Journal of Ethnopharmacology 177 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Inhibitory activities of some traditional Chinese herbs against testosterone 5α-reductase and effects of Cacumen platycladi on hair re-growth in testosterone-treated mice

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form
10 August 2015
Accepted 5 November 2015
Available online 11 November 2015

Keywords:
5α-reductase
Androgenic alopecia
Traditional Chinese medicines
Hair re-growth
Dihydrotestosterone
Immunofluorescence

ABSTRACT

Ethnopharmacological relevance: Many traditional Chinese medicines (TCM) have been used for hundreds of years for hair blackening and hair nourishing, and now many of them are commonly used in Chinese herbal shampoo to nourish the hair and promote hair growth.
Aims of the study: The present study was performed to screen 5α-reductase (5αR) inhibitors from traditional Chinese medicines, evaluate its hair growth promoting activity in vivo, and further investigate its effects on androgen metabolism and the expression of 5αR II in hair follicles.
Materials and methods: Nine TCM which were dried, ground and extracted by maceration with 75% ethanol or distilled water were used for screening 5αR inhibitors, and enzymes were extracted from the rat epididymis. The leaves of *Platycladus orientalis* (L.) Franco was used to evaluate the in vivo anti-androgenic activity. Skin color was observed daily and the hair re-growth was assessed by assigning the hair growth score. The longitudinal sections of hair follicles were used for observing follicle morphology, classifying of distinct stages of hair follicle morphogenesis and calculate the average score. The transverse sections were used for determination of hair follicle counts. Testosterone (T), Dihydrotestosterone (DHT) and Estradiol (E2) levels in serum and skin tissue were detected by ELISA kits. The immunofluorescence assay was used to detect the influence of CP-ext on 5αR expression in dorsal skin.
Results: We found the extract of *Ganoderma lucidum* (GL-ext), *Polygonum multiflori* (PM-ext), Cacumen platycladi (CP-ext) and *Cynomorium songaricum* (CS-ext) showed stronger 5αR inhibitory activity. CP-ext (5 mg and 2 mg/mouse/day) could significantly shorten the time of the dorsal skin darkening and got longhaired ($P < 0.01$), and showed high hair re-growth promoting activity. Furthermore the histological data of hair follicles in each group showed that CP-ext could promote the growth of hair follicle and slowed down hair follicles enter the telogen. What's more CP-ext significantly reduced DHT levels and down-regulated the expression of 5αRIIin skin ($P < 0.01$).
Conclusions: GL-ext, PM-ext, CP-ext and CS-ext showed strong 5αR inhibitory activity. CP-ext possesses high hair growth promoting activity in the in vivo androgen-sensitive mouse model via inhibiting the 5αR activity, decreasing the DHT levels and in turn suppressing the expression of 5αR. Our study may contribute to the development of a new generation of herbal supplements with clearer material basis of pharmacodynamic for treating androgenic alopecia (AGA).

1. Introduction

5αR is a key enzyme in the metabolism of androgens that converts testosterone (T) into the more potent androgen dihydrotestosterone (DHT) (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968). It is a microsomal enzyme localized in the membrane of target cells. Three isozymes of 5αR are already known: the isozyme type I (5αRI), the isozyme type II (5αRII) and the isozyme type III (5αRIII). 5αRI is mainly expressed in livers and non-genital skin, the optimal pH range it play a physiological role is 6–9, while 5αRIIis the predominant isozyme detectable in androgen-dependent tissues such as the prostate, epididymis, seminal vesicles, and hair follicles and the optimum pH is 5.5

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http://dx.doi.org/10.1016/j.jep.2015.11.012
0378-8741/

(Asada et al., 2001; Zhu and Sun, 2005), which suggests that it is closely related to hair loss. And 5αRIII has been identified recently, which is overexpressed in the tissues of hormone-refractory prostate cancers (Uemura et al., 2008; Iwai et al., 2013). Studies have demonstrated that the generation of many diseases such as androgenic alopecia (McPhaul and Young, 2001), benign prostatic hyperplasia (Bartsch et al., 2000), acne (Förström, 1980), and female hirsutism (Faloia et al., 1998) is closely to the abnormal metabolism of androgens or the role of androgen, in which 5αR plays an important role.

Seborrheic alopecia (SA), also known as androgenic alopecia (AGA) or male pattern alopecia (MPA) and female pattern alopecia (FPA) is characterized by greasy hair, dandruffy, itching, progressive alopecia in frontotemporal area and followed forming baldness. Although it may not be a life-threatening disorder, it has a great impact on a person's appearance, self-respect, mental health and overall quality of life. 5αR II, which converts T to DHT is the predominant form in hair follicles and plays a more critical role in the pathogenesis of SA. Although androgens mediate their activities by binding to the same androgen receptors (AR), the affinity of DHT is fivefold higher (Ellsworth et al., 1998). AR belongs to the nuclear receptor superfamily and functions as a transcription factor. Upon binding ligand, AR undergoes a conformational change, translocates to the nucleus, and binds to the specific DNA sequence of target genes. These result in positive (Promote hair growth) or negative (Inhibit hair growth) regulation of gene expression. Therefore, inhibition of 5αR II would thus be a good target for investigation of efficacy in SA. In fact, in recent years, many steroidal and non-steroidal 5αR inhibitors have been synthesized. Finasteride, a specific 5αRII inhibitor, has been used to treat androgen-related disorders and achieved outstanding results. However, these synthetic inhibitors often come with a lot of adverse reactions, for example: erectile dysfunction, abnormal sexual function, gynecomastia, impairment of muscle growth, and so on (Lacy et al., 2008).

Recently years, the researchers are focus on looking for new, effective and low toxicity 5αR inhibitors from natural plants for the treatment of SA, which is of great significance. And many plants were proved to have the potential to inhibit 5αR and promote hair growth, for example: *Piper nigrum* leaf (Hirata et al., 2007), Puerariae Flos (Kazuya et al., 2012), *Carthamus tinctorius* and *Phyllanthus emblica* (Kumar et al., 2012), and *Rosmarinus officinalis* Leaf (Kazuya et al., 2013).

Traditional Chinese medical theory believes poliosis and lapsus pilorum are related to the weakness of liver, kidney and blood vigor. Therefore, the cure must rely on the reinforcement of the factors above. In China, many TCM have been used for nourishing the liver and kidney, promoting blood generation, cooling blood and blackening hair for hundreds of years (Huang et al., 2007; Zhang, 2010). Modern medical theory proves that many of them could promote hair follicle growth (Chen et al., 2010; Gu et al., 2007; Wu et al., 2006), which were used for the treatment of hair loss and applied in Chinese herbal shampoo.The Leaves of *Platycladus orientalis* (L.) Franco is a commonly used TCM in the treatment of hair loss, and previous experiments revealed that it mainly comprises volatile oils, flavonoids and tannins and could promote hair growth in mice (Chen et al., 2010; Shan et al., 2013). However, the possible mechanisms involved in their treatment of hair loss have not yet been elucidated. In this manuscript, we first report the results of the screening of the 5αR inhibitors from those crude drugs. In addition, an in vivo AGA model suppressed by testosterone was used to evaluate its hair growth promoting activity, and further investigate its effects on androgen metabolism and the expression of 5αR II in hair follicles. These crude drugs, their traditional efficacy and their applications are shown in Fig. 1 and Table 1.

Fig.1. Traditional Chinese medicines we used in the experiment.