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Research Article

Development and Validation of Androgen-induced Hair Loss and Anagen Induction Mouse Models for Pharmacological Evaluation of Anti-Androgenic Agents

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Abstract

Background and objectives: Androgen-induced hair loss and anagen induction mouse models are commonly used in Androgenetic alopecia (AGA) studies. This paper aims to verify the feasibility of these models for evaluating the androgen receptor (AR) antagonist *in vivo* and investigated its pharmacological mechanism via biomarker study. **Methods:** We compared various doses and administration routes of dihydrotestosterone (DHT) and testosterone propionate (TP) to optimize the androgen-induced hair loss model. The efficacy of Minoxidil and Pyrilutamide (KX-826, Kintor Pharma, Suzhou China) was evaluated using the optimized androgen-induced hair loss model and the anagen induction mouse model. We investigated the pharmacological mechanism of Pyrilutamide via biomarker studies. **Results:** Subcutaneous administration of 30 mg/kg of DHT slowed hair growth, and no apparent skin lesions were observed, making it the optimal dose for efficacy experiments. Both Minoxidil and Pyrilutamide significantly promoted hair growth in androgen-induced hair loss and the anagen induction mouse models. Minoxidil and Pyrilutamide both decreased the AR protein level and enhanced the Wnt/β-Catenin signaling pathway. **Conclusions:** The anagen induction mouse model and the optimized androgen-induced hair loss model are suitable for evaluating anti-androgenic agents. These models can be used to study the efficacy and pharmacological mechanisms of novel AGA treatments.

Keywords: Androgenetic alopecia; Mouse model; Androgen receptor; Anti-androgenic agents

Abbreviations: AGA: Androgenetic Alopecia; AR: Anti-Androgenic Drugs; DHT: Dihydrotestosterone; TP: Testosterone Propionate; HF: Hair Follicles; DPC: Dermal Papilla Cells

Introduction

Androgenetic alopecia (AGA), the most common type of hair loss for both men and women, is characterized by a gradual loss of terminal hair and follicular miniaturization to vellus hair fibers in a characteristic distribution [1]. Although the mechanisms of AGA remain largely unknown, the involvement of androgens and the androgen receptor (AR) is well known [2]. Higher levels of mRNA or protein of 5α-reductase and AR are found in the scalp balding area [3]. There is no balding seen in individuals with non-functional AR (e.g., patients with androgen insensitivity syndrome) [4]. Previous studies revealed that 5α-reductase converts testosterone into dihydrotestosterone (DHT) in hair follicles (HF), and AR enters the nucleus of dermal papilla cells (DPCs) in response to DHT binding and acts as a transcription factor to regulate gene expression [4]. Studies showed that DHT drives DPCs to secrete paracrine factors like TGF-β1, IL-6, and DKK1 to promote apoptotic death and suppress cell proliferation, which is proven could push anagen phase entering into catagen phase earlier in shaved mice [4-7]. In addition, DHT is closely linked to the Wnt/β-catenin signaling pathway, which contributes to initiating and maintaining anagen [8, 9]. These findings imply that AR-5α-reductase-DHT complexes and their transactivation activity are responsible for the miniaturization of HFs in AGA.

AR and AR-related signaling pathways are proven to be involved in the pathogenesis of AGA. Topical AR antagonists or degraders are promising strategies with that there are limited treatment options at present. Minoxidil and Finasteride are the only Food and Drug Administration (FDA)-approved drugs for the effective treatment of AGA, and both of them show multiple disadvantages [9]. Topical Minoxidil requires lifelong dosing, and its pharmacological mechanism is unclear [10]. Orally administered Finasteride therapy is limited due to adverse sexual side-effects resulting from systemic inhibition of 5α-reductase [11]. Currently, a variety of topical AR antagonists or degraders are under extensive investigation, and they have shown promising results both in pre-clinical and clinical studies [12]. For example, Pyrilutamide (KX-826, Kintor Pharma, Suzhou China) showed a convincing efficacy and safety profile in a phase 2/3, randomized, double-blind, multicentre, dose-ranging clinical trial (CTR20201655) for AGA patients [12].

A well-established and verified animal model is critical for the development of new therapies in AGA. The commonly used mouse model for AGA study can be roughly divided into the androgen-induced hair loss model and the anagen induction mouse model [13]. The androgen-induced hair loss model uses androgens as modelling agents to slow down hair growth. The model is characterized by a shortened anagen and is generally used in medical research due to biological and behavioural similarities to human AGA. However, the modelling methods are diverse, such as the agent used, the agent dosage, and the administration route, among others [14, 15]. On the other hand, the anagen induction mouse model, without androgen-inducing, is based on the natural HF growth cycle of mice, which includes anagen, catagen, and telogen stages. Mice around the telogen stage are mainly used to study the effect of various plant extracts and non-androgen-related small molecule compounds on promoting HF entry into the anagen stage to induce hair growth [16-19].

