety and efficacy.

On the other hand, the usual side effects of these drugs, such as dizziness, nasal congestion,

asthenia, impotence, orthostatic hypotension, and ejaculation disorders, can be problematic if patients also suffer from age-related conditions, such as diabetes, cardiovascular diseases, metabolic syndrome, sexual disorders, and hypertension [3]. Although the etiology of BPH remains undetermined, it is dependent on two important factors: dihydrotestosterone (DHT) from testosterone by type-II 5α-reductase and older age [4]. If the amount of DHT in the body is too low, BPH will not appear, while if the amount of DHT is high, the prostate will grow. Elderly age is very effective in the development of BPH [5].

Some studies have suggested the major contributions of metabolic syndrome, hormonal changes, oxidative stress, and inflammation to BPH development [6-8]. Acute and chronic inflammation can result in a series of events, which cause proliferation in the prostate tissues. Subsequently, prostate inflammation leads to the generation of free radicals and induction of oxidative stress [9]. Inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin-6 (IL-6), trigger the expression of cyclooxygenase-2 (COX-2) in the prostate epithelium, followed by the excessive expression of BCL-2 anti-apoptotic gene and increased cellular proliferation in the prostate [10, 11]. Another study suggested that inflammation in the prostate leads to high regulation of the responsible androgens and cascading genes of TGF-β1, which are clinically linked to molecular variations in BPH pathophysiology [12].

In recent years, involvement of inflammation in BPH pathophysiology has been reported [13]. In fact, the key involvement of chronic prostate inflammation in prostatic hyperplasia and specific urinary symptoms has been confirmed in some studies [14]; therefore, through treatment of prostatic inflammation, we can treat BPH [15]. Celecoxib, which is recognized as a nonsteroidal anti-inflammatory drug (NSAID), is extensively used to treat inflammatory disorders, including acute menstrual pain, reduced polyps in familial adenomatous polyposis, and rheumatoid arthritis (Steinbach et al., 2000). By connecting to its active site, celecoxib particularly inhibits COX2 (Penning et al., 1997) [16]. In a previous study, celecoxib could decrease nocturia in BPH patients [17]. In another study, reactions of prostate carcinoma cells to celecoxib included apoptosis induction, morphological changes, caspase-3 activation, and DNA damage [18].

Also, a previous study showed that inhibition of Akt pathway activation can contribute to apoptosis induction with celecoxib. By inhibiting anti-apoptotic kinase Akt/protein kinase B activation, celecoxib can induce apoptosis. Considering this mechanism, celecoxib has a greater capacity to induce apoptosis, compared to other tested COX-2 inhibitors [18]. In addition, since inhibition of COX2 expression can reduce cell proliferation in prostatic hyperplasia, we aimed to examine the effects of celecoxib on prostate hyperplasia.

Objectives

We aimed to determine the effects of celecoxib (a NSAID) on stromal cell proliferation in a rat model of BPH.

MATERIALS AND METHODS

Animals

In this study, 32 male Wistar rats (250-280 g) were supplied by the Research Center of Animal Laboratory (Zahedan University of Medical Sciences). They were kept in a 12:12 h light/dark cycle (temperature, 21±3) with access to food and water under standard conditions. The weight of the animals was measured and recorded each week.

The animals were allocated to 4 groups (n, 8 per group): group I, control (no treatment); group II, sham (daily distilled water through an oral gastric tube, simultaneously with corn oil injected subcutaneously); group III, BPH (daily distilled water through an oral gastric tube, simultaneously with 3 mg of TP injected subcutaneously); and group IV, BPH+CLX (10 mg/kg of celecoxib daily through an oral gastric tube, simultaneously with 3 mg of TP injected subcutaneously) [12]. The protocol was confirmed by the University Ethics Committee (IR.ZAUMS.REC.1394.194) and Animal Research Center of Zahedan University of Medical Sciences. To produce BPH, 3 mg/kg/day of TP was subcutaneously injected for 4 weeks [19].

