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BOTANICAL EXTRACTS FOR THE TREATMENT OF ANDROGENETIC ALOPECIA

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ABSTRACT

Androgenetic alopecia (AGA), commonly referred to as male or female pattern hair loss, affects approximately 50% of the population by the age of 50. While AGA is primarily a cosmetic condition, it is associated with aging and reduced virility and hence has negative psychological and social impact on patients. A number of treatments have been proposed for AGA, many originating from botanical extracts. Though, few of these treatments have undergone rigorous scientific scrutiny, years of empirical evidence often support their efficacy. In this study, we explored the use of lauric and myristic acid in the treatment of AGA. Gas chromatography and mass spectroscopy data taken from a representative sample of popular botanical treatments in Asia, revealed that lauric and myristic acid are the most commonly used botanicals for the treatment of AGA. We conducted a novel ex-vivo assay on surgically biopsied hair follicles to demonstrate the action of lauric and myristic acid on the biochemical pathways linked to AGA. Interestingly, we found evidence to support that the mechanism of action is not direct inhibition of 5α-reductase but instead a reduction of free testosterone.

Key Words: Androgenic Alopecia, Lauric acid, Myristic acid, 5α-reductase.

INTRODUCTION

Many pharmacological drugs used today were derived from botanicals and the medicinal use of botanically derived compounds pre-dates modern medicine. A survey in 2001 found that 122 compounds used in modern pharmacology were originally derived from botanical sources (Fabricant and Farnsworth 2001). This is not surprising given the plethora of molecules readily extractable from plant species, many reflecting each plant's exposure to pathogens and individual environment. In the treatment of androgenetic alopecia (AGA), few botanical extracts have undergone rigorous scientific scrutiny; however, years of empirical evidence often support their efficacy.

AGA, commonly referred to as male or female pattern hair loss, affects approximately 50% of the population by the age of 50 (Kuster and Happle 1984; Bergfeld 1995; Tang, Chia et al. 2000; Xu, Sheng et al. 2009) While AGA is primarily a cosmetic condition, it is associated with aging and reduced virility and hence has negative psychological and social impact on patients (Schmitt, Ribeiro et al. 2012). A myriad of treatments have been proposed for AGA, many originating from botanical extracts. In this communication we interpret how the botanical extracts lauric and myristic acid (found in non-pharmaceutical hair products) affect the scientifically established pathways involved in the pathogenesis and treatment of AGA. We further demonstrate, the efficacy of these botanicals in reducing testosterone in surgically biopsied hair follicles.

Androgen Receptor Pathway in AGA

AGA is a condition that effects both sexes (Price 1999). AGA leads to the miniaturization of hair follicles that over time produces shorter and thinner hair (Price 1975; Kaufman 1996). As the name implies, the manifestation of AGA depends on the effects of androgens in genetically predisposed individuals (Price 1999; Roberts, Fiedler et al. 1999). Testosterone (T) is a steroid hormone primarily secreted in the testes of males and ovaries of females. In the hair follicle, peripheral T is converted to the more potent dihydrotestosterone (DHT) by the enzyme 5α-reductase (5αR) (Jackson 2000). Both T and DHT can bind to the androgen receptor (AR) and initiate its translocation to the nucleus and subsequent activation of genes leading to the miniaturization of hair follicles (Fig 1). However, DHT has been demonstrated to be the responsible androgen in the progression of AGA (Price 1975; Kaufman 1996; Price 1999; Roberts, Fiedler et al. 1999; Jackson 2000). Men genetically deficient in 5α-reductase have an absence of AGA (Imperato-McGinley, Guerrero et al. 1974) and an over expression of 5αR has been observed in the hair follicles of individuals with AGA (Sawaya and Price 1997).

Based on an understanding of the biochemical pathway, it is apparent that the progression of AGA can be inhibited at more than one reaction step. Most treatments have focused on diminishing the conversion of T to DHT by competitive inhibition of 5αR. For example, the commonly prescribed, US FDA approved pharmaceutical treatment for AGA, finasteride, is a competitive inhibitior of 5αR (Kaufman 2002). However, inhibition of DHT binding to AR is an equally appropriate strategy; androgen receptor antagonists such as flutamide are sometimes used in treatment of AGA (Paradisi, Porcu et al. 2011).

