

Reduction of Interleukin-6 after PRP-Exosomes Treatment in A Mouse Model of Androgenetic Alopecia

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Abstract

Purpose: This study aim to determine the role of platelet-rich plasma (PRP) exosomes as anti-inflammatory (IL-6) in a mouse model of androgenetic alopecia.

Methodology: There are four group of androgenetic alopecia mice model with no treatment group (control group, K1), group treated with PRP-exosomes (K2), group treated with 5% topical minoxidil (K3) and the last group treated with combination of PRP-exosomes and 5% topical minoxidil (K4). All of the group was evaluated clinically and immunohistochemical examination of IL-6 was performed on the 32nd day.

Results: Better hair growth results were obtained in the treatment group compared to the control group. In clinical evaluation, K4 had the best results, followed by K2 and K3. The expression of IL-6 is highest in the K1, followed by K4, K2 and K3 and statistically significant.

Applications/Originality/Value: PRP-exosomes can enhance hair growth in androgenetic alopecia mice model. IL-6 expression in the control group is significantly higher than in the treatment group.

Introduction Section

Androgenetic alopecia (AGA), also known as male or female pattern hair loss, is the most common form of hair loss worldwide. It is a progressive condition marked by the gradual miniaturization of terminal hairs into vellus hairs under the influence of androgens, especially dihydrotestosterone (DHT) (Saavedra et al., 2023). This condition can begin after puberty and affects a significant portion of the population up to 80% of men and 50% of women during their lifetime. The prevalence varies among ethnicities and geographic regions, with studies showing that about 53% of European-American men aged 40–49 are affected in the United States, and the lifetime prevalence in this population can reach up to 90%. Meanwhile, in Asia, AGA affects approximately 20% of Chinese men aged 40–49 years, and 14.1% of Korean men of all ages (with 5.6% of Korean women affected as well) (Frith and Jankowski, 2023).

AGA is a genetically predisposed condition, often inherited in a polygenic manner, and is strongly linked to the activity of androgen hormones. Patients with AGA have higher levels of DHT, increased expression of 5 α -reductase (an enzyme responsible for converting testosterone into DHT), and a greater number of androgen receptors in the affected scalp regions (typically the frontal and vertex areas in men, and central scalp in women). These molecular changes lead to shortened anagen (growth) phases and progressively finer hair shafts, ultimately resulting in visible hair thinning or baldness (Abdin et al., 2022).

Although often classified as a non-inflammatory and non-scarring alopecia, mounting evidence suggests that inflammation plays a subtle yet critical role in the pathogenesis of AGA. Early ultrastructural studies, such as those conducted by Jaworsky et al., revealed inflammatory infiltrates and changes in the perifollicular environment of balding areas. These included mast cell degranulation, fibroblast activation, and the presence of activated T cells around the bulge region of hair follicles, suggesting that immune-mediated inflammation contributes to follicular miniaturization and progression of hair loss. This concept is further supported by studies indicating elevated levels of pro-inflammatory cytokines, including Interleukin-6 (IL-6), in the affected scalp. IL-6, in particular, has been shown to inhibit hair shaft elongation and is considered a negative regulator of hair growth (Kwack, 2012).

Clinically, AGA presents differently in males and females. In males, it typically begins with a receding frontal hairline and thinning over the vertex, eventually leading to complete baldness in the affected regions. In females, it more commonly presents as diffuse thinning over the crown with preservation of the frontal hairline. Despite being a benign medical condition, AGA can have profound psychosocial consequences. Hair is closely tied to cultural and individual identity, and hair loss is often perceived as a visible sign of aging or ill health. Studies have shown that individuals with AGA may suffer from reduced self-esteem, social withdrawal, anxiety, depression, and lower health-related quality of life (HRQOL) (Aukerman et Jafferany, 2022).

Treatment of AGA remains a significant clinical challenge due to the chronic nature of the condition and the variable efficacy of current therapies. Only two medications, topical minoxidil and oral finasteride, are approved by the U.S. Food and Drug Administration (FDA) for the treatment of AGA. Minoxidil is a vasodilator that prolongs the anagen phase, while finasteride inhibits 5 α -reductase, reducing DHT levels. However, both drugs require long-term, often lifelong use, and their effects diminish upon discontinuation. Moreover, side effects such as sexual dysfunction (in the case of finasteride) and scalp irritation (with minoxidil) limit adherence and satisfaction (Huang, et al., 2021).