Tissue Samples

After the last dose of injection and gavage, the animals were sacrificed under deep anesthesia, and their prostates were removed and weighed. Then, the ventral prostate was removed, stored in 10% buffered formalin, and processed with paraffin blocks. 7 micrometer (mμ) sections were prepared for histopathological examination and immunehistochemical (IHC) analysis.

Prostate Index

The prostate index of each rat was calculated by dividing prostate weight into body weight (mg/g) and multiplying by 100. [20]

Histological Studies

For histological process, paraffin blocks were obtained from the prostate tissues and 7 micrometers sections were cut. Some of them were stained by toluidine blue to determine the level of inflammation by staining the mast cells, and the other sections were used for immunehistochemical staining.

Toluidine blue staining

The samples were placed in xylene for paraffin removal, hydrated with an ethanol series, and washed with distilled water. Afterwards, the slides were placed in a toluidine blue solution for 2 to 3 minutes and washed with distilled water. They were then dehydrated by alcohol, embedded in xylene, and mounted. Toluidine blue-positive cells (mast cells) were enumerated at 400× field magnification, using 15 random fields from 5 rats in any group [21].

Tunel Staining

The TUNEL staining was performed based on the Roche protocol. Summary, the sections were incubated by proteinase K for 30 minutes. Followed by blocking activity of the endogenous peroxidase, the sections were placed in 3% H2O2 for 10 minutes. The TUNEL reaction compound was appended and the sections were maintained for 1 hours at temperature 37°C and placed in POD (transformer) for 30 minutes at 37°C and then in diaminobenzidine substrate solution (DAB) for 10 minutes. The counterstaining was accomplished by hematoxylin. Then the apoptotic cells were enumerated at 400× field magnification in random 10 fields in each section. Finally the apoptotic index (AI) was calculated [22]: Number of TUNEL positive cells/all epithelial cells×100

Immunohistochemical detection of COX-2

After placing the tissue sections in citrate buffer (pH, 6.0; 10 mM), an autoclave was used for 5 minutes to retrieve the antigens. The tissue slides were placed for 2 hours in 5% bovine serum albumin (BSA) and phosphate-buffered saline (PBS). Then, anti-COX-2 rabbit monoclonal antibodies were added to the slides and maintained overnight at 4°C. Following that, they were rinsed by PBS and placed in the corresponding HRP-conjugated secondary antibody for 1 hour. They were then rinsed by PBS and placed in DAB for 10 minutes.

After counterstaining with hematoxylin, the tissue sections were observed using a light microscope. Distribution brown cytoplasm staining in the prostate epithelial cells was determined as positive staining by COX-2. The sections were assessed by measuring the immunohistochemical score (IHS), and estimated by summing the quantity score (immune-reactive cell percentage) with the score of staining intensity (staining intensity), as previously explained [23].

Data Analysis

The values are described as mean± SEM. For data analysis, one-way ANOVA and Tukey's test were performed. Also, Kruskal-Wallis test was applied to compare the IHS scores in the groups. The significance level was set at 0.05.

RESULTS

Effects of celecoxib on prostatic index (PI)

The control and sham groups were not significantly different regarding the prostate weight. However, the PI significantly increased (p<0.001) in the BPH group in comparison with the control and sham groups. Also, the PI significantly diminished in the CLX group versus the BPH group (p<0.05, Fig.1)
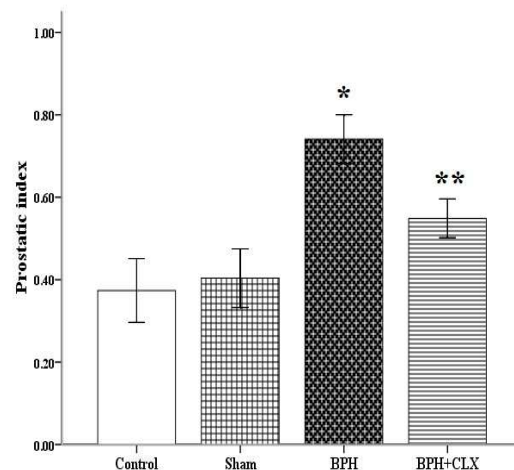

Figure.1: The effects of CLX on prostatic index (PI). * P < 0.001 vs. control group; ** P < 0.05 vs. BPH group. (PI): prostate weight/body weight × 100.