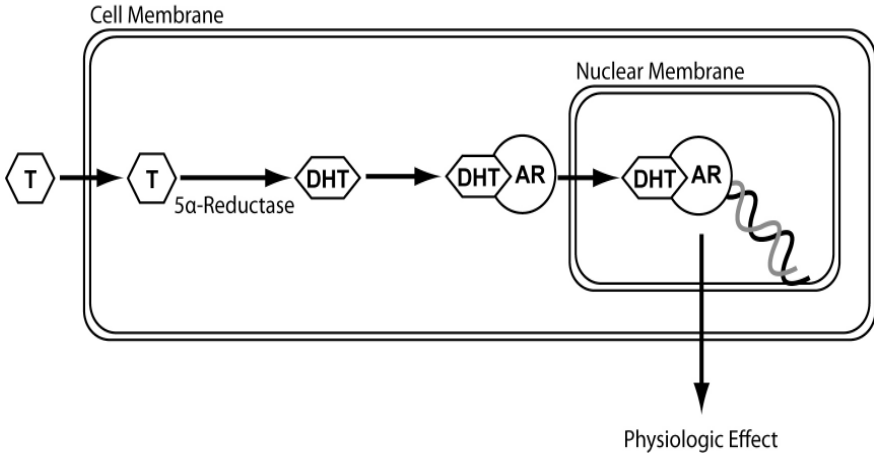

Figure 1: ***Testosterone and DHT pathway in AGA.***

Synthetic Inhibitors of 5α-Reductase

The conversion of T to DHT is accomplished by two isoforms of 5αR, type I and type II. The sythetic drug finasteride (Fig. 2) is a competitive inhibitor of 5αRII. Finasteride reduces the conversion of T to DHT in the hair follicles and thus diminishes the activation of AR by the higher affinity androgen, DHT (Stoner 1990) (Fig. 3). The efficacy of finasteride in the treatment of AGA is well documented (Kaufman, Olsen et al. 1998; Leyden, Dunlap et al. 1999). Three clinical trials with 1,879 men suffering from AGA were sponsored by Merck, Inc. The studies demonstrated that after 12 months, 58% of the patients in the placebo group continued loosing hair compared to 14% of the patients treated with finasteride (Kaufman, Olsen et al. 1998; Leyden, Dunlap et al. 1999; Price, Menefee et al. 2002). In addition, investigators assessed significant hair growth in 65% of the patients treated with finasteride vs 37% in the placebo group (Kaufman, Olsen et al. 1998).

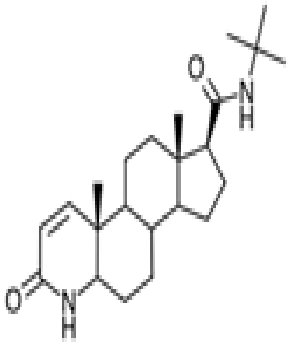

Figure 2: ***Finasteride***

Other synthetic 5αR competitive inhibitors have also been shown to be effective for the treatment of AGA. A study of dutasteride, a 5αRI and 5αRII inhibitor, on 416 men with AGA was conducted by Olsen et al. (Olsen, Hordinsky et al. 2006). At 24 weeks, 56% of patients treated with dutasteride had an increase in hair count larger then 10% compared to 0% in the placebo group.