In addition to these conventional treatments, alternative and adjunctive therapies have gained popularity in recent years. These include low-dose oral minoxidil, dutasteride, spironolactone (especially in women), low-level laser therapy (LLLT), microneedling, red light therapy, and hair transplantation. Each of these interventions has shown varying degrees of efficacy, but standardization across clinical trials remains inconsistent, making it difficult to compare outcomes or define best practices. Change in anagen hair count remains the most objective and consistent endpoint in determining treatment success (Kaiser et al., 2023).

Among emerging therapies, platelet-rich plasma (PRP) has garnered significant interest. PRP is an autologous concentrate of platelets in plasma that contains a high concentration of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF- β), which are believed to stimulate hair follicle activity and reduce inflammation. Clinical studies suggest that PRP can improve hair density and thickness in patients with AGA (Pakhomova, 2020). However, variability in PRP preparation methods and treatment protocols presents challenges for reproducibility and comparison across studies (Ntshingila et al., 2023).

Another innovative and promising treatment for AGA is the use of exosomes. Exosomes are nanosized extracellular vesicles derived from stem cells that facilitate intercellular communication and are rich in signaling molecules, including proteins, lipids, and RNAs. These vesicles have shown regenerative properties in various tissue repair models and are thought to modulate immune responses, stimulate angiogenesis, and enhance cellular proliferation and differentiation (Devjani et al., 2023). Because of their size and content, exosomes can penetrate the scalp and influence hair follicle stem cell niches. Their ability to regulate inflammation, including the downregulation of IL-6 and other pro-inflammatory cytokines, makes them a compelling candidate for AGA therapy (Oiwoh, et al., 2024).

Combining PRP with exosomes represents a novel therapeutic approach that harnesses the regenerative capacity of PRP and the potent signaling ability of exosomes. The rationale for this combination lies in their complementary mechanisms of action—PRP supplies a reservoir of growth factors, while exosomes act as biological messengers that can enhance and prolong the effects of PRP. Moreover, both agents may exert anti-inflammatory effects, potentially modulating the low-grade inflammation observed in AGA and improving the scalp microenvironment (Kidagazhiathmana and Santhosh, 2022).

In the context of the inflammatory hypothesis of AGA, targeting IL-6 represents a meaningful therapeutic goal. Given the emerging understanding of IL-6 as a suppressor of hair growth, therapies that can reduce IL-6 expression or activity in the scalp may restore follicular homeostasis and promote hair regeneration. Although previous studies have focused on either PRP or exosomes alone, there is limited literature evaluating the combined effect of PRP-exosomes specifically on inflammatory markers like IL-6 (Krefft-Trzcieniecka, et al., 2023).

Therefore, the objective of this study was to investigate the role of PRP-exosomes in reducing IL-6 expression in a mouse model of androgenetic alopecia. By evaluating both clinical and histological outcomes, this research aims to provide insight into the anti-inflammatory mechanisms of PRP-exosome therapy and support its development as a potential treatment for AGA. Understanding how this novel combination affects the inflammatory cascade in AGA may open new avenues for effective, targeted interventions for this prevalent and psychologically burdensome condition (Nestor, et al., 2021).

Method

This study used a post-test only with control group laboratory experimental design which aimed to determine the role of PRP exosomes as anti-inflammatory (IL-6) in the androgenetic alopecia mouse model.

There were four groups of mice, all groups were induced with subcutaneous testosterone injection of 0.1 cc on the back skin area of mice measuring 2x2 cm for 10 days. The first group (K1) was the negative control group without further treatment, the second group (K2) was treated with PRP-exosomes, the third group (K3) was treated with 5% minoxidil and the fourth group (K4) was treated with a combination of PRP-exosomes and 5% minoxidil. Furthermore, for 21 days, the second group received 0.1 cc exosomes PRP injection on days 11, 18, and 25, the third group was given topical 5% minoxidil with a dose of 2x spray every day until the 31st day and the combination group received treatment like the second and third groups.

The mice was evaluated clinically and skin tissue sampling for immunohistochemical examination of IL-6 was performed on the 32nd day.

