Chapter I

Introduction

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1.1 Background information

Androgenic alopecia (AGA) is a major type of scalp hair loss in both males and females, which is becoming a worldwide issue. It is caused by androgens, namely, testosterone (T) and 5α-dihydrotestosterone (5α-DHT). T is the major circulating androgen and is converted to 5α-DHT by the 5α-reductase enzymes (5α-R) [EC 1.3.99.5] which is present in two isoforms i.e., 5α-reductase type 1 (5α-R1) and 5α-reductase type 2 (5α-R2) [1-6]. Both androgens binds to the androgen receptor (AR), forming a receptor-ligand complex which is translocated to the nucleus where it acts as a transcription factor in regulating androgen-sensitive genes [7]. However, 5α-DHT exhibits five times higher binding affinity and 10-fold higher potency than T does in inducing androgen-sensitive genes. The products of these androgen-sensitive genes act as growth factors, affecting the hair growth [3,8]. During AGA, over production of 5α-DHT causes either the up- or down-regulation of growth factors resulting in the shortening of anagen/growth phase. This causes the miniaturization of large, thick-pigmented terminal hair to small, fine, un-pigmented vellus hair with a diameter of less than 0.03 mm [1-2].

Currently, minoxidil and finasteride are the two FDA-approved synthetic drugs used to treat AGA [9]. Minoxidil, a vasodilator and potassium channel opener, prolongs the anagen phase and converts vellus hair to terminal hair [10-11]. However, minoxidil is only effective on 30-35% of patients, and the treatment must be continued over lifetime [9]. In addition, the side effects of 2% and 5% minoxidil solution includes scalp irritation, pruritus, dryness, scaling, itchiness, redness, contact dermatitis and hypertrichosis [12-13]. Finasteride, a synthetic azo-steroid, is a 5α-R2 inhibitor which binds irreversibly to the enzyme and inhibits the conversion of T to 5α-DHT, thereby reducing the serum 5α-DHT concentration by 68% [3]. However, finasteride is effective in only 48% of the patients [12], with the observed side effects of impotence, abnormal ejaculation, abnormal sexual function, myalgia, testicular pain and gynecomastia [14].

Because only two synthetic drugs are currently used, a search for new drugs in treating AGA is necessary. Among various sources, natural products provide an abundance of diverse chemical structures, representing a rich source for lead structures in drug development [9]. Recent studies have primarily focused on medicinal plants, and many natural product groups have been demonstrated to have 5α-R inhibitory activity. These products include a sterol from *Cuscuta reflexa* [15]; fatty acids namely: oleic, lauric, myristic and linoleic acid from *Serenoa repens* [16]; a

triterpenoid, ganoderic acid from *Ganoderma lucidium* [17]; the saponins, soyasaponin I and kaikasaponin III from *Pueraria thomsonii* [18]; and a catechin, epigallocatechin-3-gallate [EGCG] from *Camellia sinensis* [19]. In addition, various well-known flavonoids, such as myricetin, quercitin, alizarin, kaempferol, genistein and daidzein, have also been reported to exhibit inhibitory activity [19]. However, none of these reports have used dermal papilla cells in their cell-based or enzyme-based (source of 5α-R) assay systems. The dermal papilla cells surrounded by the extracellular matrix make up the dermal papilla, which is the main regulator of hair growth and without which the hair follicle cannot exists. In addition, dermal papilla cells are the only site of androgens action within the hair follicle and also express 5α-R [1, 9]. Therefore, inhibition of 5α-R activity in this specific type of cells should have a direct effect in the treatment of AGA. Therefore, to obtain an inhibitory assay system for this type of hair loss, it is most relevant and reliable to use hair cells specifically dermal papilla cells, from humans, namely human hair dermal papilla cells (HHDPCs).

Inhibition of 5α-R within HHDPCs leads to the decrease of T to 5α-DHT conversion and thereby reducing the formation of the receptor-ligand complex, hence affecting the expression of androgen-sensitive genes of the growth factors. This effect is known as the anti-androgenic activity of a potential 5α-R inhibitor. Therefore, potential natural products or extracts should have the ability to reverse the subsequent effects of androgens on various relevant growth factors produced by HHDPCs, such insulin-like growth factor 1 (IGF-1), fibroblast growth factor-7 (FGF-7), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) [20-21]. As the receptor-ligand complex acts as a transcriptional factor, the anti-androgenic activity must be tested at the transcriptional level, i.e., the mRNA expression levels within HHDPCs using RT-PCR [21].

In this study, a non-radioactive HHDPC-based assay system, which is highly relevant to the AGA type of human scalp hair loss, combined with non-radioactive thin layer chromatography (TLC) detection technique was developed and used to screen forty-one Thai medicinal plant extracts for the 5α-R inhibitory activity. The most potent crude extract was further tested for the anti-androgenic activity through the potential of overcoming the effects of androgens on the expression of mentioned growth factors. In addition, activity-guided fractionation was conducted in order to isolate active compound(s) with 5α-R inhibitory activity and their anti-androgenic activities was also evaluated. Structure elucidation of the active compound(s) was also carried out.