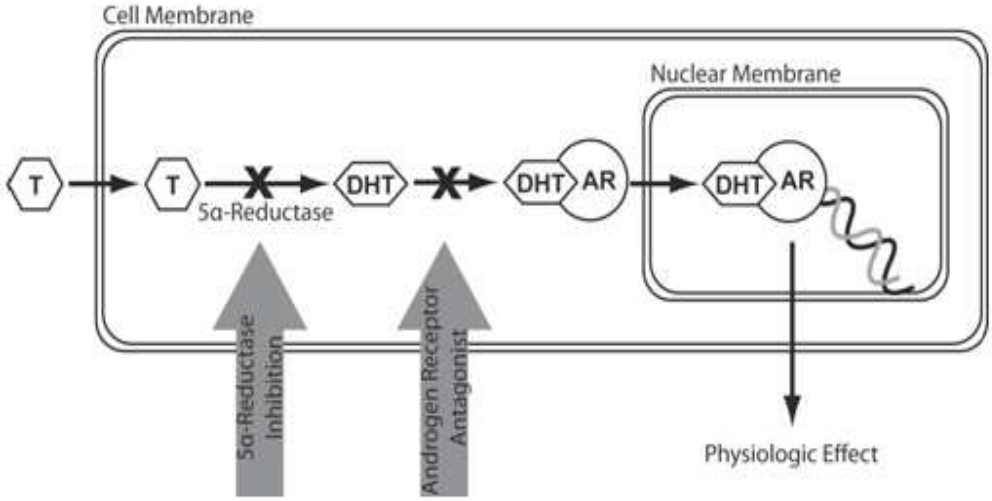

Figure 3: ***5α-Reductase inhibitors mode of action***

Botanical Inhibitors of 5αR

Botanical inhibitors of 5αR have been studied in the treatment of Benign Prostatic Hyperplasia (BPH). DHT is a mediator of prostate growth and 5αR inhibitors provide an effective treatment for BPH (Nickel, Gilling et al. 2011; Schmidt and Tindall 2011). To gain deeper understanding of natural treatments for BPH, Liu et al (Liu, Shimizu et al. 2009) investigated the affect of naturally occurring fatty acids on the inhibition of 5αR activity. Fatty acids with carbon chain lengths between 12 and 16 exhibited more then 50% inhibition of 5αR at 1.3 mM. Among the fatty acids tested, lauric acid and myristic acid exhibited the most significant 5αR inhibition. Lauric acid is derived from botanicals such as coconut oil. It is a saturated fatty acid with a chain of 12 carbon atoms (Fig. 4). Lauric acid has been shown to be a 5αR I and II inhibitor (Raynaud, Cousse et al. 2002). Myristic acid is derived from botanicals such as nutmeg butter and coconut oil. It is a saturated fatty acid with a chain of 14 carbon atoms (Fig. 5). Myristic acid has been shown to be 5αR II inhibitor (Raynaud, Cousse et al. 2002).

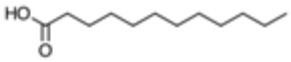

Figure 4: Lauric acid.

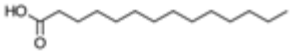

Figure 5: Myristic acid.

These studies support the claim that the botanicals myristic acid and lauric acid may provide effective non-pharmaceutical treatments for AGA. Hundreds of non-pharmaceutical botanically derived products are marketed as treatment for AGA. Due to the scarcity of scientific reviews, the majority of these products are dismissed by the medical community.

In this study, we have chosen to investigate further the compounds lauric and myristic acid based on gas chromatography and mass spectroscopy data obtained from several botanical products marketed for the treatment of AGA by Svenson Hair Centers - Asia. These ingredients were determined to be the most commonly deployed botanicals for the treatment of AGA from Svenson, a large distributor of traditional Chinese herbal remedies in Asia. Subsequently, we preformed an ex-vivo ELISA assay to examine the efficacy of lauric and myristic acid in reducing the amount of T to DHT conversion in surgically biopsied hair follicles.

MATERIAL AND METHODS

Gas Chromatography Mass Spectroscopy Analysis

Gas chromatography mass spectroscopy (GC/MS) analysis of 17 hair and scalp products marketed by Svenson Hair Products in Asia was performed at an accredited lab (Hwayo Tech & Lab Co., Ltd. - Taiwan). The products analyzed were Svenson's Advanced Factor R Yellow, AP23, Corrective Scalp Drops, Corrective Shampoo, Diachol II / Diachol Lotion, Eugenol Scalp Drops, Factor R Drops, Follicular Nourishing Scalp Serum, Hair Root Intensification Drops, H Lotion, J Lotion, JH Lotion, Juniper Shampoo, Morning Tonic, Night Tonic, SVSO Lotion, and Trichsolve Scalp Drops.