Results and Discussion

In this experimental study on a mouse model of androgenetic alopecia (AGA), clinical differences in hair regrowth were evaluated among four groups: untreated AGA (K1), PRP-exosomes treatment (K2), 5% minoxidil treatment (K3), and the combination of PRP-exosomes and 5% minoxidil (K4). As shown in **Figure 1**, group K4 demonstrated the most significant hair regrowth, characterized by denser and darker hair coverage. K2 and K3 also showed improvement compared to the control group, with K2 (PRP-exosomes alone) exhibiting better outcomes than K3 (minoxidil alone). These findings align with the theory that a combinational approach targeting both regeneration and follicular activation yields superior clinical outcomes (Gupta et al., 2023).

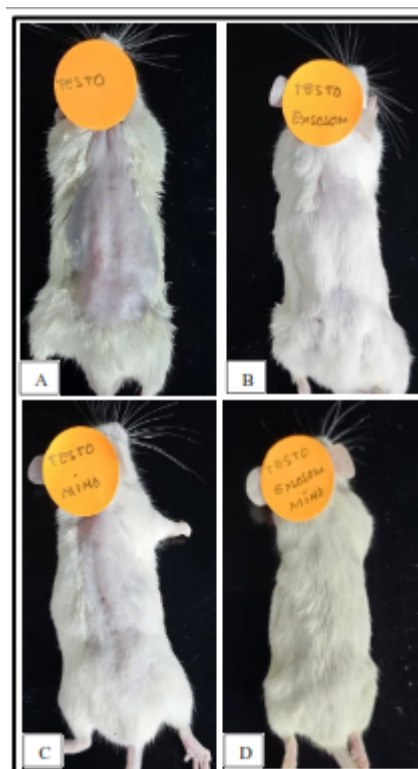


Figure 1. Clinical picture of the mice model. A. Androgenetic alopecia mice model without treatment (K1) B. Androgenetic alopecia mice model after treated with PRP-exosomes (K2) C. Androgenetic alopecia mice model after treated with 5% minoxidil (K3) D. Androgenetic alopecia mice model treated with combination of PRP-exosomes and 5% minoxidil (K4).

In this study, better hair growth results were obtained in the treatment group compared to the control group seen in [Table 1](#). In clinical evaluation, K4 had the best results, followed by K2 and K3 (Figure 1). Exosomes, which are extracellular vesicles secreted by various cells, are known to mediate intercellular communication and promote tissue repair. In the context of hair regeneration, exosomes derived from mesenchymal stem cells (MSCs) have demonstrated the ability to modulate signaling pathways involved in the hair cycle. Specifically, they play a pivotal role in promoting the transition from telogen to anagen phase by transferring mRNAs, microRNAs, and proteins to dermal papilla cells (Gupta et al., 2023). PRP, or platelet-rich plasma, provides an additional regenerative stimulus by delivering a concentration of growth factors such as VEGF, PDGF, and IGF-1, which support follicular health and vascularization (Huang et al., 2021).

Minoxidil, on the other hand, is a well-known potassium channel opener and vasodilator that has long been used for AGA therapy. It prolongs the anagen phase of the hair cycle and increases follicular size (Kang et al., 2019). Additionally, it reduces perifollicular microinflammation and may upregulate vascular endothelial growth factor (VEGF), contributing to improved perfusion of the follicular microenvironment (Wasitaadmadja et al., 2019). Thus, while minoxidil works primarily by enhancing blood supply and follicular responsiveness, exosomes and PRP have a more regenerative and anti-inflammatory action.

Table 1. The expression of IL-6 in the research group.

IL-6	Mean \pm SD	Minimum	Maximum
Group 1	12.01 \pm 3.79	7.13	17.08
Group 2	2.85 \pm 4.50	0.25	12.87
Group 3	2.67 \pm 2.18	0.56	6.38
Group 4	3.42 \pm 2.25	1.33	6.31

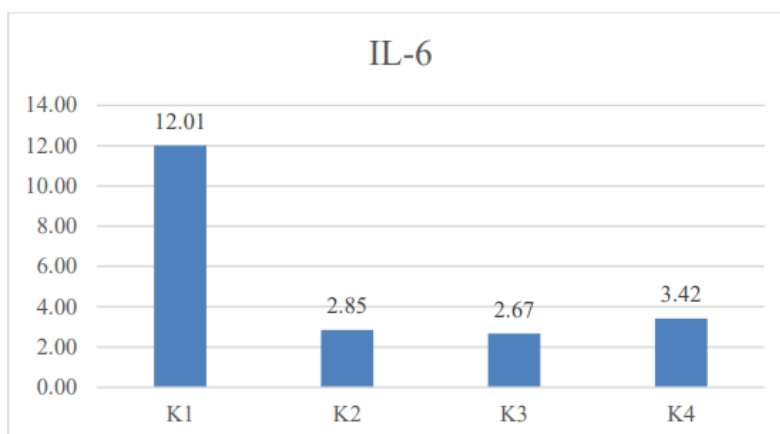


Figure 2. The chart of IL-6 mean values between research groups.