Effects of celecoxib on mast cell infiltration in BPH

As seen in figure 2, toluidine blue staining was applied for imagining mast cell infiltration in the stromal cells of all tested groups. BPH resulted in an increase of mast cell count, compared to the control and sham groups (p<0.001). On the other hand, compared to the BPH group, celecoxib diminished mast cell infiltration (p<0.05) (Fig2E).
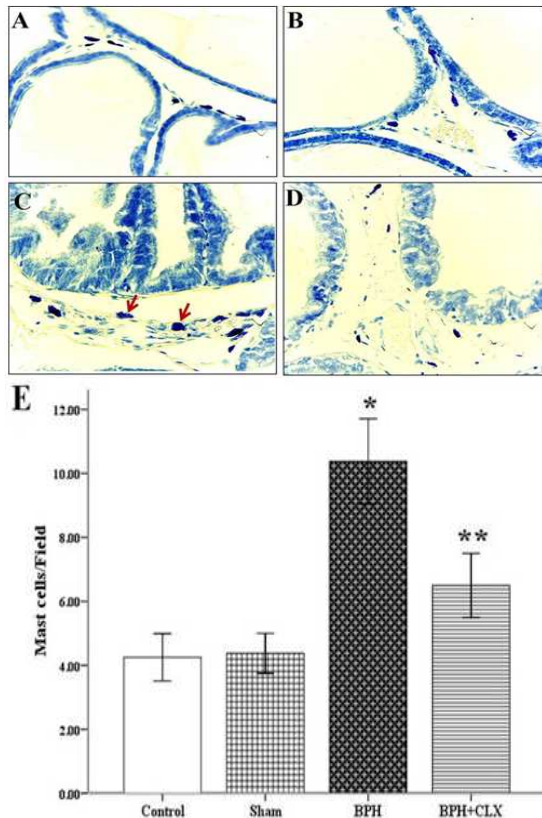

Figure 2: Toluidine Blue Staining of the Rat Ventral Prostate in the (A): control,(B):sham, (C):BPH,(D): BPH+ CLX groups (X400). The histogram, E shows the number of mast cells in the stromal cells of the prostate. * P < 0.001 vs. control group; * P < 0.05 vs. BPH group.