Considering the increasing needs for more appropriate preclinical animal models for drug screening, we attempted to optimize the androgen-induced hair loss mouse model and explore the feasibility of using anagen induction mouse model for evaluating AR antagonists *in vivo*. The pharmacological mechanism of Pyrilutamide was also investigated. We sought to provide preferences on hair loss mouse model usage for translational research on AR antagonists or AR degraders.

Materials and Methods

Study design

Various doses and administration routes of DHT and TP were compared for optimizing the androgen-induced hair loss model. The efficacy of Minoxidil and Pyrilutamide was evaluated using the optimized androgen-induced hair loss model and the anagen induction mouse model. The pharmacological mechanism of Pyrilutamide was also investigated via biomarker examination.

Standard of Care Drugs and Lab Animals

The standard of care drug Minoxidil was obtained from Zhejiang Wanma Pharmaceutical Co., Ltd., (Zhejiang, China). Pyrilutamide, a clinically proven AR antagonist developed by Suzhou Kintor Pharma (Suzhou China; patents WO2022199577A1 and US20140315957A1; [20, 21], was produced in house.

Six-week-old male C57BL/6 mice were purchased from Slac Laboratory Animal Co., Ltd (Shanghai, China) or Vital-Star (Beijing, China). The mice were allowed to adapt for one week with ad libitum access to food and water. The mice were housed in a controlled barrier facility with a temperature of 23 ± 2°C, humidity of 35–60%, and a 12-hour light:12-hour darkness cycle. The study was reviewed and approved by the Institutional Animal Care and Use Committee of Simcere Pharmaceutical Co., Ltd., China.

Establishment of Androgen-Induced Hair Loss Model

To establish the androgen-induced hair loss model, the dorsal area (2 cm in width and 3 cm in length) of seven-week-old male C57BL/6 mice was shaved using animal clippers and depilated with hair removal cream which is produced from Veet, Oxy Reckit Benckiser (Chartes, France). For the model optimization experiment, the mice were randomly divided into seven groups of 5 to 7 mice each. The mice received subcutaneous administration of DHT (15, 30, or 60 mg/kg, 100 µL/20 g), subcutaneous administration of TP (25 mg/kg, 100 µL/20 g), intraperitoneal administration of DHT (1 mg in 160 uL per mouse), or the solvent alone (control group) once daily. DHT and TP were purchased from Energy Chemical, (China). DHT is dissolved in DMSO, and then corn oil was added (DMSO/corn oil, 5:95, v/v). The solvent of TP is corn oil. For the efficacy experiment, the mice were given subcutaneous injection of DHT (30 mg/kg) in a volume of 100 µL/20g once daily for four weeks. 0.5% Pyrilutamide (dissolved in ethanol/propylene glycol, 7:3, v/v) or Minoxidil (20 uL per cm^2) was applied to the skin twice daily. The mice were photographed and scored during the dosing period, and at the end of the experiment, all mice were sacrificed.

Generation of Anagen Induction Mouse Model

Male C57BL/6 mice were shaved with electric razors (2 cm in width and 3 cm in length) at seven weeks of age, at which time all of the hair follicles were synchronized in the telogen phase. 0.5% Pyrilutamide (dissolved in ethanol/propylene glycol, 7:3, v/v) or Minoxidil (20 µL per cm^2) was applied to the skin twice a day. The mice were photographed and scored during the dosing period, and at the end of the experiment, all mice were sacrificed.

Scoring method for hair re-growth

The hair re-growth was assessed by assigning a hair growth score, as follows: score 0=no growth observed; 1=up to 20% growth; 2=20-40% growth; 3=40–60% growth; 4=60-80% growth; and 5=80% to full growth observed [17, 22].

Western Blotting

AR protein level was tested by Western Blotting to investigate the pharmacological mechanism of Pyrilutamide. The harvested back skin was frozen with liquid nitrogen and grounded into powder. The powder was lysed in ice-cold RIPA lysis buffer, and proteins were extracted using a protein extraction buffer. Protein concentrations were determined using a protein assay reagent (Bio-Rad, USA). Forty micrograms of total protein from skin lysates were subjected to 4-12% Precast sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto Immobilon-P Transfer membranes. Antibody-antigen complexes were visualized using the ECL system, and the results were analysed using MF-ChemiBIS 3.2 (DNRBio-Imaging Systems Ltd., Israel). Western blotting was performed following the manufacturer's instructions for the antibodies. The antibodies AR (EPR1535(2)) was obtained from Abcam.

Quantitative PCR Analysis

The mRNA levels of TGF-β1, β-catenin, Wnt3a were tested by Quantitaive PCR investigate the pharmacological mechanism of Pyrilutamide. Total RNAs were extracted from the skins using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Sample cDNAs were amplified using the Model 7700 Sequence detector (Applied Biosystems, Perkin Elmer Japan, Chiba, Japan) with the following primer pairs: TGF-β1, 5'-GCCCTGGACACCAACTATTG-3' (forward) and 5'-GTCCAGGCTCCAAATGTAGG-3' (reverse); β-catenin 5'-GGCAGCGGCAGGATACACGG (forward) and 5'- CAGGACACGAGCTGACGCGG(reverse); Wnt3a 5'-TCTGCAGGAACTACGTGGAGATCA (forward) and 5'-TCCCGAGAGACCATTCCTCCAAAT (reverse). The thermal cycler conditions were as follows: 10 min at 95°C for denaturation, followed by 40 cycles of 10 s at 95°C for denaturation, and 34s at 60°C for annealing and extension. The mRNA levels of TGF-β1, β-catenin, Wnt3a were normalized to those of GAPDH from the same sample.