1.2 Objectives

1.2.1 To evaluate the properties of HHDPCs for the suitability of this study

1.2.2 To develop a novel AGA-relevant HHDPC-based assay system in a 96-well plate format

1.2.3 To identify the most potent 5α-R inhibitory crude extract from the forty-one Thai medicinal plant extracts using the developed assay system

1.2.4 To observe the anti-androgenic activity of most potent 5α-R inhibitory crude extract on androgens treated and un-treated HHDPCs

1.2.5 To identify active compound(s) with 5α-R inhibitory activity from the most potent crude extract through activity-guided fractionation and further investigate their anti-androgenic activities

1.3 Hypothesis

As AGA is caused due to the over-production of 5α-DHT which results in the shortening of the anagen phase, therefore one potential target for finding new hair growth promoters is to inhibit the formation of 5α-DHT by inhibiting the 5α-R within HHDPCs. An additional increase in the expression of the growth factors produced by HHDPCs in presence of androgens would prolong the anagen/growth phase of the hair cycle, resulting in hair growth.

1.4 Scope of study

1.4.1 To identify the presence of enzymes and AR in HHDPCs

1.4.2 Screening of forty-one Thai medicinal plant extracts for 5α-R inhibitory activity using a newly developed HHDPC-based assay system

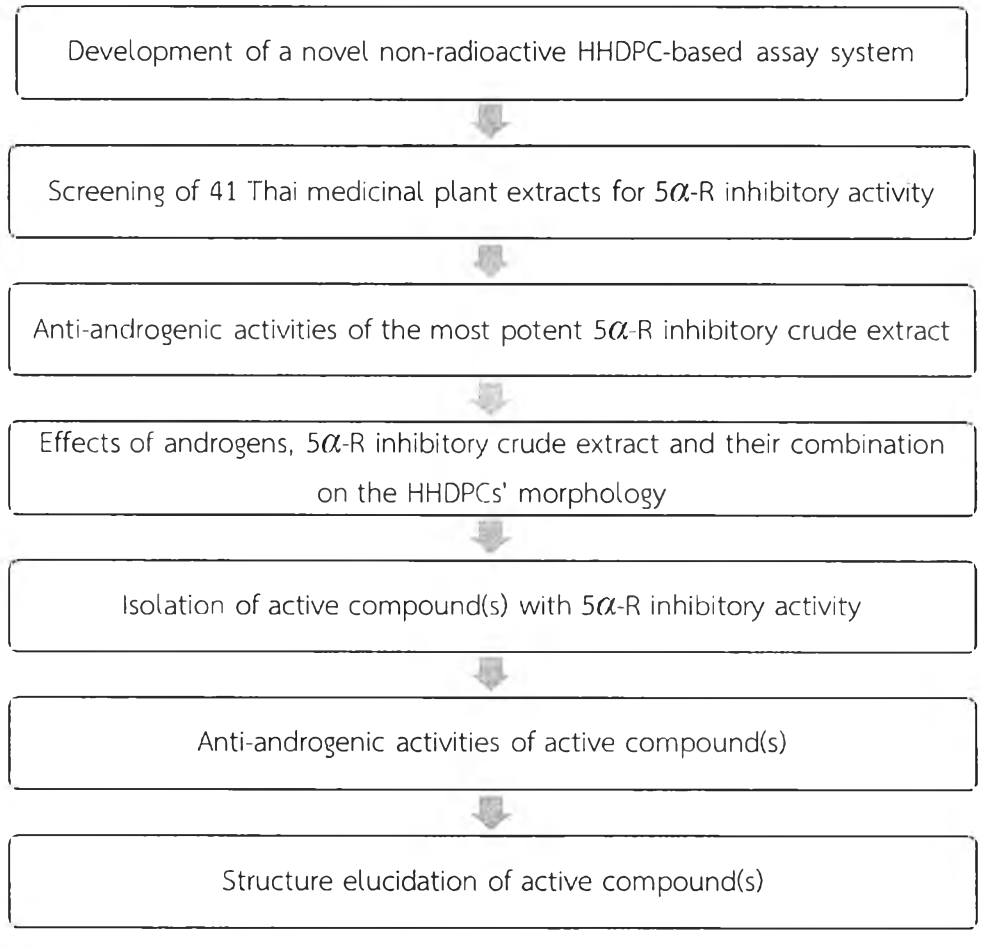
1.4.3 Studying the effect of androgens and the most potent 5α-R inhibitory crude extract on the expression of growth factors produced by HHDPCs

1.4.4 Observation on the changes in HHDPCs' morphology when treated with androgens and the most potent 5α-R inhibitory crude extract

1.4.5 Isolation of active compound(s) from the most potent 5α-R inhibitory crude extract possessing 5α-R inhibitory activity and further investigate their anti-androgenic activities

1.4.6 Characterization of the active compound(s)

1.5 Conceptual framework



1.6 Benefits

Purified compound(s) isolated from the most potent crude extract possessing 5α-R inhibitory and anti-androgenic activities might have the potential in treating AGA patients.

1.7 Thesis organization

The thesis is organized into five chapters starting from introduction, literature review, materials and methods, results and discussion and conclusions.