Effects of celecoxib on apoptosis in BPH

We found that celecoxib could diminish the proliferation of prostate cells in BPH through immunohistochemical staining for TUNEL (fig3D). In the prostate, few TUNEL-positive cells were found in the control, sham, and BPH groups (fig3E). CLX treatment enhanced the apoptotic index, compared to the BPH group (p<0.05).
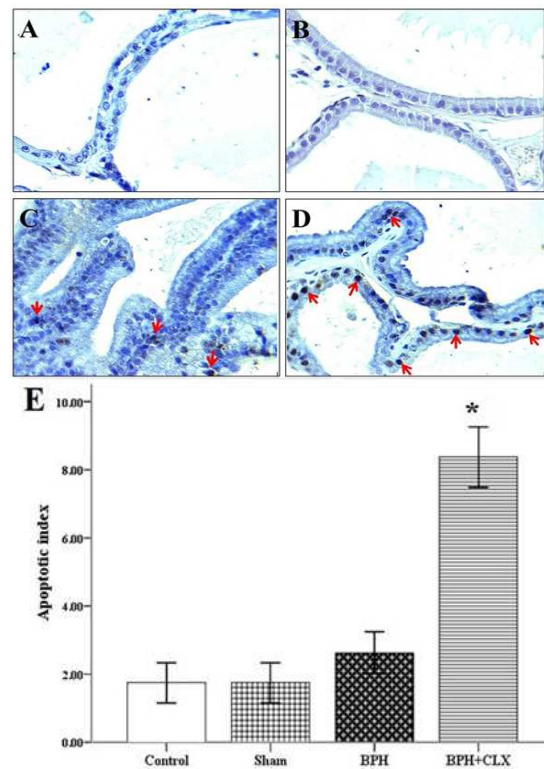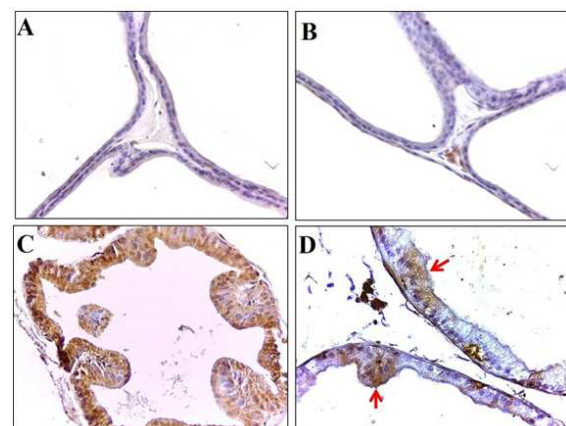

Figure 3: TUNEL Staining of the Prostate tissues in the (A): control, (B): sham, (C): BPH, (D): BPH+ CLX, groups. The red arrows show TUNEL positive cells (X400). The histogram (E) shows the number of apoptotic cells. * P < 0.001 vs. BPH group.

Effects of celecoxib on COX-2 expression in BPH

COX-2 expression was slightly reported in the control and sham groups. However, COX-2 showed major expression in the prostate epithelium of BPH rats when compared with control group (p<0.001) (fig4C). Also, upregulation of COX-2 diminished in the CLX group (p<0.05) (fig 4D&E).
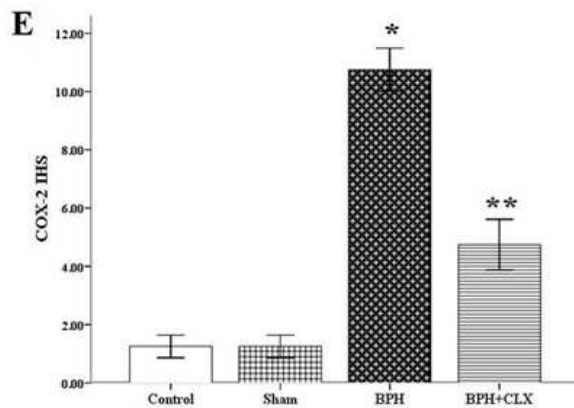
Figure 4: Immuno-histochemical Staining and Immuno-histochemical Score (IHS) for COX-2 in the Prostate Tissues of (A): control, (B): sham, (C): BPH, (D): BPH+ CLX, groups. The histogram (E) shows IHS score in the groups. * P < 0.001 vs. control group; ** P < 0.05 vs. BPH group. Red arrows indicate the COX-2 cytoplasm staining (in brown).