Enzyme Linked Immunosorbent assays (ELISA)

Blood samples collected from mice were centrifuged at 1,000 x g for 30 min. Serum was separated and stored at -70°C until assayed. Serum testosterone levels were measured by ELISA (R&D Systems, Wuhan, China, Cat. # KGE010). Briefly, each sample (100 µl each in duplicate) was placed in the 96 well plate, and 50 µl of Testosterone Conjugate was added and incubated for 3 h. After washing three times with wash buffer, 200 µl of substrate A and B mix were added and incubated for 30 min. The reaction was then terminated by the addition of 50 µl stop solution, and the absorbance was measured at 450 nm using a Spectra Max i3x Multi Mode detection platform (Molecular Devices, CA, USA).

Pathology and HE Staining

The number of terminal hairs and vellus hairs were counted at the end of the efficacy experiment of anagen induction mouse model. The dorsal skin of each animal was dissected and fixed in 4% paraformaldehyde. After 24 hours, the skin samples were processed for hematoxylin and eosin (H&E) staining. A light microscope was used for overall histological assessment. Transverse sections were used to determine the HF count. The number of terminal hairs (diameter larger than the inner hair root sheath diameter, with a darker hair shaft pigment) and vellus hairs (diameter smaller than the inner hair root sheath diameter, with light or no hair shaft pigment) were counted under a microscope (×100). The ratio of terminal hair to vellus hair was calculated.

Three visual fields with the most HFs were selected for each specimen, and the visual fields could not overlap. The number of terminal hairs and vellus hairs were counted separately.

Statistical Analysis

The results were expressed as mean ± SEM (standard error of the mean). Differences between group means were statistically analysed using two-way analysis of variance (ANOVA) followed by Post Hoc Tukey's tests. Statistical significance was defined as P<0.05.

Results

Development and optimization of androgen-induced hair loss models

Given the diverse modelling approaches for androgen-induced hair loss, we evaluated the most commonly used agents TP and DHT, along with the effect of the dosage and administration route (subcutaneous and intraperitoneal) in androgen-induced hair loss model development. The schematic illustration of the S.C. (subcutaneous) administration experiment is showed in the Figure 1a. Dose dependent hair loss effect was observed in DHT treated mice. Among the tested approaches, the subcutaneous administration of 30 mg/kg of DHT significantly slowed hair growth without causing apparent skin lesions. Administration of 25 mg/kg of TP and 15 mg/kg of DHT subcutaneously also delayed hair growth, albeit for a shorter duration. Notably, administration of 60 mg/kg of DHT resulted in skin damage, rendering it unsuitable for subsequent use (Figures 1b and 1c). In comparison, intraperitoneal administration of DHT was found to be less effective compared to subcutaneous administration (Figures 2a-2c). The body weights were stable in all modelling groups (Figs.1d and 2d). Overall, the subcutaneous administration of 30 mg/kg of DHT was considered as optimal approach and was used for the subsequent efficacy experiment.