Ex-Vivo 5αR Activity Assay

Anagen VI hair follicles were micro-dissected from temporofrontal and occipital human scalp skin of AGA patients. The 5αRI isoform is present throughout the hair follicle, while, the 5αRII isoform has been localized primarily in the dermal papilla and connective tissue sheath (Asada, Sonoda et al. 2001). Accordingly, each hair follicle was dissected further to isolate the follicular bulb from the remaining bulk of the hair shaft. This was done to remove as much as possible 5αRI.

Isolated bulbs were placed in 100 uL of buffered saline containing 50 pg of testosterone (IBL USA). Myristic and lauric acids were obtained commercially (Cayman Chemicals, Chicago, Il) and dissolved in ethanol. 10 uL of the acid containing ethanol were doped into each sample as indicated. 10 uL of ethanol was added to each control sample. Enzymatic activity was measured at 16 hours. Testosterone metabolism was determined by an ELISA assay (IBL USA CAT# IB79303) following the protocols of the manufacturer. Optical densities were measured at 450nm on a Shimadzu UV-1700 spectrophotometer.

RESULTS

Botanical Extract Actives

Gas chromatography and mass spectroscopy revealed that 15 of the 17 products contained myristic or lauric acid. Of the 15 products, 7 contained myristic acid, 13 contained lauric acid,

and 5 contained both lauric and myristic acids (see Table 1). The two remaining products did not contain any known 5αR inhibitors; however, they contained eugenol, a molecule shown to be an AR antagonist (Ogawa, Akamatsu et al. 2010). In addition, some of the products contained botanical ingredients that have been demonstrated to be prostaglandin D2 inhibitors, a newly discovered pathway for promoting hair growth currently under investigation (Garza, Liu et al. 2012). These product ingredients will be investigated in future studies.

Botanical Actives Delivery

14 of the 17 products we reviewed contained a significant percent of ethanol. Solutes dissolved in ethanol have been proven to penetrate the hair follicle readily. Pharmaceutical products such as topical minoxidil penetrate more effectively at higher ethanol concentrations (Tata, Flynn et al. 1995). Furthermore, we confirmed that lauric acid solubility in ethanol is extremely high (~50% w/w at 20^{o}C (Ralston 1942)). The solubility of myristic acid in ethanol is half that of lauric acid (~25% w/w at 20^{o}C (Ralston 1942)), but is sufficiently high to allow for solute to reach the hair follicle.

Ex-Vivo ELISA Assay of Human Hair Follicle

The botanicals lauric and myristic acid have been previously reported to block the conversion of testosterone to DHT by 5αR in samples of fresh rat liver (Liu, Shimizu et al. 2009). In order to test if myristic and lauric acid would have similar DHT reducing efficacy on humans, we measured the consumption of testosterone by human hair follicles using an ELISA assay. Samples of single dissected hair follicles were incubated overnight in saline solution containing 50 pg of free testosterone and botanicals where indicated (Fig. 6). Active enzymes in each hair follicle reduce testosterone to DHT. Surprisingly, the samples with added myristic and lauric acid were found to have less testosterone after incubation than the control sample with no botanical added. We believe that this data suggests that lauric and myristic acid interact directly with testosterone forming a steroid ester. Because the ELISA test is specific for testosterone and not DHT this result could also be interpreted as proof that lauric and myristic acid are 5αR agonists, improving the conversion of T to DHT. However, this is highly improbably given the strong evidence presented by Liu et. al. (Liu, Shimizu et al. 2009) supporting the opposite conclusion. Experiments to test directly the presence of steroid esters suggested by these data will be performed in future studies.

Table 1: ***Botanical Content of Sampled Products***

Product	Myristic Acid	Lauric Acid	Eugenol
1	-	X	-
2	X	-	-
3	X	-	-
4	-	X	-
5	X	X	-
6	-	-	X
7	-	X	-
8	-	X	-
9	-	X	-
10	X	X	-
11	X	X	-
12	X	X	-
13	-	X	-

14	-	X	-
15	-	X	-
16	-	-	X
17	X	X	-

Products: (1) Advanced Factor R Yellow, (2) AP23, (3) Corrective Scalp Drops, (4) Corrective Shampoo, (5) Diachol II / Diachol Lotion, (6) Eugenol Scalp Drops, (7) Factor R Drops, (8) Follicular Nourishing Scalp Serum, (9) Hair Root Intensification Drops, (10) H Lotion, (11) J Lotion, (12) JH Lotion, (13) Juniper Shampoo, (14) Morning Tonic, (15) Night Tonic, (16) SVSO Lotion, (17) Trichsolve Scalp Drops.