Figure 2 presents the immunohistochemistry results for IL-6 expression in the scalp tissues. The expression of interleukin-6 (IL-6), a pro-inflammatory cytokine associated with follicular miniaturization, was highest in K1 (untreated control), indicating active inflammation and disease progression. Conversely, IL-6 levels were markedly reduced in K2, K3, and K4. Among treated groups, K4 showed significantly lower IL-6 levels than K2 and K3, suggesting synergistic anti-inflammatory effects when PRP-exosomes and minoxidil are combined. These differences were statistically significant, as demonstrated by the post hoc Dunn test results in [Table 2](#) ($p < 0.05$).

Table 2. Post hoc test for IL-6 value.

IL-6		p-value
Group (mean)	Group (mean)	
Group K1 (12.01)	Group K2 (2.85)	<0.001*
Group K1 (12.01)	Group K3 (2.67)	0.003*
Group K1 (12.01)	Group K4 (3.42)	0.011*

Group K2 (2.85)	Group K3 (2.67)	0.559
Group K2 (2.85)	Group K4 (3.42)	0.314
Group K3 (2.67)	Group K4 (3.42)	0.673

Note: The post hoc Dunnett T3 test (data that is normally distributed but not homogeneous) (*) is statistically significant if the p-value < 0.05.

In this study, in the Figure 2, the expression of IL-6 is highest in the K1, followed by K4, K2 and K3. Based on the post hoc Dunn test in the table 2, group K1 (control group) shows a significant difference compared to all treatment groups (K2, K3 and K4), with a p-value <0.05. This indicates that all therapeutic treatments administered have a significant effect in reducing IL-6 levels in mice models of androgenetic alopecia.

Androgenetic alopecia is a dihydrotestosterone dependence of male pattern baldness. Circulating androgens, including dihydrotestosterone, enter the follicle via the dermal papilla capillaries. IL-6 is upregulated in balding dermal papilla cells compared with non-balding dermal papilla cells (Kwack et al, 2011). IL-6 has previously been implicated in the pathophysiology of AGA. According to Kwack et al. (2011), IL-6 expression is markedly upregulated in dermal papilla cells of balding scalp compared to non-balding areas. IL-6 promotes premature catagen entry and inhibits keratinocyte proliferation, contributing to follicular regression. Therefore, suppression of IL-6 is a desirable therapeutic target in the treatment of AGA (He et al., 2022).

AGA is a polygenic disorder with complex androgen-dependent and androgen-independent pathways. Twin studies have established a heritability of up to 80% in men, with the androgen receptor (AR) gene on the X chromosome being one of the most well-studied loci (Hillmer et al., 2005). Moreover, genome-wide association studies (GWAS) have identified more than 100 SNPs associated with AGA, including polymorphisms in the EDA2R, HDAC9, SRD5A2, and WNT10A genes. Many of these genetic variants affect pathways involved in stem cell activation, extracellular matrix remodeling, and hormonal signaling (Liu et al., 2021).

Importantly, some of these loci regulate inflammation and immune cell infiltration, thereby linking genetic susceptibility to the observed upregulation of cytokines like IL-6. The integration of PRP-exosomes into therapy may help counteract this genetic predisposition by modulating the inflammatory microenvironment and promoting regenerative signals at the molecular level (Pakhomova and Smirnova, 2020).

Understanding the dynamics of the hair cycle is crucial for interpreting the therapeutic effect of PRP-exosomes. The hair follicle undergoes a cyclical pattern composed of three phases: anagen (growth), catagen (regression), and telogen (rest) (Kang et al., 2019). During the anagen phase, mesenchymal-epithelial interactions between dermal papilla cells and hair follicle stem cells drive active hair shaft production. The dermal papilla is a key signaling hub, orchestrating the proliferation and differentiation of matrix keratinocytes (Wasitaadmadja, 2019).