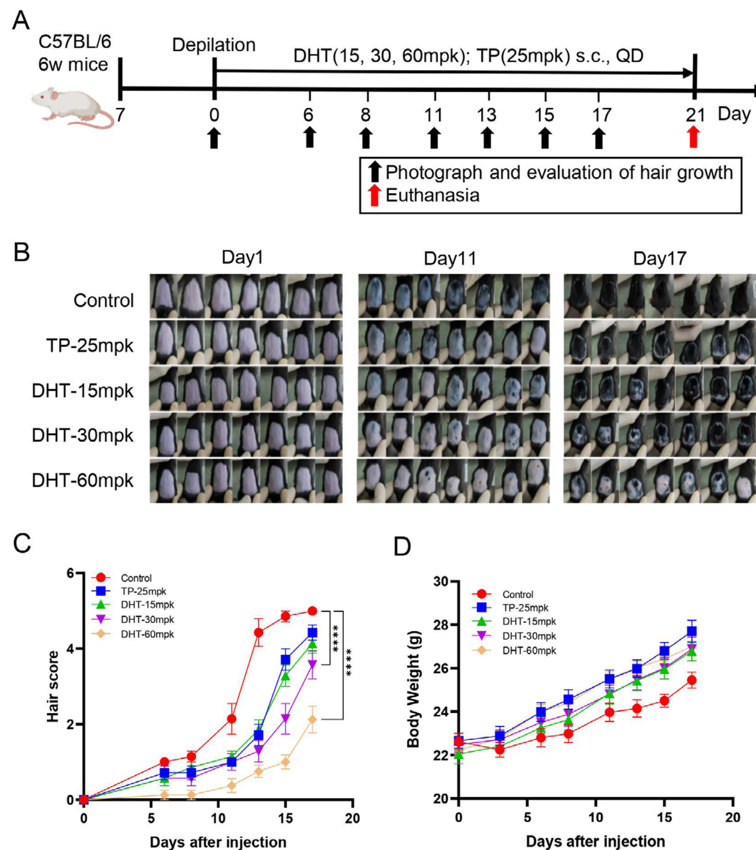


Figure 1: Development of the androgen-induced hair loss models using S.C. administrated DHT and TP. (A) Schematic illustration of establishment of the androgen-induced hair loss model. (B) Photographs of mice subjected to subcutaneous (S.C.) modelling, indicating inhibitory effects of DHT and TP on hair regrowth. (C) Hair scores of mice subjected to S.C. modelling. (D) Body weights of mice subjected to S.C. modelling. Significant difference from control group, ****P < 0.0001.

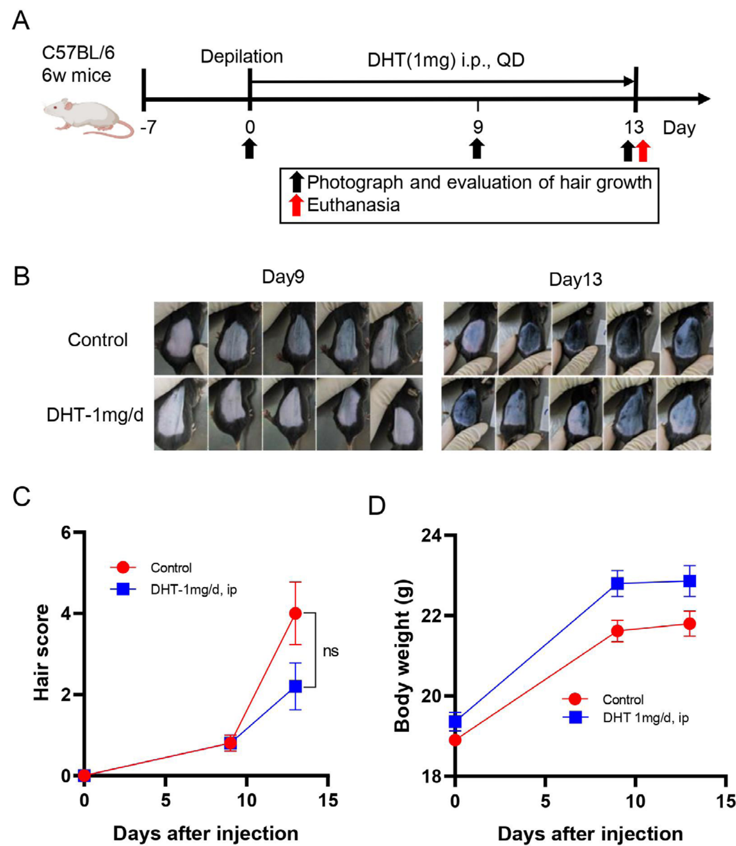


Figure 2: Development of the androgen-induced hair loss models using I.P. administrated DHT. (A) Schematic illustration of establishment of the androgen-induced hair loss model. (B) Photographs of mice subjected to intraperitoneal (I.P.) modelling. (C) Hair scores of mice subjected to I.P. modelling, illustrating inhibitory effects of DHT on hair regrowth. (D) Body weights of mice subjected to I.P. modelling.

Hair Growth-Promoting efficacy of Pyrilutamide and Minoxidil in Androgen-induced hair loss model

The AGA model generation procedures and experimental design are illustrated in Figure 3a. The optimized androgen-induced hair loss model was employed to evaluate the efficacy of Pyrilutamide and Minoxidil (Figure 3a). The results showed that both Minoxidil and Pyrilutamide promoted hair growth of AGA model, with Minoxidil being slightly higher efficacious than Pyrilutamide. Minoxidil group is showing statistical significance compared to the vehicle group ($P<0.01$) (Figures 3b and 3c). The body weights were stable for both Pyrilutamide and Minoxidil groups during the treatment period (Figure 3d).