DISCUSSION

We assessed the effects of celecoxib, an anti-inflammatory agent, on BPH induced by testosterone in rats. Treatment with celecoxib reduced the prostate weight and COX-2 expression, while increasing prostate cell apoptosis in comparison with the BPH group. The findings of this study also showed that celecoxib reduced the number of mast cells and proinflammatory cells in the BPH model. Also, this study reported a decline in the mast cell count, which increased during inflammation. In many recent studies, therapeutic application of NSAIDs has been considered in cancer treatment. Although the mechanism of apoptotic activity is still undetermined in NSAIDs, COX-2 inhibition may be involved. PC-3 and LNCaP cells express different COX-2 levels. Considering the molecular basis of COX-2 inhibitors inducing apoptosis, stimulation of ceramide generation [24] or downregulation of Bcl-2 expression is triggered [25, 26]. The present study indicated the antioxidant and antiproliferative effects of celecoxib in a BPH model of rats. Many factors involved in creating BPH for example aging and androgens. Testosterone is the main androgen related to prostate growth [27]. A change in the testosterone level is usually causes changes in the growth of the prostate [28]. Testosterone is transformed into DHT in the prostate cells. The DHT connected to the androgen receptors and causes protein synthesis, differentiation, and growth in prostate cell [29]. Researchers have shown that serum testosterone and DHT levels increase in BPH cases and are associated with the size of the prostate [30]. In our study, the prostate weight and prostate index (PI) were calculated. Finally, we saw that the prostate weight was enhanced in the BPH model that demonstrated our findings agree with above studies. Celecoxib has a novel mechanism and an apoptotic pathway, which blocks the activation of Akt pathway. An active Akt pathway prevents apoptosis by celecoxib in the cancer cells of the prostate [31]. Therefore, celecoxib can decreased the prostate weight and consequently it decreases IP. In line with the above study, in present study was showed that celecoxib lead to decrease the prostate weight. Consequently, it cause decrease IP in the treated with celecoxib group when compared to the BPH group. Celecoxib performs an apoptotic activity more than that of other COX-2 inhibitors tested. Celecoxib may regulate Akt downstream of phosphoinositide (PI) 3-kinase or include PI 3-kinase-independent pathways.

Recently, it has been confirmed that C2-ceramide can prevent the Akt pathway activation, regardless of the activity of PI3-kinase [31]. The fact that celecoxib-induced apoptosis is not hindered by the expression of Bcl-2 in PC-3 cells suggests the importance of therapeutic implications [18]. The overexpression of Bcl-2 has been attributed to the development of prostate cancer [32-34]. The upregulation of this anti-apoptotic protein increases the apoptosis threshold, thus improving apoptosis resistance in form of cellular disorders. Therefore, the independent function of Bcl-2 advocates the application of celecoxib in the treatment of prostate cancer.

A previous study, which introduced a mechanism to determine the apoptotic activity of COX-2 inhibitors, reported contradictory findings to our study regarding the correlation between COX-2 prostanoid-mediated application and apoptotic effects of COX-2 inhibitors. Another study showed that COX-2 expression was not significant in the normal epithelial cells of the prostate, and apoptosis was not induced by celecoxib [18]; our study also confirms this finding.

According to some evidence, the capacity of COX-2 inhibitors in apoptosis may be unrelated to COX-2 enzymatic activity, and celecoxib cannot be an intermediate between apoptosis and COX-2 [18]; this finding is contradictory to our study. In another study, various COX-2 inhibitors with

similar IC50 values exhibited various effects on apoptosis and different apoptotic mechanisms were indicated in these molecules [18]. On the other hand, the major effects of chronic inflammation on the symptoms of LUTS and prostate hyperplasia progression have been described in many articles [35-38]. Also, Chuang reported higher serum C-reactive protein (CRP) levels in prostate hyperplasia patients, compared to those without the symptoms. Therefore, it was suggested as an anti-inflammatory drug for BPH treatment [39].

Few studies have been conducted on the impact of anti-inflammatory factors on BPH. In a previous study, it was explained that treatment with celecoxib can diminish nocturia [17]. In addition, the symptoms of BPH improved using rofecoxib, as a COX-2 inhibitor [40]. Other studies have also demonstrated that diclofenac and indomethacin can diminish nocturia [41, 42]. Moreover, the present study revealed that celecoxib by decreasing inflammation in the epithelial cells of prostate tissues causes a decline in cellular proliferation. In another study, it was revealed that a combination of celecoxib/terazosin produces better treatment reactions than terazosin alone in diminishing the signs and prostate volume [43]. It was shown that chronic prostate inflammation is correlated with the greater volume of the prostate gland [35], and treatment with terazosin alone could not decrease the prostate volume [43].