DISCUSSION

The medical community by and large does not employ botanical treatments for AGA. However, this is not necessarily due to lack of efficacy, but instead to lack of a systematic clinical review of botanical actives. Many patients have little alternative to the use of systemic hormone modulating drugs; use of these drugs can often result in severe side effects (Irwig and Kolukula 2011). In an attempt to elucidate the potential effectiveness of botanical extracts for the treatment of AGA, we studied a representative sample of popular botanical treatments in Asia. GC/MS data confirmed that most of these products contained the botanicals lauric and myristic acid.

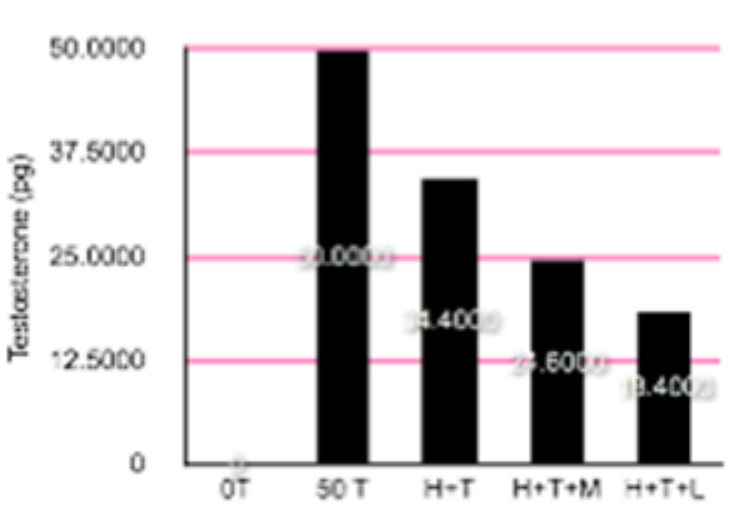

Figure 6: ***Testosterone conversion of incubated hair follicles. The labels are: 0T - no hair control with 0 pg testosterone, 50 T - no hair control with 50 pg testosterone, H+T - hair with 50 pg testosterone, H+T+M - hair with 50 pg testosterone and myristic acid, and H+T+L - hair with 50 pg testosterone and lauric acid.***

When immersed in ethanol these products should be easily absorbed in the scalp and hair follicle. Evaluation of the lauric and myristic acid concentrations when used in accordance with the manufacturer's recommend application, yields a dosage of approximately 0.1 mg - 0.2 mg daily to the scalp. In contrast, a pharmaceutical like finasteride delivers a dosage on the order of 0.001 mg daily to the scalp. Finasteride is approximately three order of magnitudes more potent an inhibitor of 5αRII then the botanical fatty acids; however, it has been demonstrated that finasteride is effective in many patients at dosages of 0.2 mg daily (Roberts, Fiedler et al. 1999) i.e., 20% of the daily recommended dosage approved by the US FDA. Therefore, it stands to reason that the botanical fatty acids, when used in accordance with the manufacturer's recommend daily application, could provide an efficient inhibition of follicular 5αR at the concentrations indicated by the manufacturer. In addition, other botanicals present in each product may enhance further the efficacy by acting on different biochemical pathways. Botanicals acting on these other chemical pathways will be the subject of future studies.

Our ELISA assay of testosterone conversion by incubated human hair follicles confirms that lauric and myristic acids have an appreciable effect on the level of testosterone. These results interpreted together with previous evidence (Liu, Shimizu et al. 2009), suggest that these fatty acids interact directly with testosterone. This interaction would lower the amount of testosterone available for conversion to DHT and benifit treatment of AGA. As such, myristic and lauric acids could provide an alternative to pharmaceutical treatments for AGA.

ACKNOWLEDGEMENTS

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