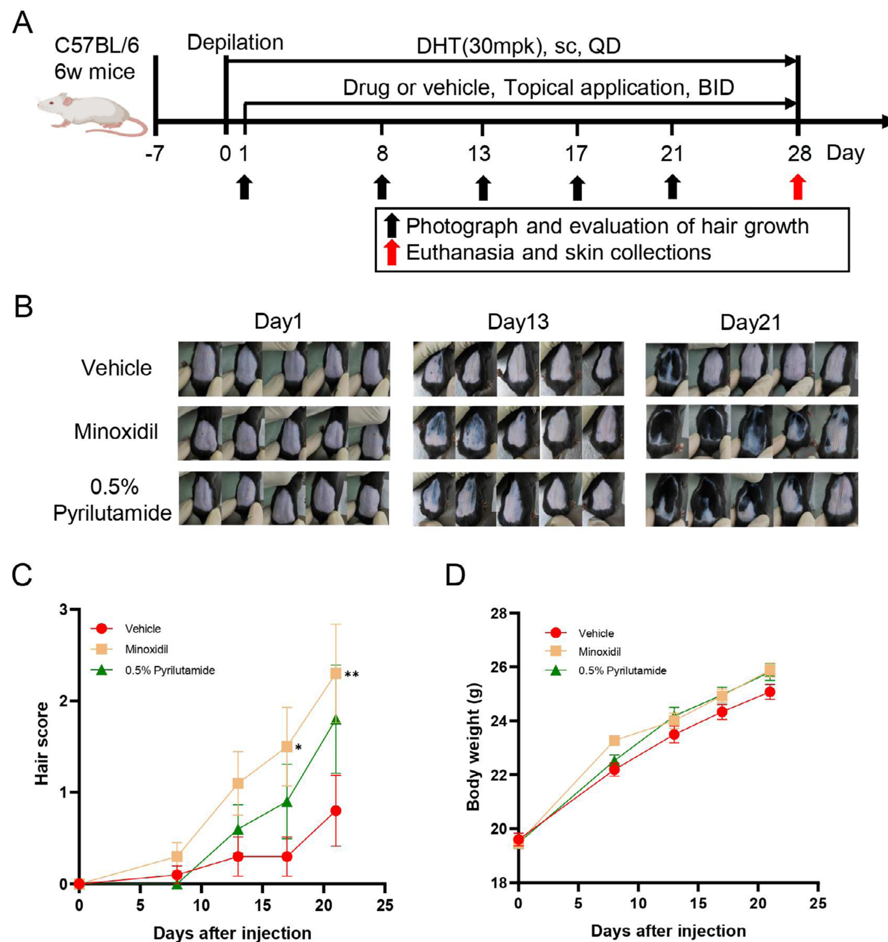


Figure 3: Effects of Pyrilutamide and Minoxidil on hair re-growth in the androgen-induced hair loss model. (A) Schematic of the efficacy experiment conducted on the androgen-induced hair loss model. (B) Photographs of mice in the efficacy experiment. (C) Hair scores of mice in the efficacy experiment. (D) Body weights of mice shown in (C). Significant difference from vehicle group, * P<0.05, **P<0.01.

Pharmacodynamic mechanisms of Pyrilutamide and Minoxidil in Androgen-induced hair loss model

The pharmacodynamic mechanisms of Pyrilutamide and Minoxidil were investigated using skin samples collected on day 28 of treatment. The AR protein level slightly decreased in the Pyrilutamide group, consistent with previous study [23]. The Minoxidil group also exhibited a decrease in AR protein levels, (Figure 4a), which was similar to the findings from literature [24]. As expected, mRNA levels of TGF-β1 were decreased in both treatment groups. Additionally, the mRNA level of Wnt3a was increased in both treatment groups, which is in line with expectations, and 0.5% Pyrilutamide group is showing statistical significance compared to the vehicle group (P<0.05) (Figure 4b). These results suggest that both Minoxidil and Pyrilutamide inhibit AR and activate the Wnt/β-catenin signaling pathway to promote hair growth in the androgen-induced hair loss model.

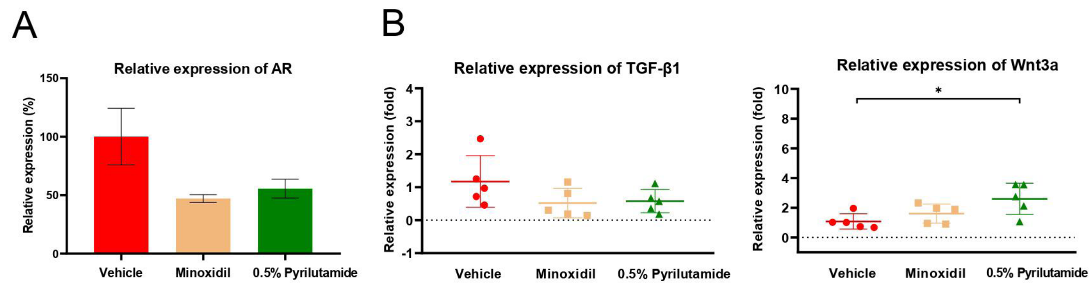
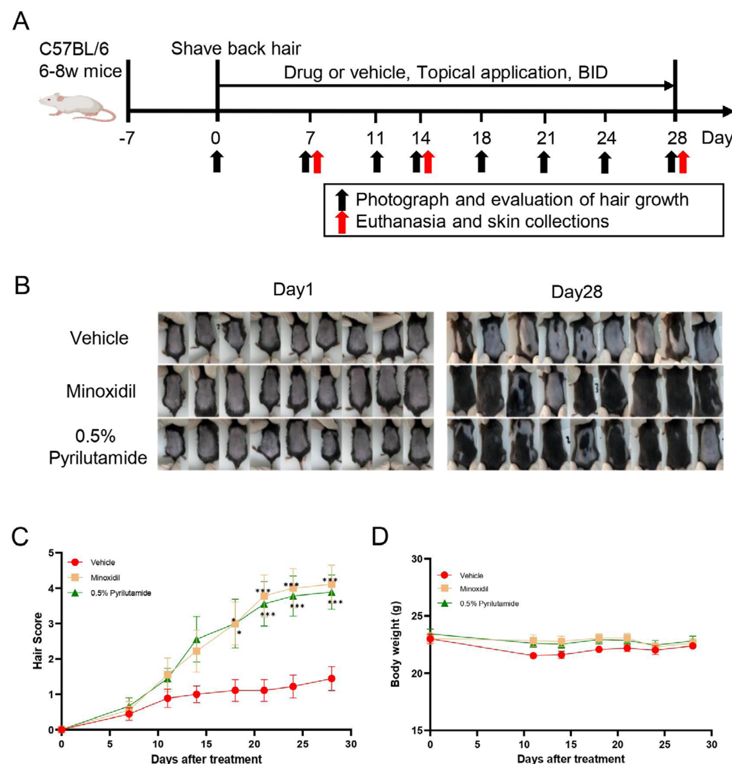