The pathogenesis of BPH is still unclear, although many studies have shown the involvement of inflammatory mediators in BPH development [14]. Mast cells are involved in the pathogenesis of inflammatory disorders [44]. They also contribute to the development of many tumors (e.g., prostate cancer) and are important regulators of inflammatory diseases [45]. According to a previous study, tumor-infiltrating mast cells improve the microenvironment for the growth of tumor through mediation of stem cell factor (SCF) of tumor and its receptor c-kit. Tumor-infiltrating mast cells are activated at a high density of SCF. This factor is expressed by several proinflammatory factors and it enhances the expression of IL-17 in tumors. Also, the function of NF-κB and AP-1 pathways improved in the inflammatory microenvironment (increased by mast cells) in tumor cells. Moreover, SCF-mediated mast cells increased tumor immunosuppression through secreting adenosine and enhancing regulatory cells and increased the repression of natural killer cells and T cells in tumors. This finding indicates that tumor cell–released SCFs can initiate the improvement of tumor microenvironment, and mast cells, as major inflammatory and immunosuppression regulators, contribute to the tumor microenvironment. Also, mast cells inhibited apoptosis in tumor cells via proinflammatory agents including TNF-α [46].

In the present study, the BPH group showed a higher mast cell count compared to the controls. On the other hand, the number of these cells reduced in the celecoxib group versus the BPH group. This finding indicated that our study agrees with the abovementioned studies. In another investigation, it was expressed that mast cells release proteases, angiogenic factors, and cytokines. The increase of the cytokines causes cell growth, differentiation, and cyclooxygenase-2 expression [10]. In another study, mast cells were shown to be involved in prostate cancer progression, and it was possible to treat tumors by inactivating the mast cells [47]. Also, the number of mast cells in the ventral prostate increased in a BPH model [48]. These studies also agree with the findings of our study. Similarly, several studies have shown that treatment by celecoxib diminishes the mast cell count in comparison with the BPH group [40, 49]. Therefore, what was mentioned in the mentioned study can be one of the mechanisms of COX-2 inhibition, which diminished cellular proliferation in our study. Also, prostaglandins have main role in urine manufacture [50]. Consequently, this drug can cure LUTSs by decreasing the urine manufacture by the renal and these agents can have an indirect effect on peoples' sleep quality via diminish in nocturnal symptoms [17]. In addition, in a study has been showed that celecoxib leads to atrophy and diminish in Leydig cells. Consequently, by reducing production of testosterone causes diminish the prostate volume [43]. Since celecoxib can prevent COX-2 expression without inducing any major effects on COX-1, occurrence of gastric, renal, or hemorrhagic dysfunctions is limited [42, 43, 49]. Also, many patients with prostatic hyperplasia could not tolerate or did not react to anti-inflammatory agents; therefore, celecoxib may be helpful for these patients. In our study, celecoxib was used to induce apoptosis in prostate epithelial cells via TUNEL staining. We observed that celecoxib enhances apoptosis in epithelial prostate cells in BPH rats, which can be attributed to the pro-apoptotic impact of celecoxib by hindering BCL-2 expression. Consequently, our

results showed that celecoxib could inhibit oxidative stress, inflammation, COX-2 expression, and apoptosis induction and might be used to treat BPH. Therefore, this drug should be considered in patients with BPH. However, more investigations are necessary to specify the therapeutic effects of celecoxib in BPH patients. Also, further studies are needed, using other COX-2 inhibitors, to examine the effects of COX-2 inhibitors on changes in the inflammatory biomarkers of BPH patients.

Acknowledgements

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