In our study, the increased anagen activity observed in K2 and K4 suggests that exosome treatment can enhance these interactions, possibly by stimulating Wnt/ β -catenin and Sonic hedgehog pathways known to be crucial for anagen initiation. Exosomes have also been shown to promote angiogenesis and increase fibroblast activity, both of which contribute to improved follicular health and hair regeneration (Devjani et al., 2023).

Psychosocial Impact and Clinical Relevance

Although AGA is not life-threatening, it exerts a substantial psychosocial burden. Studies show that men and women with AGA report reduced quality of life (QoL), anxiety, and depressive symptoms. However, a recent systematic review noted that while there is a mild to moderate impact on QoL, clinically significant psychiatric morbidity is rare and possibly overstated in commercial research (Kranz, 2011; Lundh et al., 2017). Many patients experiencing distress are also those actively seeking treatment, which introduces selection bias.

Despite this, the aesthetic and emotional consequences of AGA cannot be ignored. Hair is closely tied to self-image and social identity, and visible hair loss can impair confidence and social functioning (Huang et al., 2021). Clinicians must consider both the physical and psychological dimensions of AGA when proposing treatment plans. The incorporation of regenerative therapies such as PRP-exosomes not only addresses follicular pathology but also improves patient satisfaction by offering novel, biologically active alternatives (Fritz and Jankowski, 2023).

Ethical and Practical Considerations

The therapeutic landscape for AGA is expanding, with an increasing focus on personalized, minimally invasive, and regenerative approaches. However, this also raises concerns about cost, accessibility, and long-term efficacy. PRP and exosome therapies are not yet standardized and are often expensive, which may limit their widespread clinical use. Moreover, outcome variability due to differing preparation techniques (e.g., centrifugation speed, source of exosomes) underscores the need for protocol standardization in future research (Oiwoh et al., 2024).

From an ethical standpoint, it is vital to provide patients with realistic expectations. While treatments like minoxidil and PRP-exosomes show promise, complete hair restoration is rarely achievable. Time should be dedicated to counseling patients about treatment duration, maintenance therapy, and the expected degree of improvement. In cases where psychological distress is apparent, referral to mental health professionals should be considered, especially as stress is a known trigger for telogen effluvium and other hair disorders (Ntshingila et al, 2023).

Limitations and Future Directions

This study is limited by its animal model design and short observation period. Although the mouse model provides valuable mechanistic insights, human trials are necessary to confirm the therapeutic potential of PRP-exosomes and their anti-inflammatory effects. Furthermore, the subjective nature of visual hair regrowth assessment necessitates the inclusion of quantitative measures such as follicular density, hair shaft diameter, and dermoscopic imaging in future research.

It is also important to explore the molecular content of exosomes used in therapy. Identifying key miRNAs and proteins responsible for the observed effects could pave the way for next-generation exosome engineering to enhance efficacy. Lastly, longitudinal studies are required to assess the durability of treatment outcomes and the need for maintenance therapy.

Conclusions

This study demonstrates that PRP-exosomes therapy significantly enhances hair regrowth in a mouse model of androgenetic alopecia (AGA). Among all groups, the combination of PRP-exosomes and 5% minoxidil (K4) showed the most promising results, followed by PRP-exosomes alone (K2), and minoxidil alone (K3), with the untreated control group (K1) showing minimal or no hair regeneration. The enhanced hair regrowth observed in the PRP-exosome-treated groups suggests that this therapy promotes the anagen phase of the hair cycle and supports follicular regeneration.

Moreover, the expression of interleukin-6 (IL-6), a pro-inflammatory cytokine implicated in hair follicle miniaturization was significantly elevated in the control group compared to all treatment groups. This reduction in IL-6 expression in the PRP-exosome and combination therapy groups suggests that these treatments not only stimulate hair growth but also exert anti-inflammatory effects at the molecular level, contributing to the reversal of follicular damage and prolongation of the anagen phase.

These findings highlight the dual role of PRP-exosomes in promoting follicle regeneration and modulating local inflammation. The ability to suppress IL-6 may offer a new therapeutic target in managing AGA, especially in cases with inflammation-associated follicular regression.

Given the promising results, further research is needed to explore the long-term effects of PRP-exosome therapy, the optimal dosage and frequency, as well as its application in humans. Larger-scale clinical trials and molecular profiling of exosome contents could provide deeper insight into the mechanisms involved and help standardize treatment protocols. In conclusion, PRP-exosomes represent a novel and potentially effective therapeutic approach for androgenetic alopecia with regenerative and anti-inflammatory benefits.

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