Figure 4: Effect of Pyrilutamide and Minoxidil on expression of AR and AR-regulated genes. (A) Inhibitory effects of Pyrilutamide and Minoxidil on AR expression. (B) Effects of Pyrilutamide and Minoxidil on the mRNA levels of TGF-β1 and Wnt3a. Significant difference of Wnt3a between 0.5% Pyrilutamide group and vehicle group, * $P < 0.05$.

Hair Growth-Promoting efficacy of Pyrilutamide and Minoxidil in anagen induction mouse model

The anagen induction mouse model is commonly employed to study the effects of various plant extracts and non-androgen-related small molecule compounds on hair growth. In this study, we investigated whether this model is suitable for evaluating AR antagonists such as Pyrilutamide. The experimental design is illustrated in Figure 5a.

The results showed that both Minoxidil and Pyrilutamide significantly promoted hair growth in anagen induction mouse model ($P<0.001$) (Figures 5b and 5c). The body weights were stable for both Pyrilutamide and Minoxidil groups during the treatment period (Figure 5d). HE staining results demonstrated that both test products increased the number of terminal hairs ($P<0.001$) and reduced the proportion of vellus hairs ($P<0.0001$) (Figures 5e and 5f).



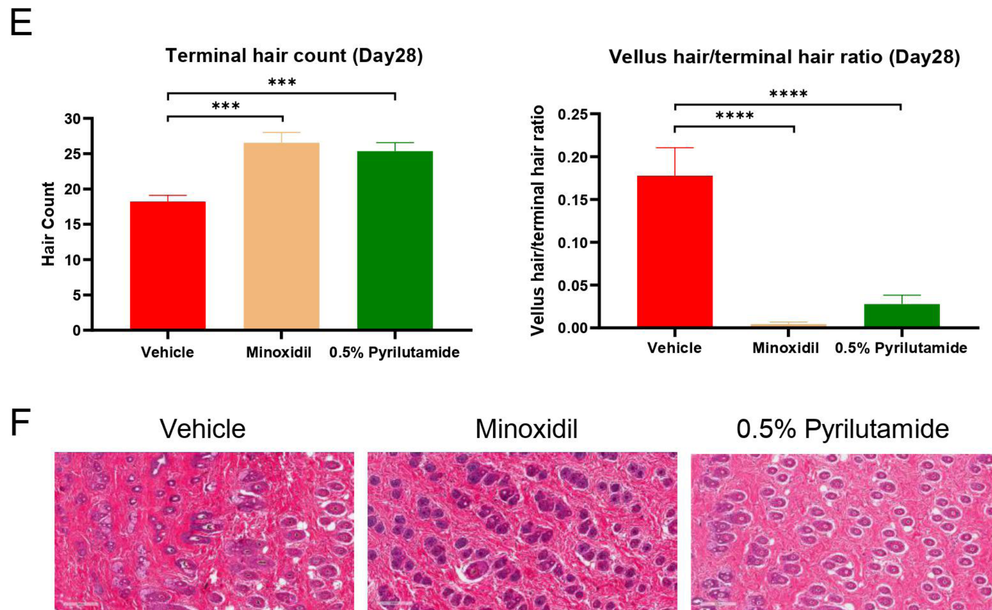


Figure 5: Pyrilutamide and Minoxidil promote hair growth in the anagen induction mouse model. (A) Schematic of the efficacy experiment conducted on the anagen induction mouse model. (B) Photographs of mice in the efficacy experiment. (C) Hair scores of mice in the efficacy experiment. (D) Body weights of mice shown in (C). (E) Statistical analysis of the number and proportion of terminal hair in mice. (F) Representative HE-stained sections (100X magnification). Statistical analysis compared with vehicle group, $^{*}P<0.05$, $^{***}P<0.001$, $^{****}P<0.0001$.

