Synthesis and bioactivity of new Finasteride conjugate

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\textbf{A R T I C L E  I N F O}

\textbf{Article history:}
Received 22 October 2010
Revised 9 March 2011
Accepted 29 March 2011
Available online 5 April 2011

\textbf{Keywords:}
Synthesis
Finasteride
Conjugates
5α-Reductase
Polimod

\textbf{A B S T R A C T}

Finasteride is a synthetic 4-azasteroid compound that acts by inhibiting type II 5α-reductase, the enzyme that converts the androgen testosterone to 5α-dihydrotestosterone. It was approved by the US FDA for the treatment of benign prostatic hyperplasia and male pattern baldness. Here the acylation product of Finasteride C-18 amide N-polimod was synthesized by employing acylation reaction with polimod amide as a pivotal intermediate. The structure of the key intermediate and target molecule was confirmed by infrared spectrum, \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra and mass spectrum, and the inhibition of the steroid 5α-reductase and the rats' benign prostatic hyperplasia by the new Finasteride conjugate was also determined. The inhibition of the Finasteride conjugate on 5α-reductase was stronger than that of Finasteride. The weight of prostates (Fig. 4) and prostate index was observed (data not shown, no significant differences in body weight were observed among different groups of the animals) between the testosterone propionate (TP) control group and the normal control group \((P<0.05)\). The increase of the size of prostate and an obvious hyperplasia exist as well through observation (data not shown). These results proved that the hypodermic injec-

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Finasteride\textsuperscript{1} a synthesized steroid chemical compound, is an idiosyncratic inhibitor of intracellular enzymes type II 5α-reductase produced in the process of androgen testosterone metabolizing into dihydrotestosterone (DHT)\textsuperscript{2} which is applied to the treatment of benign prostatic hyperplasia (BPH)\textsuperscript{3–8} Since structural modifications of steroid drugs by glycosidation and peptidation have been shown to have a dramatic effect on their physical, chemical, and biological properties biological activities and consequently benefit in their therapeutic applications\textsuperscript{9,10}, the synthesis of glycosidation and peptidation of steroidal conjugates are of significance for research and development of new steroid-based drugs\textsuperscript{9–15}. Polimod (Pidotimod), a synthetic dipetide, is an orally bioactive immune enhancer\textsuperscript{16} which promotes not only non-idiosyncratic immune reactions\textsuperscript{17} but also idiosyncratic immune reactions\textsuperscript{18}. Under the inspiration of the glycosidation and peptidation of steroid, we examined the possibility of Finasteride and polimod could be joined into a new conjugate molecule, and studied the active suppression of the steroid 5α-reductase by the new conjugate. We also observed whether the new conjugates could possess the pharmacological effect of Finasteride, and reduce its side effects.

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tions of testosterone propionate promoted the prostate hyperplasia of rats successfully.

Compared with the castrated negative control group, the growth of prostate and seminal vesicle had been greatly stimulated in TP control group (Fig. 4). After administration of treatment with Finasteride conjugate with increased dose (2, 10, 50 mg kg\(^{-1}\) d\(^{-1}\), 14 d), the prostate and seminal vesicle’s weight in the treatment group declined significantly, showing the inhibitory effect on benign prostatic hyperplasia in Finasteride conjugate group was similar to Finasteride group (Fig. 4A), while no statistical differences were found between Finasteride conjugate groups and Finasteride group although the absolute weight value appear higher in the Finasteride conjugate group of the same dosage (Fig. 4A).

We next performed histochemistry of the prostates of the animals for a closer look of the effects of the Finasteride and Finasteride conjugate treatment. The prostate gland of the rats in the normal control group arranged orderly. The columnar epithelium appeared single-layered, and a few basal cells and basement membrane were observed. There was little luminal gland secretion, and nor expansion in the gland cavity. No hyperplasia was found in interstitial tissues (Fig. 5A).

In the Finasteride group, the prostate gland of the rats arranged relatively in order, and the gland cavity recovered to the normal size. The pseudostratified epithelia reduced, and the stretched gland epithelial reduced and the hyperplasia of the interstitial tissues decreased (Fig. 5C).

Figure 1. The synthetic route of Finasteride conjugates (compound 8).

Figure 2. Lineweaver–Burk plot of Finasteride inhibition of 5α-reductase.

Figure 3. Lineweaver–Burk plot of Finasteride-polimod inhibition of 5α-reductase.
When used at same dose as the Finasteride group, the Finasteride conjugates treatment group significantly inhibited prostatopathy. The prostate gland arranged orderly similar to the animals in the normal control group. The prostate gland arranged orderly, and the pseudostratified epithelia and the epithelial protrusions disappeared. The hyperplasia of the interstitial tissues were also decreased (Fig. 5D). Taken together, these histochemistry results for the Finasteride conjugates showed slight improvement compared to the Finasteride group.

We also observed that the rats in the Finasteride conjugate group were more active than those in the Finasteride group. The rats in the castrated control group and Finasteride group were less active, their skin elasticity reduced, and their fur color was dark and gloomy. However, the rats in the treatment group had almost no such appearance (Table 1).

Prostate disease is becoming the most prominent health issue in the elderly men. Benign prostatic hyperplasia (BPH) is the most common prostate disease. It leads to symptoms of urinary hesitancy, frequent urination, dysuria, increased risk of urinary tract infections, and urinary retention. A safe, effective and relatively inexpensive treatment for BPH is in urgent need in clinical research.

In vivo, 5α-reductase is an NADPH-dependent enzyme that catalyzes the reduction of testosterone to the more biologically active dihydrotestosterone (DHT), and the accumulation of DHT in prostate tissue is main cause for BPH. The inhibition of 5α-reductase activity can reduce the DHT levels in vivo, so as to achieve the effect of inhibition of BPH. Thus 5α-reductase inhibitors can be used as an effective drug for the treatment of BPH.

Finasteride is a selecting type II 5α-reductase inhibitor which has been approved for clinical use. Polimod (Pidotimod) is a synthetic dipeptide molecule with biological and immunological activity on both the adaptive and the innate immune responses. It may thus reduce the toxic effect in where the original drug (Finasteride) effects. The results show that comparing with Finasteride,
Finasteride conjugate had better inhibition on activity of 5α-reductase in vitro and showed improved outcome in vivo, in the rat model with BPH (Fig. 4). The tissue-specific expression patterns of the 5α-reductase isozymes have been reported previously. The 5α-reductase type II isozyme was detected in the epididymis, seminal vesicle, prostate, and liver. The expression level of this isozyme in the seminal vesicle is lower than that in the prostate which may account for the fact that Finasteride and Finasteride conjugate were more effective in reducing the organ weight of the prostate than those in the seminal vesicles in the TP treated rats in our test (Fig. 4). The rats in the Finasteride conjugate group also showed better vital signs such as activity, skin elasticity, and fur color than those in the Finasteride group (Table 1). These suggest that the Finasteride conjugate may have other function on the animals than that of Finasteride, possibly with improved immune response or reduced toxicity. It would be of interest to further investigate the immune modulation activity of the Finasteride conjugate and compare it with Polimod. In addition, it is also important to study the pharmaco kinetics and metabolism of this compound and see if the Polimod moiety is cleaved in vivo. Our results warrant further research of this new inhibitor of 5α-reductase in the treatment of benign prostate hyperplasia.

**Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.102.

**References and notes**

22. Grino, P. B.; Griffin, J. E.; Wilson, J. D. Endocrinology 1990, 126, 1165.