Pharmacodynamic mechanisms of Pyrilutamide and Minoxidil in anagen induction mouse model

The pharmacodynamic mechanisms of Pyrilutamide and Minoxidil were investigated in this model on day 7 of treatment. The AR protein level decreased in both the Pyrilutamide and Minoxidil groups. Furthermore, the mRNA level of β-catenin was increased in both treatment groups, with the Pyrilutamide group showing statistical significance (P<0.01). These results indicate that both Minoxidil and Pyrilutamide inhibit AR and activate the Wnt/β-catenin signaling pathway to promote hair growth in the anagen induction mouse model (Figures 6a and 6b).

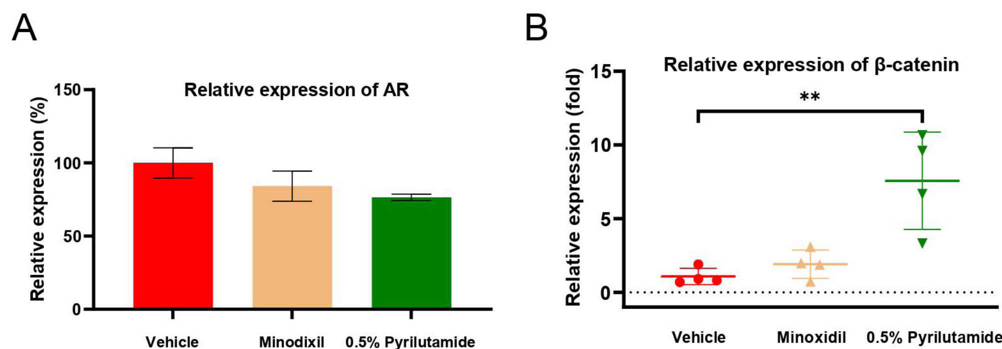


Figure 6: Effects of Pyrilutamide and Minoxidil on AR and β-catenin expression in the anagen induction mouse model. (A) Inhibition of AR expression by Pyrilutamide and Minoxidil. (B) Effect of Pyrilutamide and Minoxidil on the mRNA levels of β-catenin. Statistical analysis compared with vehicle group, $^{**}P<0.01$.

Discussion

Androgenetic alopecia (AGA) is the most common type of hair loss in both men and women, characterized by a gradual loss of terminal hair and follicular miniaturization. The involvement of androgens and the androgen receptor (AR) in AGA is well-established, with higher levels of 5α-reductase and AR observed in the balding scalp area. The conversion of testosterone to DHT by 5α-reductase and the subsequent binding of DHT to AR in DPCs play a crucial role in regulating gene expression and promoting hair follicle miniaturization. Additionally, DHT leads to the secretion of paracrine factors and inactivation of the Wnt/β-catenin signaling pathway, contributing to the progression of AGA. Current FDA-approved drugs for AGA, such as Minoxidil and Finasteride, have limitations and side effects, emphasizing the need for novel therapeutic strategies. Topical AR antagonists or degraders have shown promise in pre-clinical and clinical studies, with Pyrilutamide demonstrating efficacy and safety in a recent clinical trial. However, the development of appropriate preclinical animal models is crucial for evaluating the potential of these agents.

In this study, we optimized the androgen-induced hair loss model, a commonly used mouse model for AGA research. By evaluating different modelling approaches, we identified the subcutaneous administration of 30 mg/kg of DHT as the optimal method, which effectively slowed hair growth without causing significant skin lesions. Using this optimized model, we evaluated the efficacy of Pyrilutamide and Minoxidil. Both compounds demonstrated the ability to promote hair growth, with Minoxidil showing slightly stronger effects. Mechanistically, both compounds inhibited AR expression and activated the Wnt/β-catenin signaling pathway in the androgen-induced mouse model.

Furthermore, we explored the feasibility of using the anagen induction mouse model, a non-androgen-related model, for evaluating AR antagonists. Both Pyrilutamide and Minoxidil significantly promoted hair growth in this model, increasing the number of terminal hairs and reducing vellus hairs. The results indicated that AR inhibition and activation of the Wnt/β-catenin signaling pathway were involved in the mechanism of action for both compounds. The results of the pharmacodynamic mechanism suggest that AR may play a certain inhibitory role in the process of mouse natural hair growth cycle, which has been demonstrated in previous publications [25]. Our data confirmed the feasibility of the anagen induction mouse model for evaluating AR antagonists *in vivo*.

HF regeneration relies on multiple molecular signaling changes. The Wnt/β-catenin signaling pathways are indispensable for this process. In the adult mouse skin, AR activation by testosterone reduces β-catenin-dependent transcription, downregulates anagen induction and ectopic HF formation. The pharmacodynamic mechanism of Minoxidil and Pyrilutamide was investigated in both models. We found that Pyrilutamide reduced the protein level of AR and upregulated Wnt/β-catenin signaling in both models. Minoxidil showed the same trends but to a lesser extent than Pyrilutamide. Previous studies have shown that Minoxidil can interfere with AR and decrease AR protein stability, which is consistent with our findings here [24].

Among the mouse models used for AGA drug screening, the androgen-induced hair loss model is more relevant in terms of the pathology mechanisms. The modelling methods are diverse in many aspects of this model. Male mice at around 7 weeks are commonly used, at which time all of the hair follicles were synchronized in the telogen phase. Dorsal hair can be removed by shaving, depilation with hair removal cream, or depilation with rosin and wax mixtures before administration of androgen. Depilation with hair removal cream was chosen in our study because it can induce hair growth that could normalize the mice at the same HF growth cycle stage. We did not choose depilation with rosin and wax mixtures because it is harsh for mice skin, even though it gives synchronous hair growth. Besides the above factors, the modelling agent, dosage, and administration route were diverse in the literature. Therefore, we compared them to optimize the model. A previous study demonstrated that intraperitoneal injection of DHT inhibited hair regrowth to the same extent as subcutaneous injection, but we found that intraperitoneal injection with 1mg DHT had no significant hair growth with the control after 13 days. We suspected that the inconsistency comes from the different hair scoring methods that were used. This also suggests that appropriate scoring methods of hair growth should be used according to different hair removal methods. The area scoring method [17,22] is suitable for shaving and depilation with hair removal cream, but the overall density scoring method [21,23] is suitable for depilation with rosin and wax mixtures.

This study has limitations. A wider model window was observed in the pharmacodynamic experiment than in the modelling optimization experiment. The main difference between these two experiments is the vendor of the C57BL/6 mice. The mice of the modelling optimization experiment were more aggressive. We predicted that the serum testosterone hormone level may be higher in the mice of the modelling optimization experiment. The results showed the same as we predicted (Figure S1). The higher testosterone level may cause less sensitivity to the DHT treatment for the modelling optimization experiment mice. We suggest keeping the same vendor of mice for the experiment, even though more study is needed to confirm the concern.

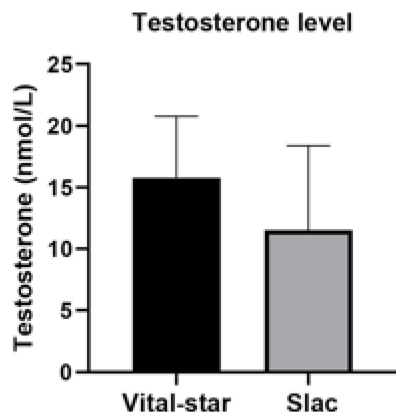


Figure S1: The serum testosterone level of Vital-Star and Slac mice.

Anagen induction mouse model is based on the natural HF growth cycle, and mice on the telogen stage were used. Telogen of C57BL/6 mouse starts at postnatal day 43-45 for males and day 58 for females and lasts for about 5 weeks [17,26]. During the telogen phase entering the anagen, Wnt/β-catenin signaling activation and HF morphological changes are the same as the natural state. However, the natural HF growth cycle is easily interfered with by mouse genetic background, age, sex, as well as environmental factors (temperature, light periods). Therefore, we suggest considering the above factors to ensure the model's stability and extend the experimental duration according to the actual observation. In addition, solvent of DHT needs to be optimized. We used corn oil to formulate DHT because it is commonly used, but it is hard to be absorbed. Thus, the skin ulceration in 60mpk DHT group observed in this study was possibly caused by the combined effects of high DHT and unabsorbed corn oil. An alternative solvent for DHT is desired.

Future Research Directions

Although the present study demonstrated that anagen induction mouse model is feasible for evaluating AR antagonist, further *in vivo* mechanism study is needed to illustrate how AR involved in the natural hair cycle.

Conclusions

We optimized the androgen-induced hair loss mouse model and confirmed the feasibility of the anagen induction mouse model for evaluating AR antagonist *in vivo*. In conclusion, our study provides insights into the optimization of the androgen-induced hair loss model and the feasibility of using the anagen induction mouse model for evaluating AR antagonists. The findings support the potential of Pyrilutamide as therapeutic options for AGA and highlight the importance of AR inhibition and Wnt/β-catenin pathway activation in promoting hair growth. Further research in this area will contribute to the development of novel therapies for AGA.

Acknowledgements

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Ethical Statement

The animal study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jiangsu Simcere Pharmaceutical Co., Ltd.

Author Contributions

X.Y., and M.C. made equal contributions to this work. Conceptualization, W.Y.; methodology, X.Y., M.C., X.S., SQ.Z., F.T., J.T., C.A. and W.Y.; data curation, X.Y., M.C., X.S., SQ.Z., F.T., J.T., C.A.; writing—original draft preparation, X.Y., M.C., X.S. and W.Y.; writing—review and editing, SQ.Z., F.T., L.X., SW.Z., F.W. and W.Y. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author reports no conflicts of interest in this work.

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